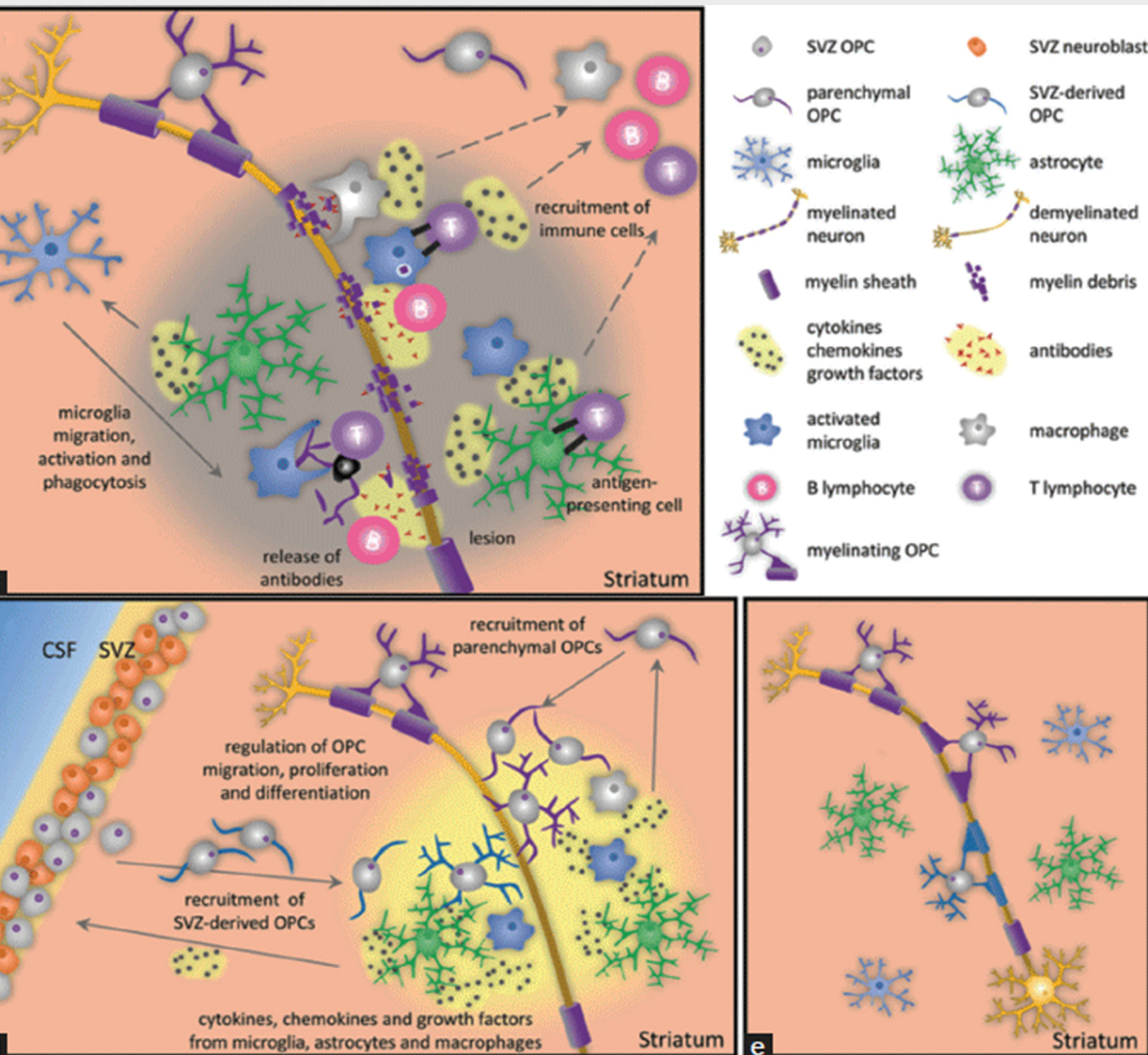


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Targeting glioblastoma with oncolytic adenovirus delta 24

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ONCOLYTIC VIRUSES

Oncolytic viruses have been introduced in cancer treatment in the last two decades, offering new opportunities and hopes for ultimate therapy. Earlier studies had also tried to take advantage of the antineoplastic activity of natural viral species, but it was genetic engineering that led to the production of modified viral strains selectively infecting and killing neoplastic cells.^[1] The two major characteristics of oncolytic viruses are their tropism for malignant cells and their augmented cytotoxicity. The strategies employed to achieve oncotropism involve mainly genetic manipulation of viral vectors in order to either turn them against cells expressing specific surface markers or make them capable to multiply only using the machinery of malignant cells.^[2] Despite the capability of specifically targeting neoplastic cells, oncolytic viruses are, usually, not capable to completely demise all malignant cells neither *in vivo* nor *in vitro*.^[3-5] This inherent limitation of oncolytic viruses is caused principally by restrictions in viral infection due to differential expression of surface viral receptors on different types of malignant cells,^[6] by barriers in viral infection in the organism^[7,8] as well as by the proliferation of noninfected malignant cells and the development of resistance.^[9] In this content, significant effort has been invested to increase the viral cytotoxicity. Thus, genetic engineering has been used to modify viral genome in order to produce molecules toxic for cancer cells, in order to increase the cytopathic effect.^[10] Furthermore, genes have been employed, causing an augmented immune response against virally infected cells,^[11] meanwhile other strategies involve

the modulation of vessel permeability for viruses^[8] and degradation of extracellular matrix.^[12]

DEVELOPMENT OF ONCOLYTIC ADENOVIRUS DELTA 24

Among tens or even hundreds of different viruses that have been used to produce oncolytic viral vectors with different genetic manipulations, oncolytic adenovirus delta 24 (Ad-Δ24) stands out due to its high specificity and toxicity for malignant cells accomplished with simple genetic engineering. Ad-Δ24 is a mutant replication-competent adenovirus containing a 24 base pair deletion in early region 1A gene (E1A), expressing a mutant E1A protein which cannot form complex with the retinoblastoma protein (Rb).^[13,14] Thus Ad-Δ24, unlike wild-type adenovirus, is unable to force the progression of infected normal cells in S phase that is required for its replication. On the other hand, the mutant virus can replicate in cells with disrupted Rb cell cycle control, like glioma cells.^[15] In 2000, the production of Ad-Δ24 was described for the first time along with a detailed description of its cytopathic effect on different glioma cell-lines.^[13] But despite its potent antiglioma effect, it was obvious even from the first studies that Ad-Δ24 would need further improvements for optimal therapeutic effect.^[16]

ADENOVIRUS DELTA 24 DERIVATIVES

A significant number of modifications have been applied on the initial Ad-Δ24 system trying to enhance its specific targeting or to augment its oncolytic potency. The most important effort that has been made to enhance glioma targeting was the insertion of an Arg-Gly-Asp peptide (RGD) in the Ad-Δ24 fiber knob, increasing its affinity with integrins that are highly expressed in gliomas and other tumor cells.^[17] Adenovirus infection in general depends on the initial binding to the coxsackievirus and adenovirus receptor (CAR) on the cell surface, followed by a secondary binding to cell surface integrins.^[6] Thus, the effect of Ad-Δ24

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10.4103/2347-8659.149392

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on glioma cells depends upon the expression of CARs on their surface. The vector produced by the insertion of RGD peptide, named Ad-Δ24-RGD, can overcome this problem since it can directly bind to cell surface integrins.^[17]

Furthermore, a number of studies have focused on augmenting the oncolytic potency of Ad-Δ24. Genetic manipulations were used in order to produce vectors expressing p53,^[18] TRAIL and Arresten,^[19] cytosine deaminase^[20] and the tissue inhibitor of matrix metalloproteinase-3.^[21] All these Ad-Δ24 derivatives are claimed to be superior to the initial vector but their efficacy awaits further confirmation.

COMBINATION TREATMENTS

Other studies have tried to improve the therapeutic effect of Ad-Δ24 and its derivatives through combining treatments with apoptotic and chemotherapeutic agents, as well as radiation. Treatment to glioma with Ad-Δ24 or its derivatives has been observed to enhance when combined with TRAIL,^[16] adenovirus expressing p53,^[22] temozolomide,^[23] radiation^[24] and topoisomerase I inhibitor irinotecan.^[25] Autophagic induced cell death and induction of apoptosis are well-characterized results of Ad-Δ24 infection giving the erratum for combination treatments.^[26]

ANTITUMOR IMMUNE RESPONSE

Another important aspect of oncolytic virotherapy is the induction of augmented antitumor immune response.^[27,28] Ad-Δ24-RGD has been shown to induce antiglioma immunity and to enhance the presentation of tumor-associated antigens to immune cells.^[29] These findings provide the base for further genetic manipulation of Ad-Δ24 in order to drive the production of immunostimulatory factors (like granulocyte-macrophage colony stimulating factor) that can possibly mediate more robust therapeutic effects.^[30]

CLINICAL TRIALS

Ad-Δ24 and its derivatives have been tested in clinical trials in patients with solid tumors, and more studies are in progress. The first results show that these agents are safe, but their antitumor efficacy remains modest.^[30,31] Ad-Δ24-RGD is also being tested in a clinical trial in patients with malignant gliomas and the results are still pending.

CONCLUSION

Ad-Δ24 is a promising agent for glioblastoma treatment. The initial vector developed 14 years ago providing

a platform for further genetic modifications and combination treatments. Since the first clinical trials have assured its safety, it is important for the future research to seek for enhancements in its genome and combining agents that could refine its effect.

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Cite this article as: Tsamis KI, Alexiou GA, Kyritsis AP. Targeting glioblastoma with oncolytic adenovirus delta 24. *Neuroimmunol Neuroinflammation* 2015;2(1):1-3.

Source of Support: Nil. **Conflict of Interest:** No.

Received: 28-08-2014; **Accepted:** 26-09-2014

Integrins and focal adhesion kinase in the malignant behavior of gliomas

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ABSTRACT

Glioblastoma multiforme (GBM) is the most common type of glioma and is associated with a very poor prognosis. The standard treatment includes radiotherapy concurrent with temozolomide, however recently the Food and Drug Administration approved bevacizumab for use in patients with progressive glioblastoma following prior therapy. The limited number of treatment options points to the need for novel effective therapeutic approaches. A promising approach is the use of tyrosine kinase inhibitors (TKIs) in GBM treatment. However, the results from the majority of clinical trials using TKIs are not very encouraging. One growing area is the development of tumor-homing peptides that resemble the integrin recognition sequence RGD. In this article, the role of integrins and focal adhesion kinase in malignant glioma is reviewed, and an experimental study is proposed that will apply a strategy for peptide-mediated delivery of compounds deep into tumor parenchyma using tumor-homing peptides.

Key words: Focal adhesion kinase, glioblastoma multiforme, integrins

INTRODUCTION

Malignant glioma (MG), including glioblastoma multiforme (GBM) and anaplastic astrocytoma, ranks among the most common primary brain tumors. Apart from maximally safe surgical resection, the first-line treatment consists of radiotherapy and concomitant systemic application of chemotherapy-usually with temozolomide-following the Stupp regimen, which represents the standard conventional treatment for GBM. Nonetheless, the median survival time of patients with MGs, and consequently their outcome, remains very poor.^[1-3]

Several mechanisms of GBM resistance to standard chemotherapy have been proposed. The use of chemotherapy has been reported as being limited, due to the fact that the blood-brain barrier restricts the accumulation of conventional cytotoxic agents to therapeutic concentrations in the tumor and the

peritumoral area.^[2-4] Other restricting factors include potential interactions between antiepileptic drugs and chemotherapeutic agents, use of glucocorticosteroids, and the implication of specific genetic transformation and characteristics of GBMs. In particular, the multidrug resistance system is considered to be mainly responsible for the development of treatment resistance.^[4]

PATHOGENETIC ASPECTS OF GLIOBLASTOMA MULTIFORMES

The pathological hallmarks of GBMs include rapid progression, neovascularization, necrosis, and intense apoptotic resistance. Common genetic alterations associated with malignant phenotypic characteristics are commonly found in tumors. However, the molecular mechanisms leading to these phenotypic features are as yet vaguely defined, mainly due to genetic heterogeneity, even within the same tumor.^[5] However, there are some known mutations, deletions, or alterations in gene expression that have been linked to the genesis of GBMs.^[3,6] Several signaling pathways leading to malignant behavior in MGs, induction of cell migration, and tumor invasiveness have also been implicated. These pathways are regulated by amplification and/or overexpression of several growth factor receptors linked with tyrosine kinases, such as the epidermal growth factor receptor (EGFR), insulin-like

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10.4103/2347-8659.149395

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growth factor receptor, platelet-derived growth factor receptor (PDGFR), vascular endothelial growth factor receptor (VEGFR), and hepatocyte growth factor/scatter factor receptor. Several hormones and cytokines are also involved in the regulation of molecular pathways related to GBM development.^[5] Recent data implicate the inflammatory interleukins (IL)-1 β , -6 and -8 in GBM pathophysiology. It has been found that these cytokines are upregulated in GBM cell lines as well as in patients' samples while some of them have high prognostic potential.^[7]

Angiogenesis is considered to be the key regulating factor of vascular development in tumors and especially for GBMs. The development and growth of MGs seem to be dependent on angiogenesis since microvascular proliferation can only be observed in high-grade gliomas.^[2,8-10] Apart from growth factors and their receptors, other molecules that significantly contribute to angiogenesis in gliomas and growth of GBMs are the integrins and focal adhesion kinase (FAK).

INTEGRINS

Integrins are cell surface receptor glycoproteins, mediating various intracellular signals through interaction with the extracellular matrix (ECM). Integrins also significantly contribute to the attachment of cells to the ECM through the formation of cell adhesion complexes consisting of integrins and cytoplasmic proteins. There are many types of integrins, which are obligate heterodimers containing two distinct chains called α and β subunits. The combination of α and β subunits determines the ligand specificity.^[11-13]

Integrins are crucial molecules in glioma because of their contribution to enhanced invasion capacity in glioma cells. This phenotype can be defined by three attributes. The cells at the invasive edge of the tumor are able to: (i) detach and migrate forward; (ii) adhere via local and self-produced ECM; and (iii) degrade the local/surrounding ECM in order to clear a path for further invasion. Since integrins are integral to the process of cell adhesion and migration, these receptors have been assessed as potential contributors to glioma invasion, as have been the cooperating ECM components.^[11] Multiple integrins have been reported to be expressed on GBM in tissue biopsies, including $\alpha_v\beta_3$, $\alpha_v\beta_5$, $\alpha_5\beta_1$, $\alpha_2\beta_1$, $\alpha_3\beta_1$, $\alpha_6\beta_1$, and $\alpha_v\beta_1$.

Functional studies with blocking antibodies directed toward the β_1 integrin subunit have shown an inhibition of adhesion, motility, and invasion of cultured glioma cells plated on multiple ECM substrates (laminin, collagen type IV, fibronectin, and vitronectin), suggesting a role for one or more β_1 integrins in neoplastic glial

cell migration into the brain.^[12] Another study utilizing neutralizing antibodies directed toward the α_v or the β_1 integrin subunits reported complete inhibition of GBM cell migration by most substrates, suggesting the α_v and β_1 integrins play a crucial role in GBM tumor cell infiltration into the normal brain.^[12]

Integrins modulate several functions of GBM cells-including survival, adhesion, and migration-through interaction between growth factors and their receptors with subsequent formation of complexes. There is robust evidence suggesting the formation of analogous complexes in different types of cells, such as complexes of various types of integrins with VEGFR-2, PDGFR- β , and EGFR.^[14,15]

FOCAL ADHESION KINASE

Focal adhesion kinase has been recently established as a key component of the signal transduction pathways triggered by integrins. FAK not only acts directly on the plasticity of cytoskeletal structures at focal adhesions, but also mediates effects on gene expression that indirectly alter the ability of cells to migrate and invade.^[13] The interaction of urokinase-type plasminogen activator receptor (uPAR) with integrins during cell adhesion and migration has also been proposed. uPAR binds the urokinase-type plasminogen activator (uPA) and facilitates a proteolytic cascade focused on the cell surface. uPAR has recently been recognized as a multifunctional protein that, through its interactions with integrins, initiates signaling events that alter cell adhesion, migration, and proliferation of various cancer cells, including GBM cells.^[11,12,16]

All these molecular and genetic alterations contribute to the well-established biological features of GBMs and may provide a target to enhance therapeutic responsiveness of these lethal brain malignancies. Recent advances in thorough understanding of the complex molecular pathogenesis of GBMs have led to the rational development of new treatment options targeting intracellular signaling.^[1,2] Despite these advances, most single-agent therapies targeted to growth and survival pathways have failed to demonstrate a significant survival benefit, mainly because of the complexity of the implicated signaling pathways and their interactions. Thus, targeting multiple signaling pathways by multi-target kinase inhibitors or combinations of single-target kinase inhibitors may increase treatment efficacy. Multi-targeted agents are needed to simultaneously target multiple signaling pathways that occur either at the same time or sequentially, as a compensatory mechanism to tumor growth and resistance to treatment.^[1] Currently, several

multi-target kinase inhibitors and combinations of single-targeted kinase inhibitors that simultaneously affect multiple pathways such as signaling, repair, and angiogenesis have been tested in clinical trials for their ability to effectively prolong the median survival time of patients and to establish future directions in GBM therapy. Overall, the identification of new targeted strategies for GBMs remains a very challenging area in the field, since it has the potential to positively affect patient outcome, survival rate, and quality of life.

Preclinical, as well as clinical studies with various integrin antagonists, have demonstrated their effectiveness in blocking tumor progression. Phase II clinical trials with cilengitide (Merck KGaA, Darmstadt, Germany), an $\alpha_v\beta_3$ and $\alpha_v\beta_5$ integrin antagonists, have shown clinical activity and few side effects in patients with glioblastoma.^[17] Cilengitide is a synthetic Arg-Gly-Asp (RGD) pentapeptide recognizing the RGD ligand binding motif on the integrin receptors $\alpha_v\beta_3$ and $\alpha_v\beta_5$ ^[18] and competitively blocks integrin ligand binding. It has been shown to diminish angiogenesis *in vitro*.^[19] In an important early preclinical study, cilengitide markedly suppressed tumor growth in amedulloblastoma and orthotopic glioblastoma models (i.e., when tumors were grown in the brain), whereas no growth inhibition was demonstrated in a heterotopic model (i.e., when tumors were grown in the flank of nude mice) or when an inactive peptide was used.^[20] This suggests that the brain environment is particularly susceptible to the integrin inhibition and has led to subsequent clinical investigation.^[21]

In addition, a new variant of RGD (internalizing RGD, iRGD) that combines the RGD motif with a tissue penetration element called C-end rule (CendR) has been recently presented.^[22] Like the earlier RGD peptides, iRGD homes to tumors, but exposure of the CendR motif activates a transport system through tumor blood vessel walls into the tumor core. Interestingly, it was shown that coupling of iRGD to anti-cancer drugs allowed them to penetrate deeply into tumors, effectively increasing the activity of the drugs.^[22] More specifically, intravenously injected compounds coupled to iRGD bound to tumor vessels and spread into the extravascular tumor parenchyma, whereas conventional RGD peptides only delivered the cargo to the blood vessels. iRGD homes to tumors through a three-step process: the RGD motif mediates binding to α_v integrins on tumor endothelium, and a proteolytic cleavage then exposes a binding motif for neuropilin-1, which mediates penetration into tissue and cells. Conjugation to iRGD significantly improved the sensitivity of tumor-imaging agents and enhanced the activity of an antitumor drug.

CONCLUSION

Based on the information provided above, we propose an experimental study that will apply a strategy for peptide-mediated delivery of compounds deep into tumor parenchyma using tumor-homing peptides. Selected tyrosine kinase inhibitors such as imatinib will be coupled to tumor-specific homing peptides and will be used for the treatment of various GBM cell lines. Such targeted delivery of the antitumor agent can result in higher drug concentrations in tumors, increasing drug efficacy and reducing peripheral toxicity, thus overcoming the chemo-resistance of cancer cells, which is usually mediated by membrane transporters. This initiative is expected to result in new drug candidates by obtaining reliable data on the molecular pathogenesis of GBMs and molecular-targeted treatment options for GBMs. The development of drug candidates involving a delivery system based on previous knowledge and targeting intracellular pathways would facilitate the identification of more effective treatment options, thereby positively affecting the outcome, survival rate, and quality of life in patients with GBM.

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Cite this article as: Giannopoulou E, Tzakos A, Argyriou AA. Integrins and focal adhesion kinase in the malignant behavior of gliomas. *Neuroimmunol Neuroinflammation* 2015;2(1):4-7.

Source of Support: Nil. **Conflict of Interest:** No.

Received: 01-09-2014; **Accepted:** 16-10-2014

A potential role of karyopherin a2 in the impaired maturation of dendritic cells observed in glioblastoma patients

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ABSTRACT

Aim: Patients with glioblastomas demonstrate well-documented immunological impairments including decreased numbers of mature dendritic cells (DCs). Recent data identified karyopherin a2 (KPNA2), a nucleocytoplasmic shuttling receptor, as diagnostic and prognostic biomarker for gliomas. The aim of this ongoing study is to correlate parameters of immunity and nucleocytoplasmic transport in glioblastoma patients. **Methods:** We preoperatively collected serum from 17 patients with glioblastomas and determined DC subsets (HLA DR+ Lin-, CD34-, CD45+, CD123+, CD11+ were analyzed) using a 6-color flow cytometry panel. Expression levels of KPNA2 and nuclear accumulation of p53 were evaluated semi-quantitatively by immunohistochemistry. O⁶-methylguanine DNA methyltransferase (MGMT) and isocitrate dehydrogenase-1 (IDH-1) status were assessed by pyrosequencing and immunohistochemistry, respectively. **Results:** Median expression levels for both KPNA2 and p53 were 5-10%. IDH-1-R132H mutation and MGMT promoter hypermethylation was detected in 3/16 and 1/9 patients, respectively. Mean counts of total mature DCs, myeloid DCs and plasmacytoid DCs were 9.6, 2.1, 3.4 cells/ μ L. A preliminary analysis suggests an association between low KPNA2 nuclear expression and increased numbers of mature DCs. However, this correlation did not reach statistical significance so far ($P = 0.077$). **Conclusion:** Our preliminary data may indicate a role of KPNA2 in the impaired maturation of DCs observed in glioblastoma patients.

Key words: Glioblastomas, isocitrate dehydrogenase-1, karyopherin a2, mature dendritic cells, O⁶-methylguanine DNA methyltransferase, p53

INTRODUCTION

Patients with glioblastomas demonstrate well-documented impairments of their immune system, including reduced values of mature dendritic cells (DCs).^[1-4] DCs is the most potent antigen-presenting cell population and therefore key regulators of adaptive immunity. Apart from their defense against infectious diseases they may also mediate antitumor responses.^[5,6] Their maturation/differentiation plays a pivotal role in their function. After recognizing and capturing

antigens/tumor cells they undergo maturation, i.e., they upregulate major histocompatibility complex class II and co-stimulatory molecules at cell surface, they migrate to T cell rich zones and secrete cytokines to induce an antigen-specific T cell response.^[7]

DCs maturation may be mediated by known signaling pathways/transcription factors, for instance, nuclear factor- κ B (NF- κ B), which is triggered after activation of the toll-like receptors (TLRs) by invading microorganisms.^[7] Once in the nucleus, NF- κ B may induce the transcription of various genes involving in immune and inflammatory responses.^[8] Nuclear import of NF- κ B is mediated by the karyopherin a2/importin unit 2 complex (KPNA2), a well-studied member of the family of karyopherins.^[9]

Karyopherins are nucleocytoplasmic shuttling receptors and comprise importins and exportins. They

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have been linked to signal transduction pathways and cell cycle control mechanisms.^[10-13] KPNA2 mediates the nuclear import of large molecules (> 40 kDa, most of the proteins and RNAs) by binding to a specific recognition sequence called the nuclear localization signal (NLS). After entering the nucleus, the NLS-containing macromolecule is dissociated by RanGTP and KPNA2 recycles back to the cytoplasm.

Recent data suggests a role for the nucleocytoplasmic transport, in particular for KPNA2, in gliomagenesis. We have previously identified low expression of KPNA2 as an independent prognostic factor for better overall survival (OS) and progression-free survival (PFS) in patients with infiltrative gliomas.^[14,15] KPNA2 has been also recognized as a prognostic factor in patients with meningiomas^[16] as well as in patients with other solid tumors.^[17-20]

The aim of our work is to investigate the role of nucleocytoplasmic import and of other known biomarkers in the maturation procedure of DCs. In a recent work, we analyzed the preoperative phenotype of DCs in patients with gliomas.^[4] In the present ongoing study we determined also parameters of nucleocytoplasmic import (KPNA2) as well as other glioma-associated molecular markers such as *O*⁶-methylguanine DNA methyltransferase (MGMT) promoter hypermethylation, isocitrate dehydrogenase-1-R132H (IDH-1-R132H) gene mutation status and nuclear accumulation of p53 within the tissue specimens and analyzed a putative correlation between them.^[21-23] Our preliminary results imply a possible role of KPNA2 in the known impaired maturation of DCs in patients with glioblastomas.

METHODS

Patients and clinical characteristics

We analyzed preoperatively collected fasting morning serum from 17 consecutive adult (median age: 54 years, range: 33-78 years; 58.8% male) patients with subsequently histologically confirmed *de novo* glioblastomas operated at the Department of Neurosurgery of the University Hospital of Bonn between November of 2010 and February of 2011 for DC subpopulations. In addition, surgical specimens from our patients were analyzed for expression of KPNA2 (*n* = 16) and nuclear accumulation p53 (*n* = 17) as well as for IDH-1-R132H mutation status (*n* = 16) and MGMT promoter hypermethylation (*n* = 9). Nonneoplastic brain tissues from two patients who underwent surgery for epilepsy served as controls. The circadian rhythm of the immunological parameters and the possible effect of dexamethasone administration were considered at the blood collection as previously described.^[4]

All tumors were located in the supratentorial compartment. Three (17.6%) patients had a diagnostic biopsy only due to the eloquent location of the tumor and 35.3% of resections were gross total. All patients underwent chemo- and radiotherapy after surgery. The demographics of our study population are shown in Table 1. Patients with a history of previous brain tumor or other cancer, radio- or chemotherapy or of an immunological or hematological disease were excluded from the study. The patients' samples were collected after their informed consents were obtained in accordance with the tenets of the declaration of Helsinki and approval of the study by the Ethics Committee of the Medical Faculty of the University of Bonn.

Flow cytometry

Dendritic cell and T-lymphocyte subpopulations values were determined by flow cytometry using six different fluorochromes: fluorescein isothiocyanate (FITC), phycoerythrin (PE), peridininchlorophyllprotein (PerCP), allophycocyanin (APC), PE-Cy7 (PE-Cy7) and APC-Cy7. The following surface and intracellular anti-human monoclonal antibodies were used: CD45-APC Cy7 (clone 2D1), CD4-PE (clone RPA-T4), CD3-PerCP (clone SK7), HLA-DR-PerCP (clone L243), CD11c-APC clone (S-HCL-3), lineage-FITC (lin-1 cocktail), CD34-FITC (clone 8G12), CD123-PE-Cy7 (clone 6H6, ebioscience, San Diego, CA) as well as isotype controls.

Cells were surface stained according to the manufacturers' protocols. DCs were isolated as previously described.^[4] Briefly, DCs were gated as HLA-DR (MCH class II) positive, lineage-negative, CD34 negative, and CD45 positive (HLA DR+/lin-/CD34-/CD45+). DCs were further subclassified as myeloid DCs (mDCs) or plasmacytoid DCs (pDCs) based on their reciprocal

Table 1: Patient demographics and tumor characteristics

Variable	Absolute numbers (%)
Number of patients	17
Median age (range)	54 (33-78) years
Males	10 (58.8)
Maximum tumor diameter* ≤ 3 cm	7 (41.1)
Resection**	
GTR	6 (35.3)
STR	8 (47.0)
Biopsy	3 (17.6)
Preoperative KPS: 90-100%	12 (70.5)
Postoperative KPS: 90-100%	11 (64.7)
Preoperative seizures: yes	6 (35.3)
Eloquence***: Yes	10 (58.8)
Radiotherapy: Yes	17 (100)
Chemotherapy: Yes	17 (100)

*Maximum tumor diameter has been defined as the longest (any) diameter of contrast enhancing mass area in postcontrast T1-weighted MRI datasets; **Extent of resection was classified according to the postoperative MRI (2-3 days after surgery); ***Tumors were categorized as eloquent if they were growing into the primary sensorimotor or visual cortex, Broca's or Wernicke's area/the dominant angular gyrus area, the basal ganglia, thalamus or internal capsule. MRI: magnetic resonance imaging; KPS: karnofsky performance score

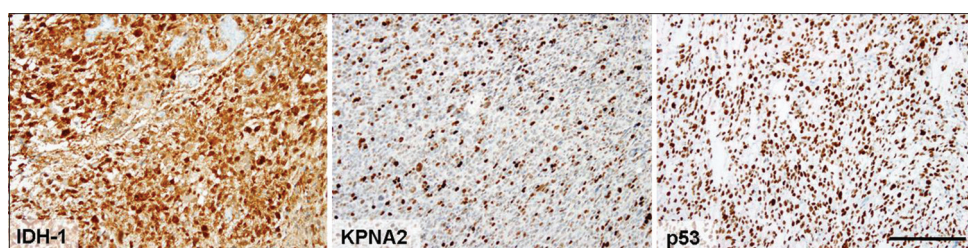


Figure 1: Exemplary images of immunohistochemical evaluation of isocitrate dehydrogenase (IDH-1 [R132H]) and karyopherin $\alpha 2$ (KPNA2) expression as well as nuclear accumulation of p53 protein. Immunohistochemical staining with antibodies against mutated IDH-1 (R132H) shows a strong cytoplasmic immunoreactivity in the tumor cells. Nuclear expression of KPNA2 is detected in a subpopulation of tumor cells. Nuclear p53 accumulation can be observed in > 50% of the tumor

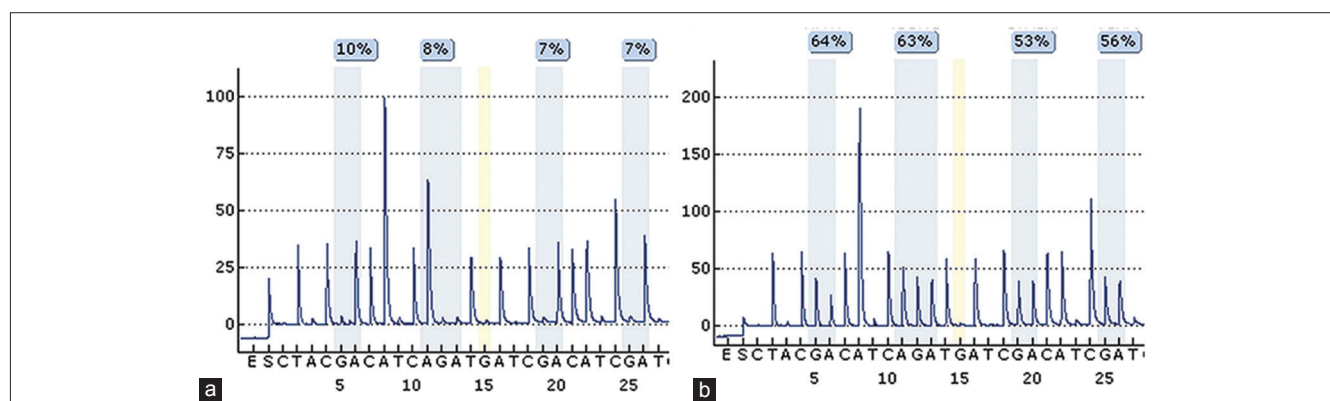


Figure 2: Pyrosequencing was used for quantitative analysis of C^5 -methylguanine DNA methyltransferase promoter methylation. Pyrogram demonstrating (a) an unmethylated and (b) methylated glioblastoma tissue. Each colored box includes one of the four studied CpG positions (CpGs 9-12). The incorporation of the bases guanine and adenine represent the methylated and unmethylated fractions, respectively. The percentages given in both pyrograms reflect the methylated fractions (fractions over 10% define a methylated sample)

expression of CD11c (a-integrin) and CD123 (IL-3 receptor α), respectively. Figures 1 and 2 of our previous publication illustrate the classification/gating steps.^[4] HLA-DR, CD11c, CD45, CD123 are co-stimulatory surface molecules, which have been identified in the relevant literature as markers of maturation.^[24-27] CD34 is a marker for all stem cells and the proportion of precursor DCs.^[28] Therefore, HLA DR+/Lin-/CD34-/CD45+ were defined as total mature DCs. HLA DR+/Lin-/CD34-/CD45+/CD123-/CD11c- represent less mature DCs, whereas HLA DR+/Lin-/CD34-/CD45+/CD123+/CD11c+ mDCs and HLA DR+/Lin-/CD34-/CD45+/CD123+/CD11c- pDCs mature DCs in advanced stages of maturation.

Immunohistochemistry

Neuropathological analysis of glioblastomas comprised hematoxylin/eosin staining as well as immunohistochemistry with monoclonal antibodies directed against the microtubule-associated protein 2 (MAP2, Sigma, Steinheim, Germany), polyclonal antibodies directed against glial fibrillary acid protein (GFAP, Sigma, Steinheim, Germany) and monoclonal antibodies directed against Ki67 (MIB1, Dako, Glostrup, Denmark).

IDH-1-R132H mutation status (monoclonal mouse antibody H09 directed against mutated IDH-1 R132H mutation, Dianova, Hamburg, Germany) and KPNA2 immunoreactivity (goat polyclonal SC6917; Santa Cruz Biotechnology, Santa Cruz, USA; dilution 1:100) were

assessed and visually scored independently by two experienced neuropathologists (PN, GHG) as previously described [Figure 1].^[14] Immunohistochemical staining with a monoclonal antibody against p53 (clone DO-7, Dako, Glostrup, Denmark), in a dilution of 1:150, were performed on the Ventana Immunostainer (Roche, Mannheim, Germany), with a closed avidin-biotin complex Ventana Detection System (Ventana). Positive and negative controls were also performed using glioblastoma tissue with p53 overexpression [Figure 1]. Tumors were assigned to immunoreactivity classes of KPNA2 and p53 based on the percentage of moderately or strongly immunopositive cell nuclei (< 1%, 1%-< 5%, 5%-< 10%, 10%-< 20%, 20%-< 50% and \geq 50%).

Pyrosequencing

The quantitative analysis of MGMT promoter methylation by pyrosequencing was performed as previously described.^[29] Briefly, the first four CpG sites are assayed for a primer extension reaction. Methylated fractions > 10% at all positions define a methylated sample [Figure 2].

Statistical analysis

Statistical analyses of the data were performed using commercially available software (SSPS 21.0, IBM Deutschland, Ehningen, Germany). Comparisons of samples were performed using standard methods (Pearson's Chi-square, Fisher's exact test). $P < 0.05$ (two-tailed) were considered to be statistically

significant. Cut-off values for nonparametric statistics were set at the median of each variable, that is, the studied subgroups were as following KPNA2: < 5% vs. ≥ 5% positive cell nuclei; p53: < 5% vs. ≥ 5% positive cell nuclei; total mature DC: < 5.9 vs. ≥ 5.9 cells/μL; pDC: < 1.6 vs. ≥ 1.6 cells/μL; mDC: < 0.6 vs. ≥ 0.6 cells/μL; age: < 54 vs. ≥ 54; preoperative and postoperative Karnofsky performance index (KPI): < 90 vs. ≥ 90.

RESULTS

The relative proportions of total mature DCs as well as subsets of mature DCs in patients with glioblastomas were as following: total DCs as proportion of WBC: 0.11% (0.05-0.16%), pDC CD123+ CD11c- as proportion of total DCs: 28.3% (16.5-40.1%), mDC CD123- CD11c+ as proportion of total DC: 15.5% (6.0-25.1%), CD123-CD11c- as proportion of total DCs: 54.5% (38.7-70.3%). Similarly, counts (mean, 95% CI, median) (cells/μL) of mature DCs were: total mature DCs: 9.6 (4.3-14.9), 5.9, pDC CD123+ CD11c-: 3.4 (1.1-5.8), 1.6, mDC CD123- CD11c+: 2.1 (0.6-3.6), 0.6 [Table 2].

Median expression levels for both KPNA2 and p53 were 5-10% [Figure 3]. IDH-1-R132H mutations were detected in 3/16 patients. All glioblastoma patients with mutant IDH-R132H experienced a sudden onset (< 3 months) of their symptoms, which imply that these patients harbored primary rather than

secondary glioblastomas. Representative results of KPNA-, p53- and IDH-1-immunohistochemistry are shown in Figure 1. MGMT promoter hypermethylation was observed in 1/9 tumors [Figure 2].

A preliminary analysis suggests an association between lower KPNA2 nuclear expression and increased numbers of mature DC. All patients with low KPNA2 (< 5%) expression and only 33.3% of patients with KPNA2 ≥ 5% demonstrated counts of mature DCs over the median (≥ 5.9 cells/μL). However, this correlation did not reach statistical significance so far (total mature DC ≥ 5.9 cells/μL: KPNA2 < 5% vs. ≥ 5%/100% vs. 33.3%, Pearson's Chi-square: $P = 0.038$, Fisher's exact test: $P = 0.077$, both two-sided; Fisher's exact test is most appropriate, since study population is limited) [Figure 4].

A trend between KPNA2 expression and IDH-R132H mutation status has been observed. Patients expressing lower KPNA2 exhibit also frequently mutant IDH-1-R132H (mutant IDH-1-R132H: KPNA2 < 5% vs. ≥ 5%/66.7% vs. 7.7%, Pearson's Chi-square: $P = 0.018$, Fisher's exact test: $P = 0.071$, both two-sided) [Figure 4]. No mutant IDH-1-R132H status was seen in patients with KPNA2 ≥ 10%. No other significant correlations between expressions of KPNA2, p53, MGMT promoter hypermethylation and IDH-1-R132H mutation status have been observed.

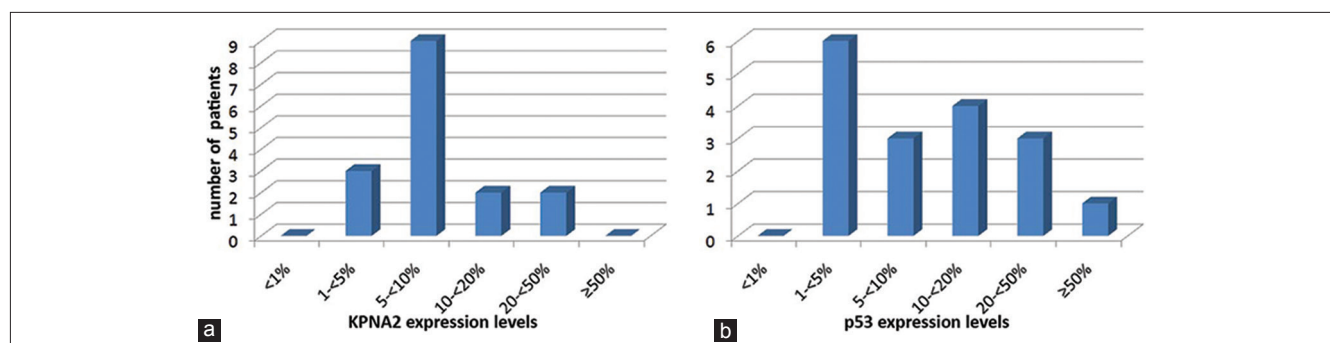


Figure 3: Frequency distribution bar diagram illustrating the nuclear expression levels of (a) karyopherin a2 (KPNA2) and (b) p53. KPNA2 and p53 expression were analyzed for nonparametric statistics as dichotomized variables with their respective median (5% < 10% for both variables) being set as cut off value. Thus, low KPNA2 and low p53 expression were defined as expression levels < 5%

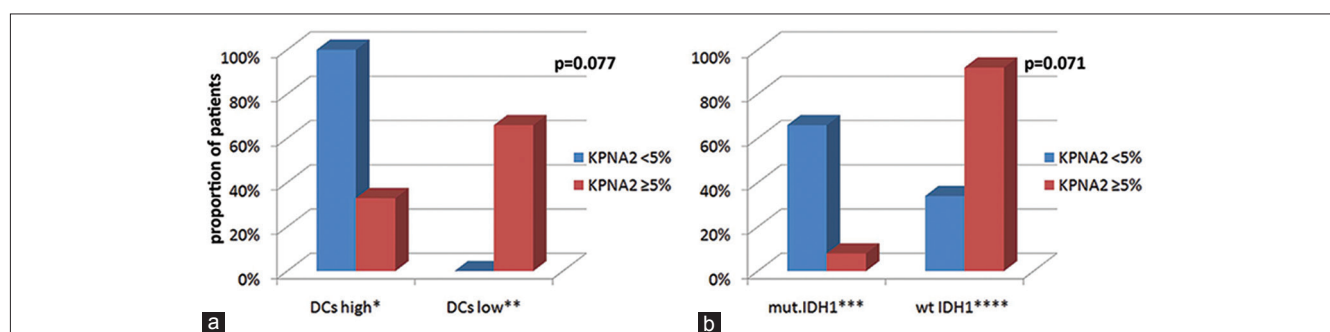


Figure 4: Correlations bar diagram. The y axis shows the relative proportion of our cohort with different karyopherin a2 (KPNA2) expression demonstrating (a) *high (≥ 5.9 (median)) vs. **low (< 5.9 cells/μL) DCs and (b) ***mutant vs. ****wild type isocitrate dehydrogenase-1 (IDH-1) R132H status. (a) All patients with low KPNA2 (< 5%) compared to only 33.3% of the patients with KPNA2 ≥ 5% demonstrated DCs high ($P = 0.077$). (b) Mutant IDH-1 status was seen more frequently in patients with low (< 5%) than in those with KPNA2 ≥ 5% (66.7% vs. only 7.7%, $P = 0.071$)

No significant imbalances between subgroups of KPNA2 and p53 expression as well as MGMT promoter methylation and IDH-1 mutation status (see statistical analysis) with respect to age, gender, type of resection (resection vs. biopsy), preoperative and postoperative KPI, tumor volume, presence of epilepsy (yes/no), neurological deficits (yes/no) and eloquence of the tumor location (yes/no) were seen [Table 3].

DISCUSSION

Patients with glioblastomas exhibit well-documented immunological abnormalities; in particular, they demonstrate an impaired cellular immunity; that is, reduced counts of effector T helper and relative accumulation of suppressive T regulatory cells.^[2,4] The APC function of DCs, as reflected by the ability to stimulate allogeneic T cells, is also altered/diminished.^[30] Patients with glioblastomas demonstrate also reduced values of mature DCs compared to patients with gliomas WHO grade I-III or healthy donors.^[4]

The maturation of DCs is the key regulator of their APC function, comprises several stages (stem cells/precursors/poorly differentiated/highly differentiated not activated/highly differentiated and activated/apoptosis) and includes the up regulation in the nucleus of MCH class II and specific co-stimulatory molecules, such as CD11c, CD45, CD83, CD86 and CD123.^[7,24-27] This up regulation may be generally triggered by transcription factors/stimuli (macromolecules > 40 kDa), which are being actively translocated from the cell surface (that is, side of “danger signal” production upon recognition of invading microorganisms/antigens) into the nucleus.^[7,8] Consequently, molecules involved in the nuclear import

of these transcription factors, such as karyopherins, may also affect the maturation procedures.

Recognition receptors of immature DCs, such as TLRs, may be triggered by invading microorganisms or endogenous inflammatory signals and on their turn they may activate signaling pathways/transcription factors, such as NF-κB, in order to foster their maturation. This pathway is among others characterized by the proteolytic processing of NF-κB p100 protein to p52 and the translocation of the latter in the nucleus.^[31,32] Lind *et al.*^[31] studied NF-κB pathway *in vivo* in alymphoplasia (Aly) and wild type (WT) mice. Aly mouse expresses mutant molecules that prohibit the induction of NF-κB pathway and demonstrate an impaired cross-presentation of antigens. Aly failed to translocate p52 to the nucleus after activation with CD40, whereas a normal nuclear p52 translocation occurs in WT.^[31] The nuclear import of p52 is mediated by members of karyopherin family proteins (α1-α5, α7).^[9,32]

Karyopherins are nuclear proteins involved in nucleocytoplasmatic shuttling and have been linked to tumorigenesis. KPNA2 has been identified as a regulator of DNA repair proteins and an activator of apoptosis pathways.^[10-13] Recent data suggested a role for KPNA2, also in gliomagenesis. We have recognized in two recent works about KPNA2 as an independent prognostic factor for OS and PFS in 94 patients with infiltrative astrocytomas WHO grade II-IV as well as in 72 patients with anaplastic gliomas (astrocytomas, oligoastrocytomas and oligodendrogliomas WHO grade III).^[14,15] KPNA2 has been also identified as a prognostic factor in patients with meningiomas^[16] as well as in patients with other solid tumors.^[17-20]

Table 2: Relative proportions and counts of T-lymphocytes, T-helper lymphocytes and DC subsets in our series

Immunological parameter	Relative proportion mean (95% CI)	Counts (cells/μL) mean (95% CI), median
T cell lymphocytes CD3+ %WBC*	6.58 (3.24-9.92)	554 (348-760), 488
T helper lymphocytes CD4+ %WBC	4.16 (1.90-6.42)	348 (199-497), 225
DCs HLA DR+/CD34-/CD45+ %WBC	0.11 (0.05-0.16)	9.6 (4.3-14.9), 5.9
pDCs CD123+ CD11c- %DC**	28.3 (16.5-40.1)	3.4 (1.1-5.8), 1.6
mDCs CD123-, CD11c+ %DC	15.5 (6.0-25.1)	2.1 (0.6-3.6), 0.6
CD123-, CD11c- %DC	54.5 (38.7-70.3)	10.1 (0.4-21.4), 2.1

*% WBC: relative proportion of WBCs; **% DC: relative proportion of DCs. DCs: dendritic cells; WBC: white blood cell; CI: confidence interval; HLA: human leukocyte antigen; DR: diabetic retinopathy

Table 3: Correlations between levels of immunoreactivity (p53 and KPNA2) and clinical characteristics

Variable	p53 < 5% vs. ≥ 5%, P	KPNA2 < 5% vs. ≥ 5%, P
Males	57.1% vs. 60.0%, n.s*	66.7% vs. 53.8%, n.s
Max tumor diameter ≤ 3 cm	71.4% vs. 25.0%, n.s	33.3% vs. 54.5%, n.s
Resection		
Cytoreductive surgery	57.1% vs. 88.9%, n.s	66.7% vs. 83.3%, n.s
Diagnostic biopsy	42.9% vs. 11.1%, n.s	33.3% vs. 16.7%, n.s
Preoperative KPS: 90-100%	57.1% vs. 22.2%, n.s	66.7% vs. 33.3%, n.s
Postoperative KPS: 90-100%	42.9% vs. 30.0%, n.s	66.7% vs. 30.8%, n.s
Preoperative seizures: yes	42.9% vs. 33.3%, n.s	66.7% vs. 25.0%, n.s
Eloquence: yes	57.1% vs. 66.7%, n.s	66.7% vs. 58.3%, n.s

*n.s: no significant; P > 0.05. KPNA2: karyopherin α2; KPS: karnofsky performance score

In the present ongoing study, we determined and correlated DC subpopulations as well as expression of KPNA2 in patients with glioblastomas. Since KPNA2 is thought to mediate the nuclear import of certain transcription factors, which may induce the maturation of DCs, a certain correlation has been expected. Indeed, our preliminary analysis suggests an association between low KPNA2 nuclear expression and increased numbers of mature DCs. However, this correlation did not reach statistical significance so far (Fisher exact test, $P = 0.077$) probably due to the limited studied population. The observed inverse correlation between KPNA2 expression and counts of mature DCs is not surprising, since higher KPNA2 expression^[14] and decreased counts of mature DCs^[4] have been both found to characterize patients with malignant gliomas. The idea of the possible role of KPNA2 also in the regulation of the immunity of glioblastoma patients is tempting; in such a case an additional therapeutic target for the immunotherapy may have been identified.

To our best knowledge, this is the first study focusing on the role of importins in the maturation of DCs. Some evidence of a role of karyopherins (only exportins) in the function of DCs has been previously elucidated.^[33] Chemnitz *et al.*^[33] studied *in vitro* the role of the exportin chromosome region maintenance protein 1/exportin 1(CRM1) in the maturation and activation of DCs. Inhibition of CRM1 by Leptomycin B down regulated the expression of the co-stimulatory molecule CD83 and abrogated the ability of allogeneic T cell stimulation.^[33]

Established prognostic molecular biomarkers, such as MGMT promoter methylation and IDH-1 mutation status were also included in our analysis. Patients with low KPNA2 expression exhibit frequently (not statistically significant) mutant IDH-1-R132H (Fisher's exact test: $P = 0.071$). The clinical history of the patients with mutant IDH-1-R132H status (sudden onset of symptoms < 3 months) does not suggest a secondary genesis of glioblastomas. However, a possible association between lower KPNA2 expression and genesis of secondary glioblastomas could not be excluded. An inverse correlation of KPNA2 expression and IDH-1 immunostaining in patients with malignant gliomas was found also previously.^[14]

Furthermore, we tested whether expression of KPNA2 and DC subpopulations correlated with clinical factors, such as gender, age, preoperative and postoperative Karnofsky Index, preoperative presence of seizures or neurological deficits, tumor diameter, tumor eloquence and degree of resection. No significant associations were found.

We presented the preliminary analysis of our ongoing study on the immunity of patients with glioblastomas. Our first results comprise a limited studied population; therefore, far reaching conclusions may not be drawn. However, our data may be taken into consideration in order to design future larger relevant studies, in particular animal models with knock out techniques that may further clarify the role of importins in the maturation of DCs and in general in the immunological abnormalities observed in patients with glioblastomas.

ACKNOWLEDGMENTS

We thank Alexandra Breuer for her excellent assistance and technical support.

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Cite this article as: Gousias K, von Ruecker A, Gielen GH, Niehusmann P, Waha A, Vatter H, Simon M. A potential role of karyopherin a2 in the impaired maturation of dendritic cells observed in glioblastoma patients. *Neuroimmunol Neuroinflammation* 2015;2(1):8-14.

Source of Support: Nil. **Conflict of Interest:** No.

Received: 20-08-2014; **Accepted:** 16-09-2014

Serum IgE levels in patients with intracranial tumors

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ABSTRACT

Aim: Several epidemiological studies have shown an inverse correlation between allergy and brain cancer. The purpose of this study was to compare the serum IgE levels between patients with gliomas and nonglial tumors and their possible prognostic role. **Methods:** A total of 84 patients with intracranial tumors were included in this study. At clinical presentation, estimation of serum IgE levels was assessed by nephelometry. Detailed information regarding the history of allergies was collected by interview. **Results:** Of the 84 cases, 42 were gliomas, 23 were meningiomas, 16 were metastases and 3 were primary central nervous system lymphomas. Patients with high-grade glioma had lower IgE levels than patients with low-grade glioma. Patients with glioma and meningioma had statistically significant lower serum IgE levels than patients with metastases. Patients with glioblastoma with serum IgE levels greater than 24 U/mL had a better survival. **Conclusion:** Patients with glioma and meningioma had lower IgE levels than patients with metastatic lesions. A prognostic role of serum IgE levels was found in glioblastoma. Further studies in larger patient series are required in order to verify our preliminary observations.

Key words: Glioma, IgE, meningioma, metastasis

INTRODUCTION

Several epidemiological studies have shown an inverse correlation between allergy and brain cancer.^[1,2] The exact mechanism of how allergy protects is largely unknown. Plasma IgE levels are a marker of allergy. Patients with gliomas have been reported to have lower IgE levels than controls.^[2] Furthermore, meningioma patients were found to have lower total serum IgE levels than controls, and an inverse correlation was also found between risk of meningioma and history of allergies.^[3] To the best of our knowledge, no previous study has examined if significant similarities or differences in serum IgE levels exist among patients with different intracranial tumors. Thus, we aimed to investigate serum IgE levels in patients with different intracranial tumors and a possible prognostic value of serum IgE levels in patients with glioblastoma.

METHODS

We prospectively studied patients who were operated on for brain tumors in our institute, between April 2010 and February 2014. Patients were included if assessment of IgE levels was performed at first presentation before receiving any treatment. Detailed information regarding the history of allergies was collected by interview using a questionnaire. Questionnaire included the following allergens: foods, dust, animals, plants, stinging or biting insects, cosmetics, drugs, and mold. A history of asthma was also recorded. Personal information, including smoking, alcohol consumption and diet was also included. Serum total IgE levels were measured for all patients by nephelometry (Immage 800, Beckman Coulter). Ethics approval was given by the review board of University of Ioannina.

Statistical analysis

Mann-Whitney *U*-test was used for two-group comparison. Chi-square and Fisher exact tests were used for categorical independent variables. Survival time was defined as the time between the date at diagnosis and the date of death for deceased patients or to the last follow-up of the surviving patients. The overall survival time was estimated using Kaplan-Meier methods, and log-rank analysis was performed to compare survival curves between groups. Patients

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who were still alive at last contact were treated as censored events in the analysis. A two-sided $P < 0.05$ was considered to be statistically significant.

RESULTS

Tables 1 and 2 summarize the patient data. Eighty-four patients (44 males, 40 females, mean age 59.3 ± 13.3) met the inclusion criteria for the study. All patients had a single space-occupying lesion and were operated. The histological diagnosis were 42 gliomas (31 glioblastomas, 4 gliosarcomas, 3 anaplastic astrocytomas, 1 Grade II astrocytoma, 1 pilocytic astrocytoma, 1 oligodendroglioma and 1 oligoastrocytoma), 23 meningiomas (20 benign and 3 atypical meningiomas), 16 metastases (10 lung cancer, 1 ovarian cancer, 2 colon cancer, 1 melanoma and 2 of unknown primary origin) and 3 primary central nervous system lymphomas (PCNSL). Among gliomas, 5 patients reported an allergic condition. In meningiomas, there were 5 patients with an allergic condition and 4 cases in patients with a metastatic lesion. The difference was not statistically significant. No difference in IgE levels was found between men and women ($P = 0.7$). In gliomas the mean IgE levels were 58.9 ± 87.9 U/mL. Patients with high-grade gliomas had lower IgE levels than patients with low-grade gliomas. However the difference was not statistically significant (median 24 vs. 74.6 U/mL, $P = 0.067$). In meningiomas, the mean IgE levels were 65.8 ± 77.7 U/mL. No significant difference was found between benign and atypical meningiomas ($P = 0.9$). In metastatic lesions, the mean IgE levels were 226.8 ± 203 U/mL. In PCNSL, the mean IgE levels were 150.8 ± 240.9 U/mL. Patients with gliomas had statistical significant lower IgE levels than patients with metastases (median 32.7 vs. 199 U/mL, $P = 0.0022$). Patients with meningiomas had statistical significant lower IgE levels than metastases (median

37.6 vs. 199 U/mL, $P = 0.01$). There was no significant difference between gliomas and meningiomas with respect to IgE levels ($P = 0.8$).

Survival analysis in glioblastoma

Two patients died in the immediate postoperative period whereas another two patients were lost to follow-up. After a mean follow-up period of 12.4 months, 10 patients were alive. Using receiver operator characteristic curve analysis a cut-off IgE value of 24 U/mL was identified as the best predicting survival with 72.2% sensitivity and 100% specificity. Patients with IgE value exceeding 24 U/mL lived longer ($P = 0.01$).

DISCUSSION

The present study investigated serum IgE levels in patients with intracranial tumors. The results showed that glioma and meningioma patients had lower serum IgE levels than patients with a metastatic brain lesion. Patients with high-grade gliomas had lower serum IgE levels than patients with low-grade gliomas. In glioblastomas, patients with serum IgE levels exceeding 24 U/mL had a better survival.

A large body of research showed that allergies may play a role in a diverse group of cancers including brain tumors, leukemia, pancreatic cancer, and other tumors.^[4] An inverse association has been reported between allergy history and cancer risk.^[4] Turner *et al.* studied 793 glioma, 832 meningioma, 394 acoustic neuroma, 84 parotid gland tumor cases and 2,520 controls and found a significant inverse association between a history of any allergy and glioma, meningioma and acoustic neuroma.^[5] Various hypotheses have been proposed to explain the associations between allergy and cancer. Single-nucleotide polymorphisms of inflammatory-related genes, such as interleukin-4, FCER1A and interleukin-10, have been associated with glioma risk.^[6] In gliomas, a large nested case-control study showed that a positive test for increased concentrations of total IgE (> 100 kU/L) was statistically significantly associated with decreased risk of glioma compared with a negative test. A lower risk for glioblastoma and glioma at least 20 years before diagnosis was found for those that tested positive for total IgE.^[7] Wiemels *et al.* found that IgE levels were strongly inversely associated with glioma and in particular, IgE to dietary allergens. A weaker association was found for respiratory allergens.^[2] Antihistamine use was strongly associated with glioma risk when allergy or asthma history existed, but not among those with a negative history.^[8] In the present study, we found that glioma patients had the lowest IgE levels. High-grade tumors had lower serum IgE levels than low-grade tumors. IgE levels should be measured at presentation since dexamethasone, which is, usually, administered

Table 1: Patients data

Patient characteristics	n (%)
Gender	
Male	44 (52.3)
Female	40 (47.7)
Age	
> 65	51 (60.7)
< 65	33 (39.3)
Reported allergy asthma	
Yes	14 (16.7)
No	70 (83.3)

Table 2: IgE levels according to diagnosis

Histology	IgE levels (U/mL)
Glioma	
High-grade	42.7 ± 47.6
Low-grade	117.8 ± 119
Meningioma	
Benign	68.2 ± 81.6
Atypical	54.3 ± 33.3
Metastases	150.8 ± 240.9

in brain tumor patients, increases IgE levels.^[9] Lin *et al.* also studied the IgE levels in glioma patients and reported that an increase in IgE levels posttreatment correlated with better survival.^[10] In the present study, we found a prognostic significance of pretreatment IgE levels in glioblastoma patients. This has not been previously reported and required further investigation.

Meningiomas are the most common benign intracranial tumor and account for nearly 30% of all intracranial tumors.^[11] Meningiomas have also been showed to have an inverse relationship with history of allergies and meningioma patients had lower total serum IgE than controls.^[3] A meta-analysis investigating the association of meningioma with the overall and specific allergic conditions found only eczema to have a significant inverse association. No significant association was found for asthma and hay fever.^[12] In their study, Schlehofer *et al.* they found that the risk of glioma was inversely correlated to allergic sensitization; however, this was not significant for meningiomas.^[13] In the present study, no significant difference in serum IgE levels was found between glioma and meningioma.

Regarding other cancers, several studies have consistently found that self-reported allergies are associated with lower risk of pancreatic cancer.^[14] Bosetti *et al.* reported an inverse correlation with history of allergy for cancer of the oral cavity, pharynx, esophagus, colon, rectum and larynx.^[15] Nevertheless, a recent study showed an increased risk of hematologic malignancies in women with a history of allergies to airborne allergens.^[16] In the present study, 2 metastatic lesions from patients with colon cancer were found. Patients with metastatic disease had significant higher IgE levels than patients with gliomas and meningiomas. This finding needs to be verified on a larger scale.

The present study has several limitations. First, the results are based on a series of 84 patients and should, therefore, be considered preliminary. Second, given that only 31 patients had glioblastoma, the reported prognostic significance of IgE levels should be dealt with caution and needs further investigation in larger studies. Another important limitation pertains to the absence of a control group. Several previous studies consistently reported lower IgE levels for brain tumor patients than controls. Thus we focused on the possible impact of tumor type on serum IgE levels.

In conclusion, the present study shows that patients with glioma and meningioma have lower IgE levels than patients with metastatic lesions. Patients with high-grade gliomas have lower serum IgE levels than patients with low-grade gliomas. In the glioblastoma, serum IgE levels are associated with tumor aggressiveness. Since serum

IgE levels can be easily measured, further research is required to validate our preliminary observations.

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Cite this article as: Alexiou GA, Kallinteri A, Nita E, Zagorianakou P, Levidiotou S, Voulgaris S. Serum IgE levels in patients with intracranial tumors. *Neuroimmunol Neuroinflammation* 2015;2(1):15-7.

Source of Support: Nil. **Conflict of Interest:** No.

Received: 19-08-2014; **Accepted:** 30-09-2014

The influence of postoperative infection in survival of patients with high-grade gliomas

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ABSTRACT

High-grade gliomas are the most common type of brain tumors. Of these, glioblastoma account for 60-70% and despite treatment carries a dismal prognosis. Postoperative surgical site infection has been associated with prolonged survival. Herewith, we present a case of glioblastoma and a case of anaplastic oligoastrocytoma that developed postoperative infection of the surgical site and had prolonged survival. A thorough literature review is also presented.

Key words: Glioblastoma, high-grade glioma, infection, survival

INTRODUCTION

High-grade gliomas are the most common type of primary brain tumors and carry a dismal prognosis.^[1] Glioblastoma is by far the most common type occurring in adults. This devastating disease is usually incurable and despite aggressive treatment the median survival time remains in the range of 15 months.^[1] Median survival for anaplastic tumors is usually 2-3 years with anaplastic oligodendroglioma having a better survival.^[2] There have been reports that patients with postoperative infection of the craniotomy site experienced long term survival.^[3,4] Herewith, we report on two cases of high-grade gliomas with confirmed postoperative infection and prolong survival.

CASE REPORT

Case 1

A 48-year-old woman underwent a brain magnetic resonance imaging (MRI) because of persisted headache and dizziness. A left frontal lesion (4.2 cm × 3.7 cm × 5 cm) with prominent peri-focal edema and mass effect was revealed. The lesion

enhanced after gadolinium administration, The Karnofsky performance scale (KPS) score was 100. The patient underwent a left frontal craniotomy with radical resection of the lesion. Histological examination revealed the presence of a glioblastoma. The postoperative KPS at the time of discharge was 100. The patient received concomitant temozolomide (TMZ) with radiotherapy (60 Gy) followed by adjuvant TMZ. Eleven months later the patient had a generalized seizure. Follow-up MRI revealed a lesion suspicious of tumor recurrence. Brain single-photon emission computed tomography (SPECT) with ^{99m}Tc-tetrofosmin demonstrated increased tracer uptake suggesting tumor recurrence. The patient was re-operated, and carmustine wafers were placed around the resection cavity. The postoperative period was uneventful, and the patient was discharged home on the 7th postoperative day. One week later the patient was hospitalized because of discharge from the surgical wound. Wound cultures were obtained. Identification and antimicrobial susceptibility of the microorganisms were performed by the automatized VITEK 2 System (BioMerieux, France). The results showed the presence of *Staphylococcus haemolyticus* (coagulase-negative *Staphylococcus*, CoNS) resistant to penicillin, oxacillin, levofloxacin, moxifloxacin and gentamycin. The patient received proper antibiotic treatment, and the infection was resolved. The patient remained free of disease for 27 months when recurrence was noted on follow-up MRI. Anti-VEGF treatment was administered; however, she died 3 months later. Her overall survival was 42 months.

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DOI:
10.4103/2347-8659.149418

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Case 2

A 22-year-old man underwent a brain MRI because of seizures. The MRI revealed a 5 cm × 3.2 cm × 4.3 cm left frontal lesion. The lesion had a heterogeneous cystic and solid appearance that enhanced after gadolinium administration. The patient underwent surgical resection, and gross total excision was performed. Histological examination revealed the presence of an astrocytoma Grade II. Postoperative radiation treatment was administered. On follow-up MRI 7 years later a lesion suspicious for tumor recurrence was noted. The patient was re-operated, and the histology revealed the presence of an anaplastic oligoastrocytoma. The patient received TMZ-based chemotherapy. Nevertheless, 28 months later the patient had uncontrolled seizures. Brain MRI demonstrated a lesion suspicious of tumor recurrence. Brain SPECT with ^{99m}Tc-tetrofosmin was positive for recurrent disease. The patient was re-operated. A new recurrence was noted 23 months later, and the patient re-operated, carmustine wafers were placed around the resection cavity. Postoperatively, the patient had fever and discharge from the wound. Brain CT showed findings consistent with infection and there was contrast enhancement. The patient was re-operated, and craniectomy was performed. Cerebrospinal fluid cultures showed the presence of *Staphylococcus epidermidis* (CoNS) resistant to penicillin, oxacillin, erythromycin and clindamycin. After proper antibiotic treatment, the patient improved. The patient did not show any sign of the tumor reappearance for 37 months until recurrence was noted. Two months later he died. The overall survival was 14 years.

DISCUSSION

High-grade gliomas are the most common brain tumors in adults and are highly malignant.^[2] Treatment includes surgery, postoperative radiotherapy, and concomitant and adjuvant chemotherapy.^[2] Nevertheless, even receiving the same treatment, the clinical outcome of patients varies significantly.^[1] Age, 1p19q deletion status and isocitrate dehydrogenase (IDH) mutational status are of prognostic significance.^[5,6]

A survival benefit for patients that developed postoperative infection in the tumor removal site has been reported.^[3,4] Postoperative infection is generally considered when wound and/or bone flap infection, cerebral abscess, or meningitis, occur within 2 months from surgery.^[2] Its incidence has been reported to range between 0.75% and 2.3% for intracranial operations.^[7] De Bonis *et al.* studied 197 patients operated for glioblastoma and found 10 cases of postoperative bacterial infection. Patients that

developed infection had a median survival of 30 months whereas patients without postoperative infection had a median survival of 15 months. The difference was statistically significant. In 5 cases there was a surgical abscess, in 3 cases abscess and wound infection and in 2 cases surgical wound and bone flap infection required surgical revision. In 6/10 cases *Staphylococcus aureus* was isolated.^[2] In the present study, the patient with glioblastoma had an overall survival of 42 months. In a previous study in our institute, the median survival of patients with glioblastoma was 15.5 months.^[8] Bowles *et al.* also reported 4 cases of malignant brain tumors with prolonged survival after postoperative infection. In those cases, *Enterobacter aerogenes* was isolated. The authors suggested that in addition to the bacteria direct oncolytic effect, an immune adjuvant responses to tumor suppression might play a role.^[4] Nevertheless, Bohman *et al.* did not find a survival advantage in 18 patients with postoperative infection after glioblastoma resection out of 382 patients included in their study.^[9]

Regarding experimental data, a recent study showed that intracerebrally implanted heat-inactivated staphylococcal epitopes mixed with 9L gliosarcoma cells in Wistar rats, resulted in significant prolong survival than controls. In one case there was complete regression of an already grown mass.^[10] William Coley, a pioneer in immunotherapy, was the first injecting a mixture of live streptococcus bacilli and subsequently heat-killed *Streptococcus* into tumors and managed to induce remission of inoperable sarcomas.^[11] This vaccine was also successful in cases of melanoma and lymphomas.^[12] According to Coley, the induction of fever was a key element.^[12] The inflammation cascade induced by bacteria and the presence of factors such as interferon-alpha, tumor necrosis factor-alpha, interleukin-2, have been considered as the cause of this effect.^[12] Tanaka *et al.* injected intratumorally an immunopotentiator, Picibanil, in 13 patients with brain tumors.^[13] Picibanil was a low virulent mutant strain of Lancefield's Type 111, Group A *Streptococcus pyogenes*. The results showed significant tumor regression in 6/12 patients for whom CT was performed.^[13] More recently, Jeys *et al.* investigated the effect of postoperative infection in patients treated for osteosarcoma, using endoprosthetic replacement and neo-adjuvant chemotherapy. The results showed that patients who developed an infection had a significantly longer survival. Furthermore, infection was an independent prognostic factor on cox regression analysis.^[14] Ruckdeschel *et al.* reported improved survival rates for patients who developed the empyema after lung cancer compared with noninfected patients.^[15]

In the present study, both patients were treated at recurrence with carmustine wafers that were placed around the resection cavity. Carmustine wafers have been shown to yield better survival rates of 1-2 months in primary high-grade gliomas.^[16] Thus, the prolonged survival of both patients cannot be attributed to the implanted wafers.

In conclusion, the role of infection provides a rationale for further research in cancer treatment. Certainly, there is a need for larger studies that may provide more accurate answers. This may also lead to a subgroup analysis that would better define if patients survival is influenced by bacterial strain, location of the infection or time to infection.

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Cite this article as: Alexiou GA, Kallinteri A, Michos E, Zagorianakou P, Priavali E, Pachatouridis D, Levidiotou S, Voulgaris S. The influence of postoperative infection in survival of patients with high-grade gliomas. *Neuroimmunol Neuroinflammation* 2015;2(1):18-20.

Source of Support: Nil. **Conflict of Interest:** No.

Received: 19-08-2014; **Accepted:** 01-09-2014

Cardiac autonomic function in patients with myasthenia gravis: analysis of the heart-rate variability in the time-domain

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ABSTRACT

Aim: Myasthenia gravis (MG) is a neuromuscular transmission disorder caused by acetylcholine receptor autoantibodies. Cardiac autonomic dysfunctions were rarely reported in patients with MG. Functional cardiac abnormalities were variable and reported in patients at severe stages of the disease and with thymoma. We investigated cardiac functions in patients with MG using Ambulatory 24-h electrocardiographic Holter-Monitoring. **Methods:** This study included 20 patients with MG with a mean age of 28.45 ± 8.89 years and duration of illness of 3.52 ± 1.15 years. The standard Holter reports include data for heart-rate, ventricular ectopies (VEs), supraventricular ectopies (SVEs), heart-rate variability (HRV), ST, QT, atrial fibrillation and T-wave alternans. **Results:** VEs, SVEs and ST-T changes were reported in 55%, 40% and 20% of patients respectively. Compared with healthy subjects ($n = 20$), HRV components including SDNN, SDANN, SDNN Index, RMS-SD and pNN50 ($P = 0.001$ for all) were reduced in patients indicating sympathetic and parasympathetic autonomic dysfunctions. HRV abnormalities were reported in 30-60% of patients. No significant correlations were identified between SDNN, RMS-SD, pNN50, and duration of illness. **Conclusion:** Depressed HRV may be an early manifestation of autonomic neuropathy in patients with MG even in milder stages of the disease. This information is useful in rating disease progression and the efficacy of therapeutic interventions.

Key words: Cardiac autonomic function, heart-rate variability, myasthenia gravis

INTRODUCTION

Myasthenia gravis (MG) is a humoral immune attack to the skeletal muscle nicotinic acetylcholine receptors (nAChRs) at the neuromuscular junction by autoantibodies.^[1] The cardinal symptoms of MG is fatigue or fluctuating weakness in voluntary muscles with repeated or sustained exertion in the course of the day, but improved by rest.^[1] MG has a prevalence of 25-125/10⁶, with female to male ratio of 3-2.^[2] About 2/3 of the patients has mild weakness, which initially involve the ocular muscles, but may progress to generalized weakness in approximately 85% of patients. Respiratory muscle weakness occurs in severe stages, which may require mechanical

ventilation (myasthenic crisis).^[3] The diagnosis of MG was based on the association between the following parameters: clinical manifestations,^[4] presence of serum anti-AChR antibody, definite clinical improvement on injection of anticholinesterase, and a decremental pattern of repetitive nerve stimulation.^[3] The thymus gland is abnormal in up to 90% of adults with MG, of which 70% have enlarged thymic hyperplasia, whereas 10-20% have benign thymic tumors or thymoma.^[5] The current treatment for MG includes acetylcholine esterase inhibitors (AChE-Is) (as pyridostigmine),^[6] immunopharmacologic drugs,^[7-12] and thymectomy.^[13]

A few studies were carried out to investigate cardiac involvement in patients with MG. Some reported nonspecific electrocardiographic (ECG) changes, Doppler imaging and conventional echocardiography,^[14] while others reported variable results, which varied from asymptomatic specific ECG changes (as abnormalities of the STI,^[15] dispersion of QT and T wave alternans^[16]), giant diffuse T waves, to clinically manifest abnormalities (as conduction disturbances, atrioventricular dissociation, wide QRS,^[17] syncopal

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10.4103/2347-8659.149419

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attacks, orthostatic hypotension, impaired heart-rate variability (HRV)^[18,19]. These significant cardiac manifestations were reported in patients with severe stages of MG and in the presence of thymoma. It has been suggested that some MG patients may develop autonomic dysfunction and other nervous system manifestations.^[18-26] It has also been suggested that the heart and skeletal muscle molecules are targets for the autoimmune process of MG.^[27-30]

This study aimed to assess cardiac functions in patients with MG using Ambulatory 24-h ECG Holter Monitoring. HRV measures are sensitive indices of cardiac autonomic function (sympathetic and parasympathetic).

METHODS

Subjects

This study included 20 (males = 6, females = 14) patients with MG without known cardiac diseases. Their age range was 16-50 years and duration of illness ranged from 1 to 4 years. Clinical grading of the patients was done based on the medical-scientific advisory board of the MG Foundation of America classification.^[4] Patients grading was based on their histories and diagnosis shown in their medical records. Patients reported histories of weakness of ocular muscles (ptosis) (class I), with mild and predominant weakness of limb muscles (class IIa) or oropharyngeal muscles (class IIb), or with moderate and predominant weakness of limbs (class IIIa). Thymectomy was done to the seven patients with thymoma. Table 1 shows the demographic and clinical characteristics of the studied group. Patients were recruited from the Out-patient Clinic of the Department of Neurology, Assiut University Hospital, Assiut, Egypt during their follow-up visits in which they were free of clinical manifestations (i.e., after resolution of active stage of the disease for at least 3 months) and were on maintenance

treatment with low doses of AChE-Is and/or steroids. The patients were admitted to the hospital for 24-48 h for the purpose of the research. Twenty healthy subjects matched for age, sex and socioeconomic status were included in this study for statistical comparisons. Control subjects were recruited from the general population. This study was accepted by the regional Ethical Committee. All patients and control subjects were briefed about the detailed information of this study and hence consented to attend this study.

Excluded were subjects (patients and controls): (1) with respiratory involvement or in crisis (i.e., severe stage of the disease); (2) with major systemic illness such as organic heart disease, diabetes, hypertension or any other disease that might affect the autonomic nervous system; (3) on medications known to affect heart-rate/rhythm such as beta-blockers, vasopressors, digitalis, theophylline, thyroid hormones, tricyclic antidepressants, antiarrhythmic drugs, atropine and its derivatives, etc.; (4) with history of febrile illness in the past 1-week; and (5) with lack of sound sleep the night prior to monitoring.

Measurements

All participants underwent conventional echocardiography and Ambulatory ECG Holter-Monitoring. Holter monitoring is the continuous 24-h monitoring of ECG activity of a patient's heart while engaged in daily activities. Ambulatory ECG was carried out using the 5-electrode Holter which is a 3-channel portable battery operated digital ECG recorder (Cardiolight FMC.A, Medset, Medizintechnik, Hamburg, Germany). The standard Holter report includes data for heart-rate, ventricular ectopies (VEs), supraventricular ectopies (SVEs), HRV, ST, QT, QTc (QT corrected for heart-rate), atrial fibrillation (A-Fib), T-wave alternans and sleep apnea.

Heart-rate variability is defined as the oscillation in the interval between consecutive heart beats as well as the oscillations between consecutive instantaneous heart-rate.^[31] The general concept of HRV is that the more the HRV, the healthier the heart, because it more readily responds to its various stimuli. Small changes in R-R (NN) variability indicate cardiac risk. However, small or large changes in variability cannot be noticed at ECG strips. Two correlated methods of calculating R-R changes have been accepted by the cardiology community, which are the time-domain and frequency. In this study, we analyzed the HRV in the time-domain.^[32] The most acceptable measurements in time-domain are: (1) SDNN (ms): standard deviation of all qualified beats (NN intervals); (2) SDANN (ms): standard deviation of the averages of NN intervals in all 5-min segments of the entire recording; (3) RMS-SD (ms):

Table 1: Demographic, clinical and laboratory characteristics of the studied groups

Demographic and clinical characteristics	Patients (n = 20)	Control subjects (n = 20)	P
Male/female	6/14	10/10	-
Age; years	16-50	20-50	-
	28.45 ± 8.89	30.22 ± 5.76	0.380
Duration of illness; years	1-4	-	-
	3.52 ± 1.15	-	-
Clinical grade			
I	0	-	-
IIa/IIb	2/10	-	-
IIIa/IIIb	8/0	-	-
IVa/IVb	0	-	-
V	0	-	-
Thymic pathology (%)			
Normal	5 (25)	-	-
Hyperplasia	8 (40)	-	-
Thymoma	7 (35)	-	-

Data are expressed as range, mean ± SD; number (%). SD: Standard deviation

the square root of the mean sum of squares differences between adjacent NN intervals; (4) SDNN index (ms): mean of the standard deviations of all NN intervals for all 5-min segments of the entire recording; (5) SDSD (ms): standard deviation of differences between adjacent NN intervals; (6) NN50 count: number of pairs of adjacent NN intervals differing by more than 50 ms in the entire recording; three variants are possible counting all such NN intervals pairs or only pairs in which the first or second interval is longer; and (7) pNN50%: percentage of differences between adjacent normal NN intervals that are greater than 50 ms computed over the entire 24-h ECG recording.

The warning signs of cardiac problems include: VEs in excess of 10/h, VE Pair, V-Runs, R on T beats, SV-Runs, A-Fib, pauses in excess of 2.5 s, QTc in excess of 460 ms, ST depressions of 1 millimeter or more (a strong indication of cardiac ischemia) and SDNN of 50 ms or less.

Statistical analysis

Calculations were done with the statistical package SPSS, version 12.0. Data were presented as mean \pm standard deviation. Unpaired two-sided Student's *t* test was utilized for comparison among means of normally distributed parameters, while Mann-Whitney *U*-test was for all other cases. Correlations were assessed using Pearson's and Spearman's methods for normally and non-normally distributed data, respectively. For all tests, values of $P < 0.05$ were considered statistically significant.

RESULTS

This study included 20 patients with MG. They had mean age of 28.45 ± 8.89 years and mean duration of illness of 3.52 ± 1.15 years. None of the patients reported symptoms related to arrhythmias as syncope, dizziness, palpitation, shortness of breath, chest discomfort, diaphoresis, or neurological symptoms such as transient ischemic attack. Conventional echocardiographic measures were normal for all patients. The total ambulatory 24-h ECG recording period was ranged between 18 and 24 h (mean: 22.00 ± 0.35). The minimum heart-rates were found during sleeping and ranged from 47 to 75 beats/min (mean: 58 ± 8) and the maximum heart-rates trends were found during awake and ranged from 112 to 145 beats/min (mean: 90 ± 12). Minimum R-R ranged from 296 to 560 ms (mean: 421 ± 47) and the maximum R-R intervals ranged from 868 to 1,618 ms (mean: $1,255 \pm 205$). VEs and SVEs were found in 11 (55%) and 7 (40%) of patients (versus none for controls) respectively. SVEs were less than 25/24 h. Depressed ST-T was reported in 4 (20%) patients. Table 2 shows the results of HRV in the time-domain. Patients had

Table 2: The results of time-domain analysis of HRV

Variable	Patients (n = 20) (%)	Controls (n = 20)	P
SDNN (ms)	12 (60)	0	0.001
	45.30-111.20	86.33-198.70	
	55.70 ± 20.23	152.75 ± 15.66	
SDANN (ms)	9 (45)	0	0.001
	44.50-116.35	94.40-180.68	
	58.19 ± 13.80	142.23 ± 12.09	
SDNN index (ms)	12 (60)	0	0.001
	42.30-138.56	92.43-166.62	
	56.46 ± 20.02	112.64 ± 16.22	
RMS-SD (ms)	8 (40)	0	0.001
	12.30-45.70	5.64-95.55	
	20.51 ± 6.06	46.57 ± 4.24	
pNN50 (%)	6 (30)	0	0.001
	0.10-14.50	0.60-23.22	
	1.40 ± 0.32	14.42 ± 2.86	

Data are expressed as number of patients with abnormalities, range, mean \pm SD. SD: Standard deviation; HRV: Heart-rate variability

reduced indices of HRV (SDNN, SDANN, SDNN Index, RMS-SD and pNN50) ($P = 0.001$ for all). No significant correlations were reported between SDNN, RMS-SD, pNN50 and duration of illness.

DISCUSSION

In this study, dynamic ECG showed evidence of subclinical autonomic cardiac dysfunction in patients with MG with mild/moderate severities. Cardiac autonomic functions were assessed by measuring the cyclic variation of the heart beat intervals (i.e., HRV). HRV reflects the complex interplay of sympathetic and parasympathetic innervation of heart. The lack of intra-individual variability over time makes the measurement of HRV an excellent tool for studying autonomic input of the heart.^[31] In the present study, components of the time-domain analysis (SDNN, RMS-SD, pNN50) were significantly reduced in patients with MG. No significant correlations were identified between HRV indices and duration of illness. Accordingly, previous studies did not report significant correlations between autonomic nervous system dysfunction and disease duration, clinical manifestations, cardiovascular risk factors and diseases activity were reported.^[19,23]

SDNN reflects overall autonomic modulation of the heart but does not provide no information regarding isolated sympathetic and parasympathetic activities, while RMSSD and pNN50 which reflect very short-term HRV are predominantly mediated by the vagus nerve.^[32] Reduced HRV is well known to be associated with susceptibility to cardiac arrhythmias.^[33] Previous studies had also shown that reduced HRV is an independent risk factor for arrhythmic sudden death after myocardial infarction.^[34] In their study, Vernino *et al.*^[19] reported impaired HRV and a low vagal tone and modified cardiac parasympathetic modulation, orthostatic hypotension and gastrointestinal manifestations (severe abdominal

cramps, early satiety and nausea) in an adult with MG associated with thymoma. These manifestations improved by AChE-Is and thymectomy. Previous studies rarely reported other autonomic nervous system dysfunctions (sympathetic and parasympathetic) in patients with MG^[19-22] including gastrointestinal, intestinal pseudo-obstruction,^[21,23] acute autonomic and sensory neuropathy and severe panautonomic failure.^[19,22] These studies reported increase in the autonomic nervous system dysfunction with the severity of MG. Meanwhile improvement of both neuromuscular and autonomic symptoms was observed with AChE-Is and after thymectomy. Furthermore, neurophysiological tests and laboratory studies also confirm the presence of autonomic dysfunction in patients with MG. For instance, augmentation in epinephrine excretion, although the nor-epinephrine excretion remains unchanged or even undergoes reduction in response to forearm ischemia or orthostasis (a sign of sympathetic deficiency), was reported in patients with MG,^[24] while both stimuli in normal subjects induce a rise in norepinephrine urinary excretion without significant changes in epinephrine excretion.

The exact mechanisms of the autonomic nervous system dysfunction in patients with MG could rely on the involvement of autonomic neuronal nAChRs by an immune mediated processes of MG.^[25,26] The structural identities between different muscle and autonomic nAChRs subunits with the possibility of cross-reactivity between different nAChRs antibodies may be contributed as a cause of immune-mediated autonomic neuropathy with MG. Additionally, a few studies reported myocarditis in patients with MG and suggested that cardiac involvement with MG could be due to the immune responses driven by muscle and cancer (e.g., thymoma) mediated expression of neuronal nAChRs antibodies.^[27-30,35,36] Recently, Suzuki *et al.*^[22] have reported myocarditis in MG patients with anti-Kv1.4 (voltage-gated potassium channel) antibodies which was further manifested by ECG findings with high frequencies of T-wave abnormality and QT prolongation, ventricular tachycardia, sick sinus syndrome, or complete atrial ventricular block and severe heart failure. These authors suggested that the heart and skeletal muscle molecules are targets for the autoimmune process of MG.^[29] Some authors suggested that autonomic nervous system manifestations of MG could result from the adverse effect of medications used for MG treatment, such as pyridostigmine.^[37] As a reversible AChE-I, pyridostigmine inhibits the enzymatic breakdown of ACh and consequently potentiating cholinergic neurotransmission with selective augmentation of the efferent parasympathetic signal. A number of

studies have indicated that pyridostigmine decreases the resting heart-rate by about 5-7 beats/min and increases the R-R interval and long-term time-domain indices of HRV in normal subjects^[38] and patients with heart failure.^[39] However, none of these studies reported marked reduction of SDNN below 50 ms or presence of VEs, SVEs or depresses ST-T as observed in this study. We suggested that although pyridostigmine could reduce HRV indices but the marked reduction observed in this study is attributed to the autonomic nervous system involvement by the disease process.

The results of this study indicate that patients with MG are considered at high risk due to the presence of autonomic nervous system dysfunction and are candidates for earlier considerations of cardioprotective medications. Thus, caution has to be considered for prescribing drugs that disturb the cardiovascular autonomic nervous system and preanesthetic evaluation before surgery.^[40,41]

Despite the strength of our findings, the main limitation of this study is the small sample size. Longitudinal and prospective studies are needed.

In conclusion, patients with MG may have specific ECG abnormalities indicating subclinical cardiac autonomic dysfunction even in milder stages of the disease. Depressed SDNN of HRV is an early warning sign of autonomic neuropathy. These results indicate the need for routine evaluation of autonomic functions in patients with MG both to identify patients at high risk for earlier consideration of cardio-protection and long-term follow up studies.

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Cite this article as: Hamed SA, Mohamad KO, Adam M. Cardiac autonomic function in patients with myasthenia gravis: analysis of the heart-rate variability in the time-domain. *Neuroimmunol Neuroinflammation* 2015;2(1):21-5.

Source of Support: Nil. **Conflict of Interest:** No.

Received: 19-08-2014; **Accepted:** 30-09-2014

Central somatosensory conduction slowing in adults with isolated elevated plasma level of homocysteine

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ABSTRACT

Aim: Elevated plasma level of homocysteine (eHcy) is a recognized risk factor for dementia. However, whether the central conduction is affected in patients with an isolated eHcy is unknown. In this study, we addressed whether central conduction is altered in adults with eHcy. **Methods:** Evoked potential studies including somatosensory (SSEP), visual (VEP) and brainstem auditory evoked potentials (BAEP), were performed to evaluate central conduction in patients with isolated eHcy. **Results:** Nine SSEP, 7 VEP, and 6 BAEP were studied in 9 patients with eHcy (age: 63.3 ± 7.5 years old, mean \pm standard deviation, male/female: 4/5). SSEP with median nerve stimulation was delayed in peak latency of N9 (5/9/55.6%, abnormal/total subjects/percentage), N13 (7/9/77.8%), N20 (6/9/66.7%), and/or interpeak latency of N9-N13 (5/9/55.6%), N13-N20 (5/9/55.6%), and N9-N20 (4/9/44.4%). There was one delayed P100 latency (1/7/14.3%) in 7 VEP. BAEP was within normal limits in all the 6 subjects tested. **Conclusion:** Our pilot study provided neurophysiologic evidence of central conduction slowness in patients with eHcy, which may be due to a large diameter fiber dysfunction within the somatosensory, but not the visual and auditory, white matter pathway. The central conduction slowing in eHcy may be relevant to the pathophysiologic background for slowing the central processing.

Key words: central conduction, evoked potential, homocysteine, hyperhomocysteinemia

INTRODUCTION

Homocysteine (Hcy) is an intermediary metabolite of amino acid, methionine, during methylation. Elevated plasma level of homocysteine (eHcy) have been observed in many neurologic and psychiatric disorders including stroke,^[1,2] cognitive impairment, dementia,^[3,4] Alzheimer's disease,^[5,6] Parkinson's disease,^[6,7] amyotrophic lateral sclerosis,^[8] depression,^[6,9-12] schizophrenia and bipolar disorders and in an animal model,^[13,14] indicating eHcy may adversely cause central nervous system dysfunction.^[15] Whether eHcy interferes with central conduction as the pathophysiologic background relevant to the central processing slowness is unknown. We, therefore, studied the central conduction in patients with eHcy.

METHODS

Neurophysiology laboratory databank of evoked potentials and the charts of subjects with a clinical diagnosis of eHcy-induced neuropathy^[16] seen in the Neuromuscular Clinic were initially retrospectively reviewed. The symptoms and signs of neuropathy included the presentation of numbness and tingling in the distal limbs with a decreased sensation in a glove- and/or stocking-like pattern. Data of clinical presentations, physical and neurological examinations, history of concomitant comorbidities; and laboratory findings including plasma levels of homocysteine, methyl malonic acid, vitamin B12 and folic acid were collected. Laboratory data included mean corpuscular volume of red blood cells, glucose, creatinine, glycosylated hemoglobin, thyroid stimulating hormone, lipids and liver function panels; inflammatory and infectious studies including erythrocyte sedimentation rate, C-reactive protein, antinuclear antibody, rapid plasma reagin, lyme titers, hepatitis profile, and human immunodeficiency virus.^[8] Subjects with an isolated eHcy who completed evoked potential studies were included. Subjects with an identifiable etiology, other

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10.4103/2347-8659.149420

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than eHcy, such as deficiency of vitamin B12 and/or folic acid, metabolic, toxic, endocrinologic, infectious, inflammatory, renal or liver diseases were excluded. Additionally, subjects with history of degenerative or inflammatory disorders, such as dementia, Parkinson's, amyotrophic lateral sclerosis, multiple sclerosis, lupus, sarcoidosis, seizures/epilepsies, cervical spondylosis or traumatic injury, were excluded. This study was approved by the Temple University Institutional Review Board.

Recording conditions and data acquisition

Conventional evoked potential studies, including somatosensory, visual, and brainstem auditory evoked potentials (SSEP, VEP, and BAEP, respectively), were performed using Viking Select 10.0.0 (Nicolet, Madison, Wisconsin). Individual peak latency, interpeak latency, interlateral latency and amplitude were analyzed.

Somatosensory evoked potential (median nerve)

Each median nerve was stimulated at the wrist with square pulses above the motor threshold using 0.2 ms stimuli. Two independent trials were performed on either nerve. The stimulus frequency was 5.1 Hz and recording time 50 ms. Filters were set at LFF 10 Hz and HFF 3 kHz. Resultants were recorded and averaged from 2,000 responses from the montage of CPc-CPi, CPi-Epc, C5s-Epc and Epi-Epc. The generators for the waveforms of N9, N13, and N20 are believed to be ipsilateral brachial plexus at the Erb's point, dorsal column and contralateral nucleus cuneatus, and contralateral parietal somatosensory cortex, respectively. Alteration in interpeak latency of N13-N20 reflects central conduction abnormality.

Visual evoked potential

Two modalities were used in VEP study: pattern reversal full field VEP's performed monocularly utilizing 28-inch checkerboard stimuli, and goggles fitted with a mosaic of light-emitting diodes. The stimulating reversal frequency was 1.1 Hz in 25 ms with a filter setting at LFF 0.5 Hz and HFF 100 Hz. Two separate trials were performed on each eye. The resultants were recorded for 250 ms and averaged from 150 recordings from LO-MF, MO-MF, RO-MF and MF-A1 derivations. P100 latency was obtained and analyzed.

Brainstem auditory evoked potential

Each ear was stimulated independently utilizing broad rarefaction and alternating clicks in a frequency of 9.7 Hz in 100 ms with a filter setting of LFF 30 Hz and HFF 3 kHz. Masking noise was delivered to the contralateral ear. For each series of responses, two trials were performed separately from each ear and resultants were recorded for 10 ms and averaged

from 4000 responses from Cz referenced to ipsilateral and contralateral ears (Cz-A2 and Cz-A1). The peak latency of waves I, III and V; interpeak latency of waves I-III, III-V and I-V; the amplitudes of wave I and V; and the ratios of the amplitudes of waves V and I (V/I) were measured. The generators for waves I, III, and V of BAEP are believed to involve the structures of the cochlear nerve, superior olive complex, and lateral lemniscus nuclei the mesencephalon, respectively.^[9]

RESULTS

From 507 records, 9 subjects who fulfilled the inclusion criteria were included (age: 63.3 ± 7.5 years old, mean \pm standard deviation, range: 51-75, male/female: 4/5). Of these 9 subjects, 9 SSEP, 7 VEP, and 6 BAEP that were simultaneously performed, were included. Their plasma level of homocysteine was elevated (16.3 ± 2.3 μ mol/L, normal: < 11.4) but with normal plasma levels of B12 (621.4 ± 322.0 pg/mL; normal: 200-1100), folic acid (15.7 ± 5.2 ng/mL; normal: > 5.4), methylmalonic acid (165.1 ± 72.8 nmol/L; normal: 73-376), and a normal mean corpuscular volume of red blood cells (90.6 ± 7.8 fl; normal: 80-100).

With respect to the recordings, delayed SSEP was observed in peak latency of N9 (5/9/55.6%, abnormal/studied subjects/percentage), N13 (7/9/77.8%), N20 (6/9/66.7%) and in interpeak latency of N9-N13 (5/9/55.6%), N13-N20 (5/9/55.6%), and N9-N20 (4/9/44.4%). No significant difference in interlateral latency was noted. There was only one delayed VEP observed (1/7/14.3%). BAEP was within normal limits in all the 6 subjects studied [Tables 1-4].

DISCUSSION

In this study, we observed central conduction slowness in approximately half of the adult patients with an isolated eHcy. The central conduction slowness preferentially affected the somatosensory, but not

Table 1: Evoked potentials

	Abnormal	Percentage
SSEP (n = 9)		
N9	5	55.6
N13	7	77.8
N20	6	66.7
N9-N13	5	55.6
N13-N20	5	55.6
N9-N20	4	44.4
VEP (n = 7)		
Delayed	1	14.3
BAEP (n = 6)		
All normal		

SSEP: somatosensory evoked potentials; VEP: visual evoked potentials; BAEP: brainstem auditory evoked potentials

Table 2: Somatosensory evoked potentials

Number/ gender/age	N9-L	N9-R	N13-L	N13-R	N20-L	N20-R	N9- N13/L	N9- N13/R	N13- N20/L	N13- N20/R	N9- N20/L	N9- N20/R
3/female/68	10.3	10.3	13.9	13.2	19	18.5	3.6	2.9	5.1	5.3	8.7	8.2
6/female/71	11.9	12.3	17.4	17.4	24.8	26.2	5.5	5.1	7.4	8.8	12.9	13.9
10/male/64	12.7	12.8	16.4	16.8	22.4	23	3.7	4	6	6.2	9.7	10.2
16/male/51	11.6	11.3	17.4	14.9	22.2	22.1	5.8	3.6	4.8	7.2	10.6	10.8
23/male/62	11.6	11.8	15.3	15.1	21.7	21.2	3.7	3.3	6.4	6.1	10.1	9.4
24/female/56	11.8	11.6	15.3	16.5	22	20.8	3.5	4.9	6.7	4.3	10.2	9.2
28/female/58	8.7	8.6	16.2	16.2	Abs	Abs	7.5	7.6	Abs	Abs	Abs	Abs
29/male/75	10.6	11.3	16.9	17.6	Abs	27.4	6.3	6.3	Abs	9.8	Abs	16.1
30/female/65	9.9	10.5	15.2	14.6	Abs	23.8	5.3	4.1	Abs	9.2	Abs	13.3
Mean ± SD	11 ± 1.2	11.2 ± 1.2	16 ± 1.2	15.8 ± 1.5	22 ± 1.9	22.9 ± 2.9	5 ± 1.4	4.6 ± 1.5	6.1 ± 1	7.1 ± 2	10.4 ± 1.4	11.4 ± 2.7
Range	8.7-12.7	8.6-12.8	13.9-17.4	13.2-17.6	19-24.8	18.5-27.4	3.5-7.5	2.9-7.6	4.8-7.4	4.3-9.8	8.7-12.9	8.2-16.1
Normal	≤ 11.3	≤ 11.3	≤ 14.9	≤ 14.9	≤ 22.1	≤ 22.1	≤ 5.0	≤ 5.0	≤ 6.8	≤ 6.8	≤ 10.9	≤ 10.9

SD: standard deviation; L: left; R: right

Table 3: Brainstem auditory evoked potentials

Number/ gender/age	I-L	I-R	III-L	III-R	V-L	V-R	Amp-L	Amp-R	I-III/L	I-III/R	III-V/L	III-V/R	I-V/L	I-V/R
3/female/68	1.78	1.5	3.7	3.7	5.52	5.54	2.4	2.16	1.92	2.2	1.82	1.84	3.74	4.04
6/female/71	1.7	1.64	3.74	3.86	5.84	6	2.06	1.2	2.04	2.22	2.1	2.14	4.14	4.36
23/male/62	1.68	1.7	3.96	3.86	5.86	5.72	1.98	1.76	2.28	2.16	1.9	1.86	4.18	4.02
28/female/58	1.38	1.42	3.52	3.54	5.42	5.38	1.07	1.08	2.14	2.12	1.9	1.84	4.04	3.96
29/male/75	1.74	1.48	4	4.08	6.18	6.24	2.05	0.974	2.26	2.6	2.18	2.16	4.44	4.76
30/female/65	1.76	1.76	3.92	4.02	5.86	5.94	1	1.97	2.16	2.26	1.94	1.92	4.1	4.18
Mean ± SD	1.7 ± 0.1	1.6 ± 0.1	3.8 ± 0.2	3.8 ± 0.2	5.8 ± 0.3	5.8 ± 0.3	1.8 ± 0.6	1.5 ± 0.5	2.1 ± 0.1	2.3 ± 0.2	2 ± 0.1	2 ± 0.2	4.1 ± 0.2	4.2 ± 0.3
Range	1.4-1.8	1.4-1.8	3.5-4	3.5-4.1	5.4-6.2	5.4-6.2	1-2.4	1-2.2	1.9-2.3	2.1-2.6	1.8-2.2	1.8-2.2	3.7-4.4	4-4.8
Normal	≤ 2.2	≤ 2.2	≤ 4.5	≤ 4.5	≤ 6.5	≤ 6.5			≤ 2.6	≤ 2.6	≤ 2.4	≤ 2.4	≤ 4.7	≤ 4.7

SD: standard deviation; L: left; R: right

Table 4: Visual evoked potentials

Number/ gender/age	PI00-P-L	PI00-P-R	PI00-G-L	PI00-G-R
3/female/68	98.5	97.5	125	130
6/female/71	122	131	131	132
23/male/62	103	114	116	112
24/female/56	101	103	80.5	77.5
28/female/58	105	99.5	99.5	105
29/male/75	99.5	91	90.5	104
30/female/65	100	104	91	108
Mean ± SD	104.1 ± 8.2	105.7 ± 13.2	104.9 ± 19.5	109.8 ± 18.3
Range	98.5-122	91-131	80.5-131	77.5-132
Normal	≤ 117	≤ 117	≤ 132	≤ 132

SD: standard deviation; P: pattern reversal; G: goggles techniques; L: left; R: right

the visual and auditory, pathways. To the best of our knowledge, there is no report on central conduction in adult patients with eHcy.

Somatosensory evoked potential evaluates the integrity of the somatosensory pathway from peripheral to the cortex. The pathway initiates from peripheral segment of the large sensory fibers whose cell bodies, the pseudomonopolar neurons, reside in the dorsal root ganglia.^[17] The central processes of the pseudomonopolar neurons enter the ipsilateral posterior column of the spinal cord, decussate and synapse at the contralateral dorsal column nucleus (cuneate nucleus) at the cervico-medullary junction where the secondary order fibers start and synapse at ventro-posteriolateral nucleus of thalamus, from which the third order fibers advance to the somatosensory cortex.^[17] It is commonly

accepted that SSEP evaluates only the large diameter fibers.^[17]

The current study provided evidence that the large fiber dysfunction occurs in central conduction, involving somatosensory pathway, in addition to the peripheral conduction delay.^[16,18] We have recently reported that eHcy is an independent risk factor for the development of peripheral neuropathy.^[16] The estimated incidence of the isolated eHcy-induced neuropathy was as low as 1.81% of peripheral neuropathy (our unpublished data). The electrophysiologic features of the isolated eHcy-induced peripheral neuropathy are a mild, large fiber sensorimotor neuropathy with mixed neurophysiologic features of mild demyelination and distal axonal degeneration, although the involvement of small diameter fibers cannot be dismissed.^[18]

Elevated plasma level of homocysteine may result from deficiency of vitamin B12 and/or folate, and genetic predispositions such as C677T polymorphism of MTHFR.^[19,20] Additionally, it may also result from various pathophysiologic conditions including aging,^[21,22] obesity,^[23,24] diabetes mellitus,^[25-27] renal function impairment,^[27] medications and/or toxic substances such as levodopa,^[7,28] anti-gastric acid agents,^[29,30] anti-epileptics,^[31,32] tobacco,^[33] and alcohol.^[34-36] Because of its excitatory property which may promote the vulnerability of neuronal cells to

excitotoxic- and oxidative-stress-induced injury, Hcy, especially eHcy, can be neurotoxic.^[4,37-39]

Elevated plasma level of homocysteine has been observed in a number of neurological disorders including stroke,^[1,2] Alzheimer's disease,^[5] Parkinson's disease,^[7] and amyotrophic lateral sclerosis.^[8] In addition, eHcy may also play a role in psychiatric disorders, such as depression,^[6,9-12] bipolar disorders and schizophrenia.^[13,14] Importantly, eHcy has been linked to cognitive impairment and dementia.^[3,4] The findings of slowness of the central conduction in our study may suggest an electrophysiologic background for the central process slowing relevant to memory and cognitive functions. Notably, VEP was performed using two different modalities with only one out of 7 subjects showing abnormality. It is not clear the reason why visual and auditory pathways were spared. It may be related to the susceptibility of eHcy-induced nerve fiber damage or the tolerability of the fibers to eHcy-induced insults. An alternative explanation is that there may be a yet to be determined protective mechanism against eHcy-induced toxicity in special sensory pathways.

Vitamin B12 deficiency is a well-documented etiology for both central and peripheral nervous system dysfunction.^[40] The current study provided evidence that the large fiber dysfunction occurs in central conduction, involving the somatosensory pathway. The findings combining our previous^[16,18] and current observations suggest that, in addition to B12 deficiency, eHcy may be a potential risk factor in interfering with peripheral and central conduction of the somatosensory pathway. In other words, it is possible that the mechanism by which B12 deficiency causes central and peripheral nervous system dysfunction maybe partly via eHcy.^[3] A clinical observation that administration of antifolate agent such as methotrexate induced a clinical phenotype compatible with that of vitamin B12 deficiency favored this notion.^[40,41] However, laboratory evidence is needed to support this claim.^[3,4,19,38,39]

As the central large fiber conduction slowing is evident electrophysiologically in some patients with eHcy, it may be relevant to the central nervous system dysfunction and raise the concern regarding eHcy-related central neurodegeneration, such as dementia.^[3] Therefore, early recognition of the condition and prompt treatment may be critical. Since there is no cure currently available for neurodegenerative disorders, the best approach in clinical practice should be on prevention by modifying acquired risk factors, including eHcy. Thus, eHcy may become a therapeutic target. The efficacy of reducing eHcy with a regimen of combined vitamin B12, folate, and B6 in preventing the development of

neurodegenerative disorders needs to be explored.^[42] Nevertheless, our finding of central conduction slowing in adults with eHcy suggests clinically relevant significance and warrants further investigation. The limitation of this report was a retrospective study with a small number of subjects.

In summary, our pilot study on adults with isolated eHcy showed neurophysiologic evidence of slow central conduction involving the large fibers in the somatosensory, but not the visual and auditory, white matter pathways. The neurophysiologic changes may occur in parallel to eHcy-induced central processing decline. Additional large cohort studies may be needed to validate the finding.

ACKNOWLEDGMENTS

This article is dedicated to Mrs. Ethel Lombard for her outstanding service to the Department of Neurology at Temple University Hospital for 60 years.

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Cite this article as: Luo JJ, Bumanlag F, Ansari R, Tang YM, Dun NJ. Central somatosensory conduction slowing in adults with isolated elevated plasma level of homocysteine. *Neuroimmunol Neuroinflammation* 2015;2(1):26-30.

Source of Support: Nil. **Conflict of Interest:** No.

Received: 18-08-2014; **Accepted:** 10-11-2014

Influence of chlorpyrifos oxon on the development and progression of Alzheimer's disease in amyloid precursor protein transgenic mice

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ABSTRACT

Aim: Alzheimer's disease (AD) is a devastating neurological disorder and the most common form of dementia. Until date, the cause of AD eludes us, but a number of hypotheses have been put forward to try and understand the mechanisms involved. A series of studies have indicated that environmental factors, such as pesticides, heavy metals, and others can contribute to the development and progression of AD. Based on these data, we determined the impact of pesticides (chlorpyrifos oxon [CPO]) on AD-like pathogenesis in amyloid precursor protein (APP) transgenic mice. **Methods:** APP mice were treated at various times with low-dose CPO (1 mg/kg/day), *in utero* (3-week of gestation), during lactation (3-week), or as young adults (continuous dosing). **Results:** Exposure to CPO at all times enhanced neuro-inflammation and exacerbated oxidative stress in the brain prior to amyloid deposition. CPO-treated APP mice showed a decrease in memory and learning compared with untreated APP mice; furthermore, analyses of brain tissue sections and extracts showed an increase in A β levels and C-terminal fragment- β levels, a decrease in soluble APP α (sAPP α) levels, and an increase in plaque load. In addition, CPO-treated APP transgenic mice showed a significant decrease in neurotrophic factor levels (nerve growth factor, brain-derived neurotrophic factor, and neurotrophin-3) compared to vehicle-treated APP transgenic animals. Treatment with galantamine attenuated the effects of CPO by reducing amyloid β levels and amyloid load. **Conclusion:** CPO accelerated and exacerbated the disease development and progression in the APP mice suggesting that pesticides may play a significant role in the pathogenesis of AD.

Key words: Amyloid beta, Alzheimer's disease, amyloid, cognition, inflammation, toxin

INTRODUCTION

Amyloid β peptide (A β) containing senile plaques are one of the neuropathological hallmarks of Alzheimer's disease (AD). Much of this work has focused on the biosynthesis of A β and factors that influence its deposition.^[1] The A β peptides are generated via internal

proteolysis of its precursor, the amyloid precursor protein (APP).^[2,3] In addition, a variety of neuronal cytoskeletal alterations is prominent features of AD neuropathology.^[4,5] Whether these abnormal features are the result or cause of neuronal loss is still controversial.^[6-10] Early onset autosomal dominant AD is directly linked to mutations in one of the several genes: APP, presenilin 1 (PS1), or presenilin 2 (PS2).^[11-13] In addition, several genes, most notably the apolipoprotein E (APOE) 4 allele, alter risk for later onset AD, and it is clear that mutation or polymorphism in several other genes can lead to similar AD phenotypes.^[14]

Recent studies have suggested that early exposure of individuals to environmental toxins, drugs, and other

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10.4103/2347-8659.149421

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agents appear to contribute to diseases that occur later in life.^[15-18] Osmond and Barker have suggested (and this has been supported by others) that there are critical periods during fetal development where programming by a stimulus or insult has a lasting or lifelong effect.^[19] We know that various tissues, including the brain, go through developmental programming to determine the number of cells (programmed cell death), types of cells, that participate to the final development of the brain.^[20] It has been suggested that exposure of tissues to insults at critical times could result in reprogramming to give rise to diseases later in life.^[18] It has been suggested that exposure of the brain to toxic agents triggers a process that will elicit the onset of AD, reprogram the brain to have AD start at an earlier age, or to exacerbate the process of AD.^[21] This can be achieved by a number of ways. Metabolic, blood flow, endocrine, nutritional changes can affect blood lipids, plasma insulin, obesity, atherosclerosis, behavior and learning in small mammals and primates.^[21-23] The application of this process to AD has been recently suggested and demonstrated in the latent early-life associated regulation model.^[24,25]

This study evaluated the effects of the pesticide chlorpyrifos oxon (CPO) on the development of AD later in life. To test this hypothesis, we determined the influence of CPO on AD by treating APP transgenic mice during gestation, during lactation and after weaning. Our data showed that CPO exacerbated the pathogenesis of AD in the mouse model and that this process may be partially mediated by inflammation and by the effects of CPO on acetylcholinesterase (AChE) inhibition. These findings validate the influence of environmental toxins on the development and progression of AD.

METHODS

Transgenic Alzheimer's disease mice

The mice used for these studies expressed the mutant form of human presenilin-1 (DeltaE9) and the mutant form of the chimeric mouse/human APP695.^[26] The mouse prion protein promoter directed the expression of both transgenes. The DeltaE9 mutation of the human presenilin-1 gene is a deletion of exon nine and corresponds to a form associated with early-onset AD. The APP695 gene harbors the K595N/M596L (Swedish) AD-causing mutations. The coding sequence of mouse A β peptide domain was humanized by replacing the three amino acids that differ between the two species with the human residues. These APP/ Δ PS1-Tg (referred to as APP) mice start developing amyloid plaques at about 3-4 months of age. These mice were on a C57BL/6J background.

Polymerase chain reaction analysis was utilized to determine the genotype of the animals as previously described.^[27] All experimental mice were male. Mice were given free access to food and water before and during the experiment.

Treatment of mice

Amyloid precursor protein transgenic mice were treated with CPO administered orally, dissolved in corn oil. During a preexperimental phase, all females were trained to drink corn oil from a syringe, to ensure proper administration of the vehicle and also to reduce stress associated with handling and exposure to a new stimulus. Following this training period, the females were paired with APP males in segregated cages, for up to a week, in order for mating to occur. The appearance of vaginal plugs was taken as evidence of successful insemination, at which time the females were separated from the males and placed in single housing in cages. Each female was randomly assigned to one of six CPO (Chem Service, West Chester, PA; 99.1% pure) dose groups: oil vehicle (at each time), 1 mg/kg bw/day for 3-week during gestation, 1 mg/kg bw/day for 3-week during lactation, and 1 mg/kg bw/day starting at 2 months of age. For the gestation and lactation groups there were 6 females in each group; this was done to ensure that potential dose effects could not be confounded with individual variation associated with a single dam and to provide sufficient numbers of offspring for statistical analysis. There were no differences in weight or reduced locomotor activity following treatment (data not shown). Animal studies were conducted according to regulations by the National Institutes of Health and as approved by the Institutional Animal Care and Use Committee at the Medical University of South Carolina and Ralph H. Johnson VA Medical Center.

Age of mice for analysis of memory deficits and biomarkers

Memory function, amyloid plaque and brain biomarkers were evaluated after significant memory deficits developed in the A β PP mice.

Spatial memory deficit

The memory deficit in the animals was measured by the Morris water maze test as we have described previously.^[27,28] Briefly, the spatial memory capability of each animal was assessed with the Morris water maze test (700-0718-4 W San Diego (SD) instruments) which evaluates memory in a swimming test. Mice were individually trained in a 1.2 m open field water maze in a pool filled with water to a depth of 30 cm and maintained at 25 °C. An escape platform (10 cm square) was placed 1 cm below the surface of the water. All animals underwent nonspatial pretraining for 4 consecutive days,

which prepared the animals for the final behavioral test to determine the retention of memory to find the platform. Two days following the nonspatial pretraining, the hidden platform was placed in the center of one quadrant of the pool, the animal was released facing the pool wall in a random fashion, the time was recorded (latency period), and the distance traveled to reach the platform was measured using video recording (Smart Video Tracking System; SD Instruments).

On the day after the last training session, the platform was removed, and a spatial probe test conducted. Each mouse was allowed to search for the platform for 60 s (memory retention) and the percent time spent in quadrant where the platform was located (northeast (NE) quadrant) and in the outer annular area were determined.

Brain amyloid plaque

Amyloid plaque load was assessed in brain sections (10 from each mouse) as we have described previously, achieved by immunohistochemical staining for A β (A β antibody 10D5, Elan pharmaceuticals).^[27,28] Brain tissues were fixed in 4% paraformaldehyde and then in 4% parformaldehyde and 30% sucrose for 24 h and each at 4 °C. Tissues were washed in buffered saline and transferred to an optimum cutting temperature medium. Cryosections were cut and blocked with normal serum, incubated with anti-A β and stained with diaminobenzoic acid (vector ABC Elite kit, vector laboratories). Bright field light microscopy imaged brain areas from which stained amyloid areas were quantitated using image analysis (NIH Image software, NIH, Washington, DC).

Brain amyloid β analysis

Brain A β analysis was conducted as previously described for transgenic AD mice.^[27,28] Briefly, animals were sacrificed and brain extracts were homogenized (1:3 weight/volume of buffer) in buffer of 5 mol/L guanidine HCl in 50 mmol/L Tris-HCl, pH 7.6, 150 mmol/L NaCl, plus protease inhibitors (Sigma). Homogenates were diluted to 0.5 mol/L guanidine and centrifuged (200,000 g for 20 min), and supernatant and pellet fractions were collected. The pellet from the brain extract procedure was sonicated in 6 mol/L guanidine and centrifuged at 200,000 g for 20 min at 4 °C, and the supernatant was diluted to 0.5 mol/L guanidine. The two supernatants were combined, and A β (40) and A β (42) (A β ₁₋₄₀ and A β ₁₋₄₂, respectively) were determined using enzyme-linked immunosorbent assays (ELISAs) kits specific for each peptide (IBL, JP27718 and JP27711). ELISAs measured A β peptides by methods previously described.^[29-33] Protein content was determined by the Bradford method.

C-terminal fragment- β and soluble amyloid precursor protein α analysis

C-terminal fragment- β (CTF- β) is generated from APP by β -secretase in the amyloidogenic pathway, and soluble APP (soluble amyloid precursor protein) is generated from APP by α -secretase in the nonamyloidogenic pathway. Western blots measured CTF- β and sAPP α brains of transgenic mice, using the same amount of protein per gel lane, performed as previously described.^[27,28] CTF- β was determined in the pellet fraction from the brain extract (antibody 8717, sigma) and sAPP α was assessed in the supernatant fraction from the brain extract (antibody 6E10, signet laboratories). Relative amounts of CTF- β and sAPP α were measured by densitometry and results were expressed as a percentage of the mean levels of CTF- β and sAPP α of the control groups (without protease gene knockouts). Control β -actin western blots (anti β -actin from Cell Signaling Technology) was conducted to monitor equal loading of the same amounts of samples (20 μ g protein) in each gel lane.

Immunohistochemistry staining

Cryosections of the right brain hemispheres were washed 3 times (5 min/wash) with Tris-buffered saline (TBS) (pH 7.4) buffer, followed by washing 1 time with 0.1% Triton X-100-TBS buffer for 5 min. Sections were then incubated in 3% H₂O₂ and TBS buffer for 30 min at room temperature to eliminate endogenous peroxidase activity. After 1 h of blocking with 5.0% serum (horse or goat), the sections were incubated overnight with primary antibodies. Primary antibody and dilutions were: glial fibrillary acidic protein (GFAP)-positive astrocytes (1:200 dilution, 2E1; BD Biosciences, San Jose, CA). The next day, sections were washed 3 times (5 min/wash) with 0.1% Triton X-100 and TBS buffer to remove excess primary antibody. Thereafter, primary antibodies were detected using horseradish peroxidase (HRP)-conjugated mouse immunoglobulin G Vectastain ABC kit and DAB/substrate reagents (vector laboratories, Burlingame, CA) according to the manufacturer's instructions.

Enzyme-linked immunosorbent assays for inflammatory markers

Brain hemispheres were weighted and homogenized with 4 volumes of phosphate buffered saline buffer (125 mg/mL) containing complete protease inhibitor cocktail (Sigma-Aldrich, Saint Louis, MO, USA). The supernatant was then collected, and total protein was determined by the BCA method (Pierce Biotechnology, Rockford, IL, USA). Tumor necrosis factor (TNF)- α , interleukin (IL)-1 β and IL-6 levels were measured with a mouse TNF- α , IL-1 β and IL-6 ELISA kits (R and D Systems, Minneapolis, MN, USA).

Protein carbonyl content

Protein carbonyl content was determined by the OxiSelect Protein Carbonyl Kit (Cell Biolabs, Inc.) and ELISA. Briefly, bovine serum albumin (BSA) standards or protein samples (10 µg/mL) were adsorbed onto a 96-well plate for 2 h at 37 °C. The protein carbonyls present in the sample or standard were derivatized to dinitrophenyl (DNP) hydrazone and probed with an anti-DNP antibody, followed by an HRP conjugated secondary antibody. The protein carbonyl content in an unknown sample was determined by comparing with a standard curve that was prepared from predetermined reduced and oxidized BSA standards.

Statistical evaluation

Experiments consisted of 10 mice in each group. Each biochemical analysis consisted of two or three replicates. Statistical analyzes and data display were conducted utilizing computer software designed for scientific data analysis (Prism 4 GraphPad, Prism, La Jolla, CA). Quantitative data are displayed as the mean and standard error of the mean. Differences between groups were determined by ANOVA analysis and Dunnett's multiple comparison tests used to determine differences between transgenic control mice and treated animals.

RESULTS

Experimental protocol

To investigate the influence of environmental toxins on AD, we used the APP transgenic mice expressing the mutant form of human presenilin-1 (DeltaE9) and the mutant chimeric mouse/human APP695 residue form (51). The mouse prion protein promoter directs the expression of both transgenes. APP mice start developing amyloid plaques around 3-4 months of age. This will allowed us to study the process in a relatively short period and to allow for the generation of sufficient animals for the studies. APP mice were exposed to either 1 mg/kg CPO via ingestion by suspending

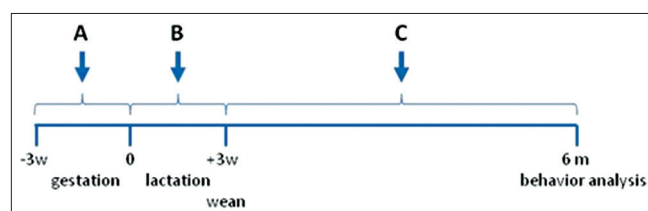


Figure 1: Experimental protocol for treatment of mice with chlorpyrifos oxon. This figure illustrates the protocol for the treatment of transgenic Alzheimer's disease mouse model utilized in this study. (a) Treatment of pregnant female amyloid precursor protein (APP) mice for 3-week during gestation; (b) treatment of female APP mice after birth and for 3-week during lactation; (c) treatment of APP mice following weaning until termination of the study at 6 months. All animals were assessed behaviorally prior to sacrifice. -3w, indicates time of plug following when male and female APP mice were placed together; 0, indicates time at birth; +3w, indicates time of weaning of APP mice

the toxin in corn oil (CPO) or corn oil alone. For these studies, three experimental paradigms were employed [Figure 1]. (1) Pregnant APP female mice were fed CPO daily for 3-week from the beginning of gestation; (2) female APP mice were fed CPO daily for 3-week from the beginning of lactation; and (3) weaned APP offspring were fed CPO daily for up to 6 months of age. CPO has been shown to cross the placental and is secreted into the milk following treatment of rodents.^[34,35]

Administration of chlorpyrifos oxon exacerbates memory deficits in the amyloid precursor protein mice

The effect of the CPO (1 mg/kg) on the various paradigms was assessed for nonspatial pretraining for four consecutive days to learn the location of the hidden platform. Analyzes by the Morris water maze test on each day of the training period showed that the mice do learn, indicated by the reduced latency time for the mice to reach the hidden platform during the training period [Figure 2]. By the 4th day of training, the control APP in all groups showed the shortest latency period, representing enhanced learning, compared to the CPO APP mice. Mice administered CPO under each paradigm showed a worsening of the latency time suggesting that the treatment with CPO perturbs the learning process.

Two days following training, mice were subjected to the final behavioral Morris water maze test to determine the memory deficits. CPO-treated mice exhibited substantial worsening of memory deficits, assessed by the latency period and distance traveled, which is the time and distance, respectively, that it took the animal to swim to the submerged platform [Figure 3]. The shorter time and distance traveled indicates better memory. The CPO treatment

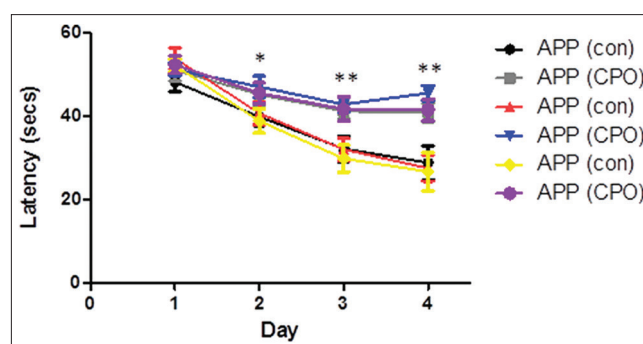


Figure 2: Treatment of amyloid precursor protein (APP) mice with chlorpyrifos oxon (CPO) results in diminished memory acquisition. APP mice (control, corn oil alone) and APP mice treated with CPO (CPO) from the different groups at 6 months of age were trained in the Morris water maze test on each of 4 consecutive days to learn the location of a submerged, invisible platform in a pool of water. The time that it took the mice to swim to the platform was recorded each day, measured as the latency period (in seconds, s), with shorter latency times indicating better memory acquisition. Latency (s) is shown as mean \pm standard error of the mean (statistical significance, * P < 0.05, ** P < 0.01, compared to APP control group; n = 12 per group)

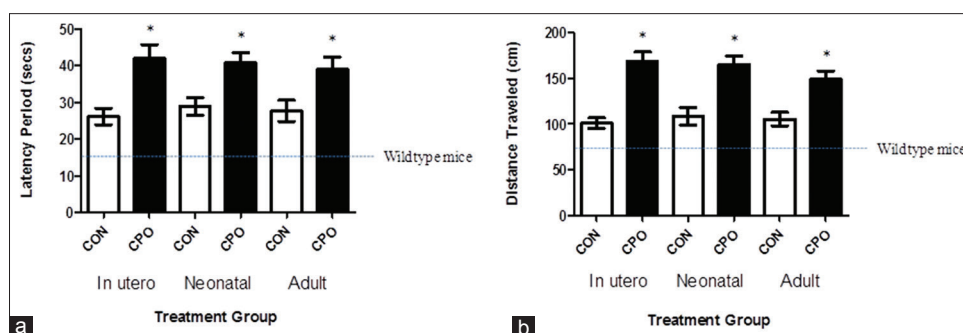


Figure 3: Treatment of amyloid precursor protein (APP) mice with chlorpyrifos oxon CPO results in exacerbated memory deficits. Memory deficits of APP mice were assessed 2 days after completion of the training in the Morris water maze test by measuring the latency period (a) and distance traveled (b) for animals to swim to the submerged, invisible platform. The shorter latency periods and shorter distances traveled indicate improved memory. APP mice (control, corn oil alone) compared to APP mice treated with CPO had shorter mean latency periods. Memory function of wild-type mice of the same strain and age is shown by the dotted line, as reported previously.^[7] Values are expressed as mean \pm standard error of the mean, and $n = 12$ per group. *Statistically significant ($P < 0.05$)

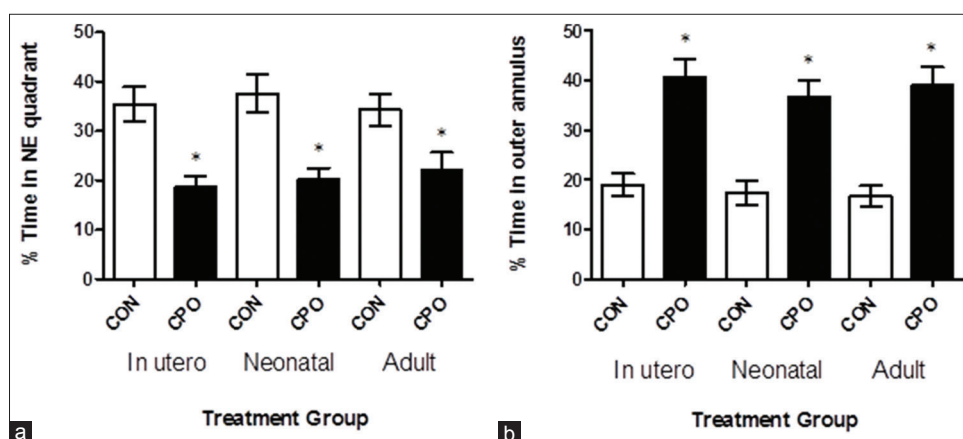


Figure 4: Treatment of amyloid precursor protein (APP) mice with chlorpyrifos oxon (CPO) results in diminished memory retention. The day after the last training session, the submerged platform was removed and the mice were allowed to swim in the pool for 60 s. The percent time each animal swam in the quadrant from which the platform had been removed (northeast [NE] quadrant) (a) and the percent time an animal swam in the annulus of the pool were recorded (b). Greater memory retention is reflected in a higher percent time in the northeast quadrant and lower percent time in the annulus. APP mice (control, corn oil alone) compared to APP mice treated with CPO had percent times in the quadrant of shorter duration. Values are expressed as the mean \pm standard error of the mean, and $n = 12$ per group. *Statistically significant with $P < 0.05$

resulted in a 160% and 167% (gestation), 141% and 152% (lactation) and 140% and 142% (weanling) increase in the latency period and distance traveled, respectively [Figure 3a and b]. The 16 s latency period, and the 72 cm distance are the time and distance for wild-type mice (nontransgenic, same strain as APP mice) [Figure 3a and b, dotted lines].

Administration of CPO resulted in substantial memory loss in the APP mice as illustrated by the reduced percent time spent in the NE quadrant (from which the submerged platform was removed), and the increased percent time spent in the outer annulus, compared to control APP mice [Figure 4]. The CPO treatment resulted in a 48% (gestation), 46% (lactation) and 35% (weanling) decrease in the percent time spent in the NE quadrant and a 215% (gestation), 213% (lactation) and 233% (weanling) increase in the percent time spent in the annulus. The APP mice (control) and APP CPO mice did not have a different swimming speed (data not shown). Thus, by the four parameters measured in the Morris water maze test, the CPO administration exacerbated the

memory deficits that develop in the transgenic APP mice.

Administration of chlorpyrifos oxon to amyloid precursor protein mice exacerbates brain amyloid plaque load

A β immunohistochemistry of brain sections showed that the treatment with CPO increased brain amyloid plaques in the APP mice [Figure 5a and b]. Administration of CPO under all paradigms resulted in an increase in amyloid plaque load in the APP mice [Figure 5c]. Quantitative image analysis of the A β immunohistochemistry showed that the CPO resulted in a significant 214% (gestation), 234% (lactation) and 215% (weanling) increase in brain amyloid plaque load relative to control APP animals [Figure 5c].

Administration of chlorpyrifos oxon alters brain biomarkers in a manner characteristic of inflammation and increase in amyloid precursor protein processing by β -secretase

Amyloid precursor protein-derived A β peptides and APP-derived cleavage products resulting from amyloidogenic and nonamyloidogenic processing of APP were evaluated in the control and CPO treated

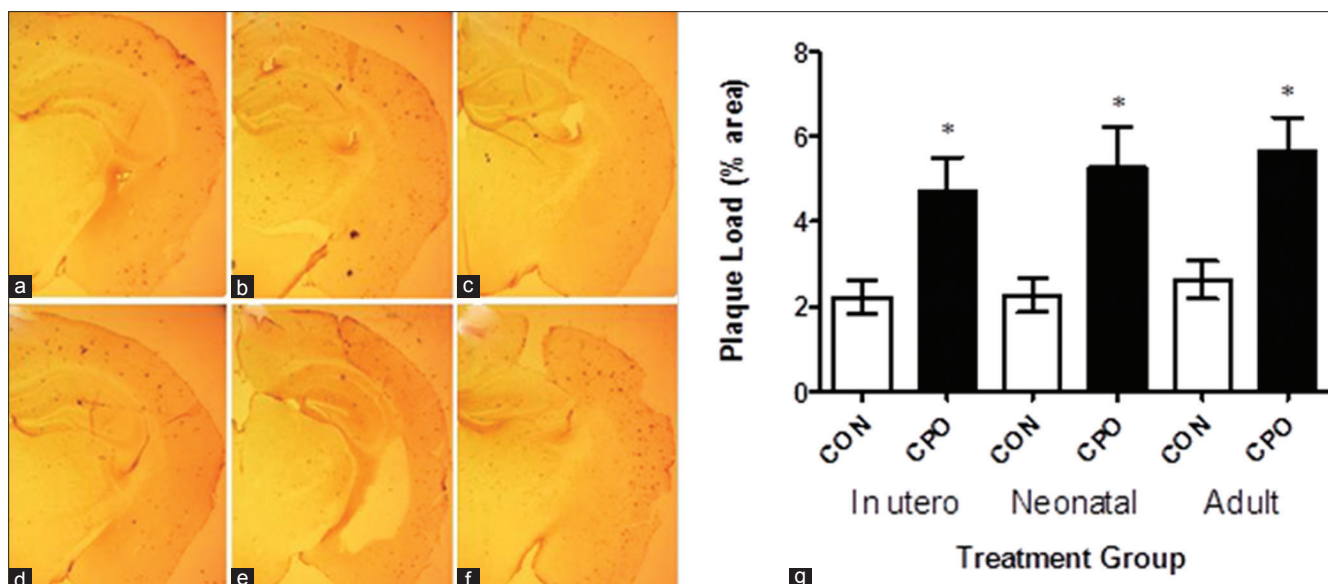


Figure 5: Treatment of amyloid precursor protein (APP) mice with chlorpyrifos oxon (CPO) results in increased brain amyloid plaque load. Amyloid plaque load was determined by immunohistochemistry and image analysis of brain sections from APP mice (control, corn oil alone) and CPO treated APP mice as shown in (a-c and d-f), respectively (representative images). Arrows indicate amyloid plaque deposits. (g) Quantitation showed that control APP mice had mean percent amyloid plaque loads lower than that of the CPO treated mice ($n = 12$ per group, values are expressed as mean \pm standard error of the mean, *Statistically significant with $P < 0.05$)

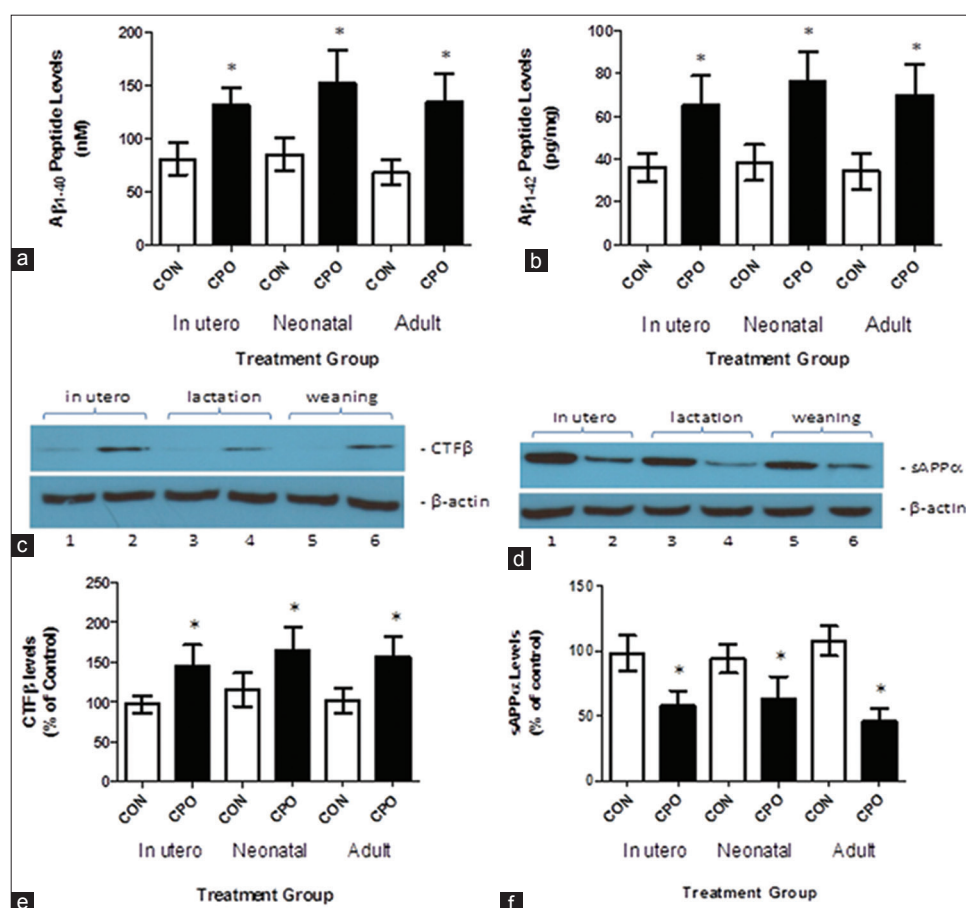


Figure 6: Treatment of amyloid precursor protein (APP) mice with chlorpyrifos oxon (CPO) results in changes in amyloid β (A β)-related biomarkers. Brain A β (40) and A β (42) (A β_{1-40} and A β_{1-42} , respectively) levels were determined by enzyme-linked immunosorbent assay. The APP mice (control, corn oil alone) compared to CPO-treated APP mice (CPO) had lower mean brain A β (40) levels (a) and mean A β (42) levels (b). Brain APP-derived CTF β , generated by β -secretase, was assessed by western blot analysis (c). Relative quantitation by densitometry showed that the APP mice (control, corn oil alone [CON]) compared with CPO treated APP mice (CPO) had lower mean brain CTF β levels (d). APP-derived sAPP α was evaluated by western blot analysis (e). Quantitation by densitometry showed that the APP mice (control, CON) compared to CPO-treated APP mice (CPO) had higher mean brain sAPP α levels (f) ($n = 12$ per group, values are expressed as mean \pm standard error of the mean, *Statistically significant with $P < 0.05$)

mice. Amyloidogenic processing of APP by β -secretase produces the CTF β fragment and A β peptides, and

nonamyloidogenic processing of APP by α -secretase results in the sAPP α fragment.^[27,31]

Chlorpyrifos oxon administration to the transgenic APP mice at all-time points increased both brain $A\beta_{1-40}$ and $A\beta_{1-42}$ compared with control APP mice [Figure 6a and b]. The CPO treatment caused an increase in brain CTF- β levels relative to controls [Figure 6c and d]. The CPO treatment reduced sAPP α levels relative to controls [Figure 6e and f].

Since CTF- β is a β -secretase cleavage product, an increase in CTF- β resulting from CPO treatment in the APP mice suggests that β -secretase activity is increased following administration of CPO. Increased production of CTF- β from APP in CPO-treated mice is likely to result in decreased APP available for α -secretase leading to a decrease in sAPP α [Table 1].

Administration of chlorpyrifos oxon enhances inflammation and oxidative stress in the brain of amyloid precursor protein mice

In order to help determine the role of CPO on AD, we determined its impact on inflammatory markers in the brain following treatment. Brain samples from the control APP and CPO treated mice were examined for the cytokines tumor necrosis factor- α (TNF- α), interleukin 1 β (IL-1 β) and IL-6 levels at the termination of the study [Figure 7a-c]. All the treatment groups showed a significant elevation in the cytokine levels compared to the control APP mice [Table 2]. In addition, weaned APP mice were fed CPO (1 mg/kg) and then examined at various times up to 12 months for TNF- α levels [Figure 7d]. As seen in the figure, animals treated

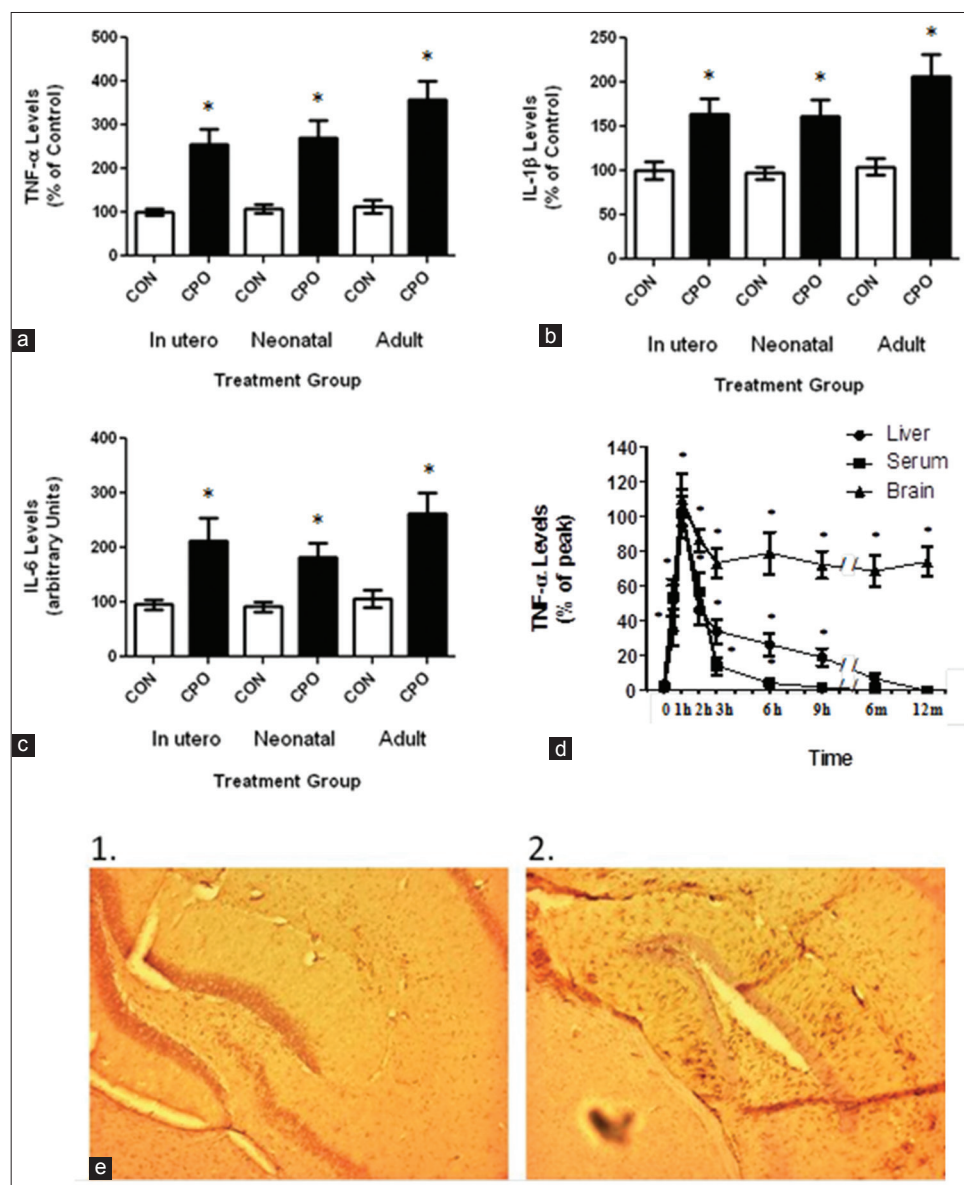


Figure 7: Treatment of amyloid precursor protein (APP) mice with chlorpyrifos oxon (CPO) results in exaggerated inflammation. Control APP mice and mice treated with CPO were evaluated for inflammatory markers. (a) Treatment of APP mice (3-week-old) with CPO showed constitutively elevated levels of tumor necrosis factor- α (TNF- α) in the brain compared to liver and serum levels. (b) Comparison of CPO treated APP mice to control APP mice showed a significant difference in the levels of TNF- α at all-time points. (c) Comparison of TNF- α (1), interleukin-1 α (IL-1 α) (2) and IL-6 (3) in the various treated groups. (d) APP mice were fed CPO (1 mg/kg) and then livers, blood and brains were examined at the indicated time points for TNF- α levels. Values are expressed as mean \pm standard error of the mean, $n = 12$ per group. *Statistically significant ($P < 0.05$). (e) Immunostaining for glial fibrillary acidic protein (GFAP) in control (1) and CPO treated (2) animals (neonates). Brain sections were stained with anti-GFAP antibody

with CPO showed a significant increase in TNF- α levels early following treatment. However, while the TNF- α levels in the liver and blood returned to baseline within several days, the levels in the brain remained elevated for up to 12 months. Finally, brain sections from the APP mice treated with and without CPO during lactation were subject to immunohistochemistry for GFAP [Figure 7e]. As shown in figure, GFAP was significantly elevated in the CPO treated mice compared with the control animals.

Furthermore, we analyzed the protein carbonyl content (a biomarker of reactive oxygen species) in the brain of the APP mice, and found that the level of oxidized proteins was significantly increased (> 50%) in mice treated with CPO than in the control APP mice [Figure 8].

Administration of chlorpyrifos oxon decreases brain neurotrophic factors

To further characterize the changes in brain following treatment with CPO, APP mice were evaluated for neurotrophic factor levels [Figure 9]. Following treatment with CPO, mice were examined for nerve growth factor, brain-derived neurotrophic factor, and neurotrophin-3 levels in the brain [Table 3]. As shown in figure, all treatments significantly reduced the neurotrophic factor levels in the brain.

Administration of galantamine partially attenuates the effects of chlorpyrifos oxon on A β peptide levels and amyloid plaque load

As a potential therapeutic approach, we determined the impact of galantamine (a competitive and reversible cholinesterase inhibitor) that can protect against organophosphorus insecticides [Figure 10].

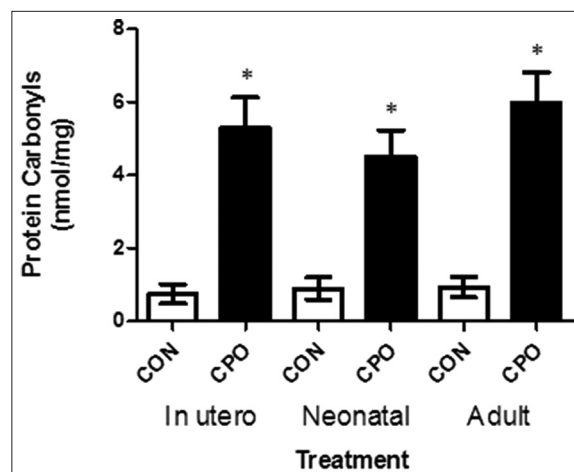


Figure 8: Protein carbonyl content in the brains of amyloid precursor protein (APP) transgenic mice treated with and without chlorpyrifos oxon. Extracts from the brains of APP transgenic mice were analyzed for the level of oxidized proteins by OxiSelect and enzyme-linked immunosorbent assay. Values are expressed as mean \pm standard error of the mean, $n = 12$ per group. *Statistically significant ($P < 0.05$)

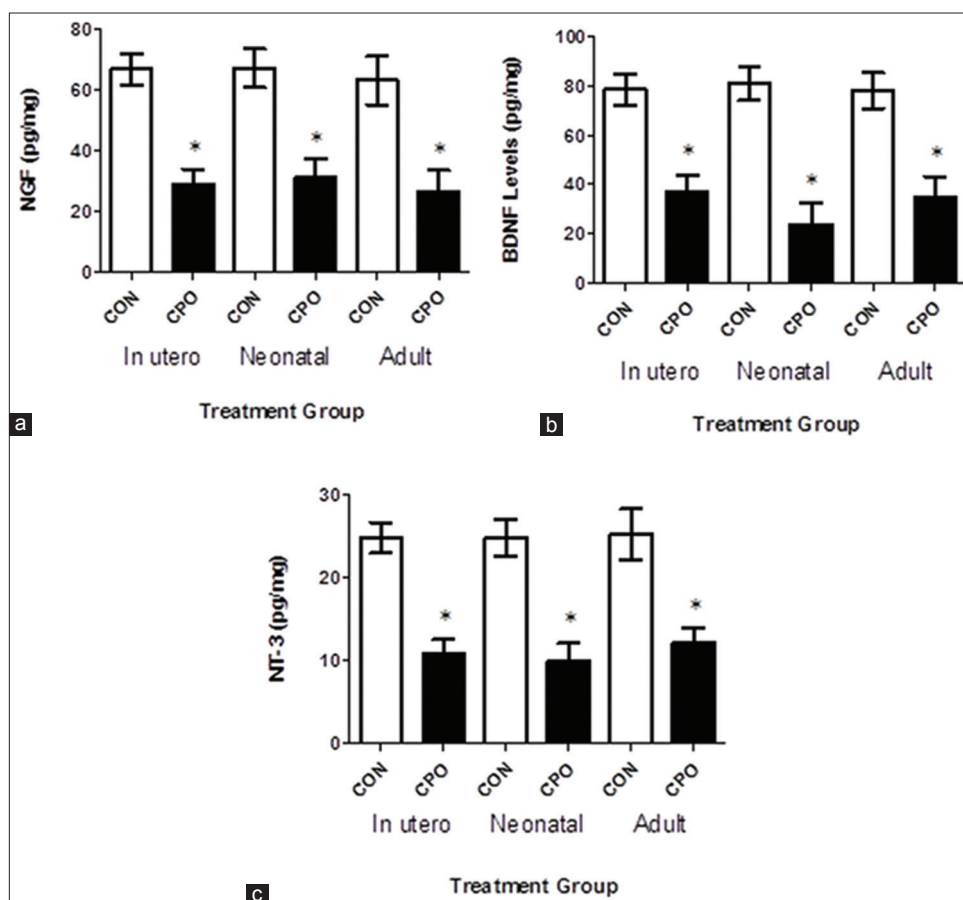


Figure 9: Treatment of amyloid precursor protein (APP) mice with chlorpyrifos oxon (CPO) results in a decrease in neurotrophic factors. Brain nerve growth factor, brain-derived neurotrophic factor and neurotrophin-3 levels (panels a, b and c, respectively) were determined by enzyme-linked immunosorbent assay in APP mice (control) and APP mice treated with CPO (CPO). Values are shown as the mean \pm standard error of the mean, and $n = 12$. *Statistically significant ($P < 0.05$)

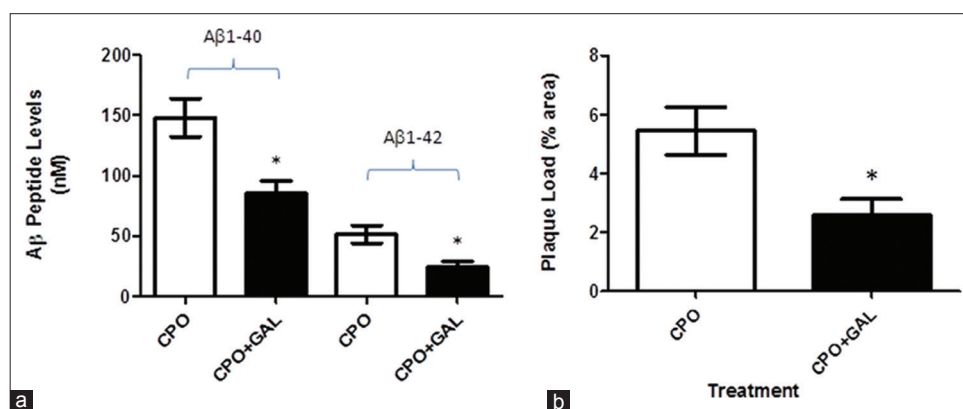


Figure 10: Treatment of amyloid precursor protein (APP) mice with galantamine partially attenuates the effects of chlorpyrifos oxon (CPO) on amyloid β ($A\beta$) peptide levels and plaque load. Weaned APP mice (3-week of age) were treated with CPO (1 mg/kg) or CPO + galantamine (2 mg/kg/day, i.m.) and examined for $A\beta$ peptide levels (a) and amyloid load (b) values are shown as the mean \pm standard error of the mean, and $n = 12$. *Statistically significant ($P < 0.05$)

Table 1: $A\beta$ -related biomarker levels in the brains of APP mice

	In utero (nmol/L)		Neonatal (nmol/L)		Adult (nmol/L)	
$A\beta_{1-40}$	81.2 \pm 14.9	132 \pm 16.3	85.3 \pm 15.3	152 \pm 30.4	68.2 \pm 11.6	135 \pm 26.1
$A\beta_{1-42}$	36.0 \pm 6.34	65.2 \pm 13.8	38.5 \pm 8.38	76.6 \pm 13.5	34.4 \pm 8.54	69.8 \pm 14.2
	In utero (% of control)		Neonatal (% of control)		Adult (% of control)	
sAPP α	98.6 \pm 13.6	58.4 \pm 11.4	94.3 \pm 11.0	63.4 \pm 17.0	108 \pm 11.4	46.2 \pm 9.59
CTF β	96.6 \pm 10.9	145 \pm 26.0	115 \pm 20.9	165 \pm 28.5	102 \pm 16.2	157 \pm 25.3

Table 2: Inflammatory marker levels in the brains of APP mice

	In utero (pg/mg)		Neonatal (pg/mg)		Adult (pg/mg)	
TNF- α	100 \pm 8.29	254 \pm 35.9	109 \pm 9.43	271 \pm 38.2	112 \pm 15.2	358 \pm 43.9
IL-1 β	99.7 \pm 10.0	164 \pm 16.8	97.3 \pm 6.74	161 \pm 19.0	105 \pm 9.23	206 \pm 25.4
IL-6	95.6 \pm 8.59	212 \pm 41.8	91.7 \pm 8.61	183 \pm 26.6	107 \pm 15.8	262 \pm 39.4

Table 3: Neurotrophic factor levels in the brains of APP mice

	In utero (pg/mg)		Neonatal (pg/mg)		Adult (pg/mg)	
NGF	66.7 \pm 5.09	29.0 \pm 4.91	67.1 \pm 6.42	31.0 \pm 6.12	63.0 \pm 7.95	26.6 \pm 6.79
BDNF	78.4 \pm 6.56	37.4 \pm 6.24	80.9 \pm 6.80	23.9 \pm 8.53	78.0 \pm 7.23	35.1 \pm 8.20
NT-3	24.8 \pm 1.82	10.8 \pm 1.72	24.8 \pm 2.23	9.96 \pm 2.13	25.2 \pm 3.09	12.1 \pm 1.88

Treatment of weaned APP mice with CPO or CPO plus galantamine (2 mg/kg/day) showed a significant reduction in $A\beta$ peptide levels ($A\beta_{1-40}$ -148.2 \pm 15.87 nmol/L vs. 85.30 \pm 10.43 nmol/L; $A\beta_{1-42}$ -51.6 \pm 7.37 nmol/L vs. 24.7 \pm 4.37 nmol/L, respectively) and plaque load (5.46 \pm 0.81% area vs. 2.57 \pm 0.56% area, respectively).

DISCUSSION

The major result of this study is that CPO (organophosphate pesticide) exacerbates amyloid pathology and enhances memory deficits in the APP transgenic mouse model. This is one of the first studies to demonstrate that pesticides result in increased AD pathology. Moreover, mice treated in utero, during neonatal development or during adulthood all developed AD-like pathogenesis to a greater extent than control animals. These significant findings suggest that environmental factors may influence AD development and progression and that developing preventatives or

therapeutics to attenuate the effects of the pesticides may limit the extent of AD and improve memory deficits of AD.

The aging United States population is leading to a growing number of individuals with neurodegenerative disorders.^[36] Since only a small proportion of the individuals with AD have a genetic predisposition to the disease and because the pathogenesis of the disease remains to be elucidated, we need to consider alternative hypotheses to determine the disease process. Over the years, a number of studies have focused on the role of environmental toxins in AD but have not been able to link the two.^[37-42] Previous studies have examined the role of heavy metals in the AD.^[43-46] For years, we have known that heavy metals affect brain development resulting in abnormalities that persist throughout life. Several studies indicated that aluminum (Al) found in antiperspirants, antacids and occupational exposure can contribute to the development of AD.^[47,48] However, just as many studies

have indicated the Al has no effect.^[48-50] The same can be said for lead (Pb), mercury (Hg), methylmercury (MeHg), iron (Fe), zinc (Zn), *etc.*^[51-54] The association between solvent exposure and AD is weak and in some cases contradictory.^[55,56] In addition, electromagnetic fields have a tenuous relationship to AD.^[57,58] Finally, various pesticides have been linked to AD as well as other neurological disorders especially Parkinson's disease. The effects of specific pesticides (organophosphates and carbamates) on the brain are well known and contribute to a number of pathological features. Tyas *et al.*^[38] and Baldi *et al.*^[40] showed that occupational exposure to defoliants/fumigants or general pesticides as statistically limited to AD. Furthermore, epidemiological studies have demonstrated that specific organochlorides (dichlorodiphenyltrichloroethane, dichlorodiphenyldichloroethylene and dieldrin) were present in the brains of both AD and Parkinson's disease patients suggesting an etiological relationship for these chemicals.^[59]

The findings of this study address the impact of environmental factors on the which exacerbates brain biochemical processes and memory deficits upon exposure in a mouse model expressing the APP transgene that is relevant to sporadic AD patients, representing more than 90% of the AD population. Environmental factors have been viewed in the field as potential targets for therapeutic intervention to prevent or attenuate pathology and memory deficits associated with AD.^[44] Identification of such pathways involved in enhanced memory deficits was accomplished in this study by using the APP mouse model of AD.^[28]

Treatment of mice in utero, during neonatal development or as adults resulted in an altered biomarker pattern consistent with enhanced AD-like activity. Biomarker analyses showed that pesticide exposure of the APP mice increased brain A β and CTF- β derived from APP by β -secretase, and reduced sA β PP α ; these changes represent an altered A β -related pattern characteristic of augmenting the processing of APP. These data demonstrate that pesticides may enhance β -secretase activity in a transgenic mouse model expressing APP or may reduce the clearance of A β peptides via proteases or other mechanisms.^[36,60] The biomarker data supports the hypothesis that exposure of the APP mice to CPO enhances memory deficits by altering the presence of A β peptides. Moreover, the data suggest that the improvement in memory deficits occurring with administration of galantamine may be due to the reversible inhibition of AChE activity.^[61]

Our data shows that certain environmental toxins regulate oxidative stress and inflammatory processes triggered by the A β peptide. The toxins enhance A β production and

exacerbate oxidative stress. Thus, the aim of this study was to determine the impact of environmental toxins on the pathogenesis of AD. The overall hypothesis is that environmental influence occurring during brain development and beyond result in damage to mitochondria, and reprogramming of the brain resulting in increased oxidative stress and inflammation.^[62,63] This process alters expression of various genes related to the development of AD (increased APP expression, increase APP processing), which further exacerbates the disease course.^[64] This susceptibility early in life exacerbates the normal process of amyloidogenesis in the aging brain, accelerating the onset of AD.^[65] Several studies have suggested in human studies or demonstrated in animal studies that environmental toxins do influence neurodegeneration and neurobehavioral function.^[44,65-68] Stewart *et al.*^[67] showed that APOE genotype and previous exposure to lead can alter behavioral aspects of aged individuals. In addition, they showed that magnetic resonance imaging analysis of these individuals showed increased neurodegeneration compared to individuals not exposed to lead.^[66] Finally, Zawia has shown that exposure of rats and mice to lead early in life can exacerbate APP processing and amyloid formation in the brain.^[44,65,68]

In conclusion, this study shows that exposure of APP transgenic mice to pesticides as different times during development results in enhanced memory deficits and altered brain APP metabolism. In addition, we show that the CPO exacerbates inflammation, oxidative stress and suppresses neurotrophic factor expression that may contribute to the disease process. Importantly, these data validate the impact of environmental toxins on the enhancement of AD pathology and suggest that this process may contribute to the development and progression of AD in people.

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Cite this article as: Yu J, Zhu H, Bhat A, El-Sayed H, Gudiz T, Gattoni-Celli S, Kindy MS. Influence of chlorpyrifos oxon on the development and progression of Alzheimer's disease in amyloid precursor protein transgenic mice. *Neuroimmunol Neuroinflammation* 2015;2(1):31-42.

Source of Support: Nil. **Conflict of Interest:** No.

Received: 14-09-2014; **Accepted:** 21-10-2014

Cardiac arrhythmia with premature ventricular contractures induced by interferon beta in a patient with multiple sclerosis

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ABSTRACT

Multiple sclerosis (MS) is an immune-mediated inflammatory and neurodegenerative disease of the central nervous system. Interferon (IFN) beta is an active ingredient of five out of twelve disease modifying treatments approved for MS. We report a case of IFN-beta-induced cardiac arrhythmia with premature ventricular contractures in a patient recently diagnosed with MS.

Key words: Cardiac arrhythmia, interferon beta, multiple sclerosis

INTRODUCTION

Multiple sclerosis (MS) is an immune-mediated inflammatory and neurodegenerative disease of the central nervous system.^[1,2] There is no cure for MS. Most MS patients are being treated with disease modifying treatments (DMTs). Selected DMTs can cause cardiovascular adverse events in MS patients. Due to a risk for bradyarrhythmia and atrioventricular blocks, patients should be monitored during fingolimod treatment initiation.^[3] Interferon (IFN) beta is an active ingredient of five out of twelve DMTs approved for relapsing-remitting form of MS. We are reporting a case of IFN-beta-1a-induced cardiac arrhythmia with premature ventricular contractures (PVCs) in a patient recently diagnosed with MS.

CASE REPORT

The patient is a 22-year-old female without any previous history of cardiac disease. In 2002, she developed her first neurological episode of decreased sensation in the right upper and lower extremities. The symptoms subsided after a 5-day course of intravenous steroids.

In June 2008, the patient developed vertigo, dysarthria, ataxia in the right hand and right hemiparesis. Her brain magnetic resonance imaging (MRI) revealed numerous ovoid lesions adjacent to lateral ventricles and a T2 hyperintense lesion adjacent to the fourth ventricle involving the right middle cerebellar peduncle. Several of these white matter lesions were contrast-enhancing. On her cervical and thoracic spine MRI, there were two separate contrast-enhancing lesions at C4 and C5-C6 level on the right side. The patient improved symptomatically after a course of intravenous steroids and was diagnosed with clinically definite relapsing-remitting MS in 2008 based on McDonalds criteria.^[4] She was initially started on Glatiramer acetate (20 mg subcutaneously daily) in August 2008. However, she had experienced two MS exacerbations (left optic neuritis and myelitis) within 7 months after starting this medication. MRI done in March 2009 revealed new contrast-enhancing lesions in the cervical spine. Therefore, Glatiramer acetate was discontinued on March 19, 2009 due to the lack of clinical efficacy. She did not take any medication between March 19 and April 7. The patient agreed to try IFN-beta-1a 3 times a week and received her first subcutaneous injection of medication (11 µg, or 25% of the full dose) on April 7, 2009. Three hours after injection, she started to feel irregular heartbeats. An electrocardiogram (ECG) performed on April 8, 2009 revealed PVCs with the heart rate 72 beats/min [Figure 1]. IFN-beta was discontinued. The patient was followed clinically; her symptoms of irregular heartbeats disappeared in 4 days

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10.4103/2347-8659.149422

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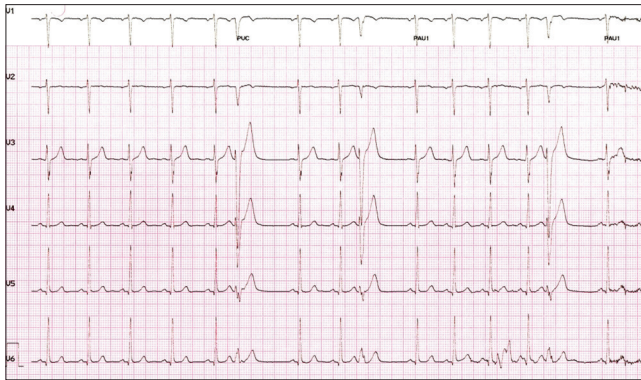


Figure 1: Electrocardiogram depicts a rhythm strip recorded from leads V1-V6 (10 s) with three premature ventricular contractures (PVCs). The heart rate was 72 beats/min. The study was performed on April 8, 2009. This EKG was done on the next day after a single IFN-beta-1a injection (11 µg SC). Two additional rhythm strips (10 s each) revealed 5 more PVCs (not shown)

after IFN-beta-1a injection. The repeat ECG on April 14, 2009 revealed normal sinus rhythm [Figure 2]. The patient was evaluated by a cardiologist. It was concluded that cardiac arrhythmia with PVCs was secondary to IFN-beta-1a treatment. The patient was switched to another (non-IFN-based) DMT for MS and has had no cardiac symptoms in the subsequent 5 years.

DISCUSSION

We describe cardiac arrhythmia with PVCs in a 22-year-old MS patient who received her first dose of IFN-beta-1a. The medication had been in her system for 3 h by the time the patient started experiencing palpitations, and it took 4 days for symptoms to resolve. The pharmacokinetics of Rebif® (IFN-beta-1a) in people with MS has not been evaluated. In healthy volunteer subjects, a single subcutaneous injection of 60 µg of Rebif®, resulted in a peak serum concentration of IFN-beta in approximately 16 h. The mean serum elimination half-life was 69 h.^[5] There were no previously described cases of early onset cardiac arrhythmia in IFN-beta-treated patients. Kastalli *et al.* reported a case of cardiac arrhythmia in a 35-year-old MS patient who was diagnosed with complete left bundle branch block after 5 years of IFN-beta-1a treatment.^[6] In contrast to IFN-beta, other Type I IFNs such as IFN-alpha subtypes are well-known for causing cardiac arrhythmia, dilated cardiomyopathy, and symptoms of ischemic heart disease, including myocardial infarction and sudden death.^[7] Ectopic beats, including PVCs were reported in patients treated with IFN-alpha-2b.^[8] Cardiotoxic side-effects in patients treated with IFN-alpha, usually, manifested within 4-12 weeks of therapy, but in some cases they could occur within 1-7 days of initiating the drug treatment.^[7,9] In most of the cases lowering the dose of IFN-alpha or discontinuing this drug helped to reverse cardiac arrhythmia (usually in the matter of 3-7 days).^[7,9]

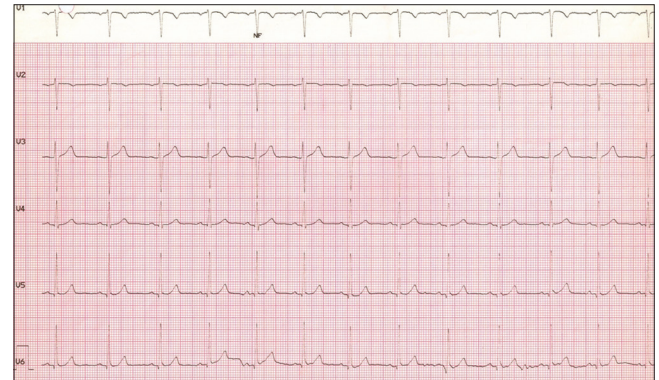


Figure 2: Electrocardiogram depicts a rhythm strip recorded from leads V1-V6 (10 s) without premature ventricular contractures (PVCs). The study was performed on April 14, 2009 and revealed normal sinus rhythm with the heart rate 71 beats/min. This EKG was done 7 days after a single IFN-beta-1a injection (11 µg SC). Two additional rhythm strips (10 s each) revealed no PVCs as well (not shown)

Although the IFN-alpha subtypes and IFN-beta interact with a common receptor, IFN-alpha receptor (IFNAR), which comprises high-affinity (IFNAR2) and low-affinity (IFNAR1) components, they nevertheless exhibit functional differences.^[10] One may suggest that difference in ligand-receptor affinity is one of the possible explanations for these variations. Even within the IFN-alpha species, individual subtypes may differ by over 10,000 fold in their biological activity.^[11] Recently, it was shown that IFN-beta binds to IFNAR1 independently of IFNAR2.^[12] Therefore, it is not surprising that the induction of cardiac arrhythmia may be less frequent in IFN-beta-treated compared with IFN-alpha-treated patients. However, more studies need to be done to understand the cause and prevalence of cardiac arrhythmia symptoms in IFN-beta- and IFN-alpha-treated patients. Nevertheless, as IFN-beta is one of the most prescribed DMT for MS, the knowledge about this adverse effect is deemed to be important for neurologists treating MS patients.

ACKNOWLEDGMENTS

We would like to thank Joan Moore for technical support.

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Cite this article as: Sobol I, Sobol M, Balashov KE. Cardiac arrhythmia with premature ventricular contractures induced by interferon beta in a patient with multiple sclerosis. *Neuroimmunol Neuroinflammation* 2015;2(1):43-5.

Source of Support: Biogen-Idec and TEVANEuroscience. **Conflict of Interest:** No.

Received: 22-08-2014; **Accepted:** 14-10-2014

Multiple autoimmune antibody limbic encephalitis: a case in a pregnant woman

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ABSTRACT

Autoimmune limbic encephalitis is most commonly associated with antibodies against the N-methyl-D-aspartate receptor (NMDAR), among other neuronal cell surface receptors. Here, a case of a pregnant female with limbic encephalitis in the presence of multiple additional autoimmune antibodies is described. The patient was a 36-year-old female who presented with 4 days of confusion, hallucinations, hypersexuality, disinhibition, and pressured speech. The patient's work-up detected the presence of anti-NMDAR antibodies, anti-glutamic acid decarboxylase antibodies, and a yet uncharacterized neuronal autoantibody. The patient was also found to be pregnant. No evidence of ovarian or other pelvic malignancy was discovered. Symptomatic control was achieved with plasma exchange.

Key words: Autoimmune, encephalitis, glutamic acid decarboxylase, limbic, N-methyl-D-aspartate

INTRODUCTION

Autoimmune limbic encephalitis is most commonly associated with antibodies against the N-methyl-D-aspartate receptor (NMDAR).^[1] Several other autoimmune antibodies have been implicated in autoimmune limbic encephalitis, but the existence of several of these antibodies in one patient has rarely been described. Autoimmune limbic encephalitis manifests as sub-acute onset of irritability, short-term memory loss, depression, sleep disturbances, hallucinations, seizures, and confusion.^[1,2] Anti-NMDAR encephalitis is the most common form, whereas other antibodies described with this syndrome are directed at amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPA), gamma-aminobutyric acid beta receptor (GABA-BR), leucine-rich glioma inactivated (LGI1) gene, and contactin-associated protein 2.^[3] Anti-glutamic acid decarboxylase (GAD) antibodies, although most commonly associated with autoimmune-mediated Stiff-Person syndrome, have also

been described in association with late-onset cerebellar ataxia, epilepsy, palatal tremor, and limbic encephalitis.^[4-6] However, the clinical significance of these antibodies in the context of encephalitis has been questioned given the small number of cases reported as well as the frequent co-presence of antibodies against cell surface antigens (e.g. GABA-BR, AMPAR, and NMDAR).^[4-6] Cases of anti-GAD receptor encephalitis described in the literature generally do not follow the clinical pattern of typical limbic encephalitis. Patients with anti-GAD receptor encephalitis present with prominent memory loss, medically refractory seizures, and language difficulty.^[2] In addition, patients with autoimmune encephalitis can also demonstrate seropositivity for other antibodies such as anti-nuclear antibody and anti-thyroperoxidase (TPO). Immunomodulatory treatment remains the gold standard of therapy, with first-line treatments consisting of corticosteroids, intravenous immunoglobulin (IVIG), or plasma exchange (PLEX); second-line treatment includes cyclophosphamide and rituximab.^[1] Here, we describe a case of a pregnant female found to have anti-NMDAR encephalitis with the presence of multiple autoimmune antibodies.

CASE REPORT

A 36-year-old female presented with 4 days of confusion, hallucinations, hypersexuality, disinhibition, and pressured speech. Initial evaluation showed a thyroid

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stimulating hormone level of < 0.01 , free thyroid hormone level (T4) of 3.53 positive anti-TPO and thyroid stimulating immunoglobulin, suggesting hyperthyroidism-induced psychosis. Methimazole treatment resulted in a euthyroid state, but no symptomatic improvement. On day 4, brain magnetic resonance image (MRI) showed no acute intracranial process. Due to concerns that the MRI was performed too early to detect changes, it was repeated 1-month after presentation. This second scan was also unremarkable. The patient's electroencephalogram showed no epileptiform activity during sleep or wakefulness.

Cerebrospinal fluid (CSF) analysis on day 8 yielded lymphocytic pleocytosis (white blood cell (WBC) count of 44 with 95% lymphocytes) with negative bacterial cultures, fungal cultures, and viral polymerase chain reactions. A 3 days course of IV steroids for inflammatory encephalitis yielded no symptomatic improvement. On day 17, repeat CSF studies showed persistent pleocytosis (WBC count of 44 with 3% lymphocytes). A second trial of IV steroids for 7 days followed by oral steroid taper again failed to achieve symptomatic improvement. CSF and serum samples were sent to two laboratories for further testing, specifically for neuronal cell surface antigens and synaptic proteins such as NMDAR, AMPAR, GABA-BR, and autoantigens. One laboratory, the Josep Dalmau laboratory, uses recombinant technology, while the other, the ARUP laboratory at the University of Utah, uses immunofluorescence to identify antibodies. On day 24, the initial CSF samples returned positive for anti-NMDAR antibody (titer 1:5). The patient was thus diagnosed with anti-NMDAR encephalitis; no anti-NMDAR antibodies were detected in the serum. Scans for malignancy with whole body computed tomography images uncovered a thickened uterine endometrium but no evidence of ovarian masses; this finding was confirmed by transvaginal ultrasound. The patient repeatedly refused a pelvic MRI.

For the management of her neuropsychiatric symptoms, the patient received olanzapine, valproate, and haloperidol for psychosis control and mood stabilization. However, as she developed significant Parkinsonian features (hypertonicity, mask-like facies, tremor), haloperidol was discontinued, and benztropine and diphenhydramine were added with subsequent resolution of Parkinsonism.

As the patient failed repeated steroid treatments, alternate treatments were considered. While IVIG or PLEX would be first-line treatments for anti-NMDAR encephalitis, there was concern that the patient would not tolerate an indwelling central line given her agitation, mania, and psychosis. Thus, Rituximab therapy was

recommended. Prior to initiation of Rituximab, a urine pregnancy test (UPT) was repeated on day 36 and found to be positive (admission UPT had been negative). Subsequent plasma human chorionic gonadotropin test was positive and transvaginal ultrasound confirmed a viable fetus.

On discovery of a viable fetus, all teratogenic medications were discontinued, folate supplements were started, and the plan for rituximab therapy was abandoned. At this time, the patient's psychosis and agitation were more controlled. She seemed to exhibit more restraint due to knowledge of her pregnancy and her care for the child's health. Thus, she tolerated the central line well. After extensive discussions with the patient, family, and consulting services, the patient underwent seven cycles of PLEX over 2 weeks (days: 44-58), with a resolution of psychosis and bizarre behavior. During this time, additional CSF and serum analyses came back positive for anti-GAD antibody (titers unavailable), and a yet unidentified neuronal autoantibody found in CSF only; no other antibodies were identified (e.g. AMPAR, GABA-BR). Clinically, the patient did not demonstrate sequelae of Stiff-Person syndrome. The patient was largely asymptomatic by day 58 and discharged home on prenatal vitamins, folate, and levothyroxine. At 4-month follow-up, the patient was symptom-free and found to have subclinical hyperthyroidism.

DISCUSSION

Three cases of anti-NMDAR encephalitis in pregnant women have been described in the literature,^[7] but to the best of our knowledge, this is the first with multiple autoimmune antibodies present. Our patient demonstrated two distinct antibodies, anti-NMDAR and anti-GAD, as well as another neuronal autoantibody that has not yet been characterized. Both the anti-NMDAR and anti-GAD antibodies have been described in association with autoimmune limbic encephalitis, though our patient's titer of anti-NMDAR antibody (1:5) was significantly lower than previously described cases ($> 1:80$).^[7] It is unclear whether one of the antibodies was the dominant cause of encephalitis or if the three worked synergistically. Our patient was atypical for anti-NMDAR encephalitis in that she had less severe symptoms and never progressed to autonomic instability or central hypoventilation. Although she likely experienced a few seizures, she never experienced the typical movement disorders of anti-NMDAR encephalitis. Her disease also manifested much earlier in the pregnancy (5 weeks of gestation) compared with previous gravid patients who presented at 8, 14, or 17 weeks of gestation.^[7] She responded well to PLEX, with significant symptomatic improvement by the fifth cycle. She was also atypical for anti-GAD

encephalitis; never developing cerebellar ataxia, epilepsy, or palatal tremor.

The role of autoimmune disease in pregnancy is brought to question in this case. Not only did our patient develop symptoms of anti-NMDAR encephalitis much earlier in pregnancy than in previously described cases, but she also began having symptoms coincidentally close to conception. Based on ultrasound dates, the patient likely conceived within days of presenting to our facility. Her symptoms of autoimmune encephalitis also began 4 days prior to presentation. In this case, there exists a temporal relationship between the time of conception and development of the patient's disease process. Although, there is evidence showing that patients with autoimmune diseases-such as multiple sclerosis and rheumatic disease-may have fewer symptoms during pregnancy, anti-NMDAR encephalitis in our patient could potentially have been precipitated by pregnancy. Further studies will be needed to determine if this is simply a correlation or if there is true causation.

This case also illustrates the need for an extensive search for the etiology of a patient's neuropsychiatric symptoms. Prior to the discovery of the described neuronal autoantibodies, a patient presenting with Hashimoto's thyroiditis and neuropsychiatric symptoms would have been diagnosed with Hashimoto's encephalopathy.^[5] However, even with anti-TPO antibodies, a more exhaustive search for other antibodies is required in order to gain full appreciation of the patient's disease. Furthermore, Hashimoto's encephalopathy is responsive to steroids. Steroid-refractory disease should lead clinicians to

investigate other etiologies of a patient's symptoms. Autoimmune limbic encephalitis should be considered in patients with psychosis refractory to medications and no identifiable cause.

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Cite this article as: Goyal M, Gildersleeve KL, Tomko SL, Kass JS. Multiple autoimmune antibody limbic encephalitis: a case in a pregnant woman. *Neuroimmunol Neuroinflammation* 2015;2(1):46-8.

Source of Support: Nil. **Conflict of Interest:** No.

Received: 19-08-2014; **Accepted:** 30-09-2014

Inflammation of the cerebral arteries: lifting the veil on the pathobiology of intracranial aneurysms

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Cerebral aneurysms (CAs) are the most common cause of spontaneous subarachnoid hemorrhage (SAH).^[1] Despite significant improvements in microsurgical and endovascular aneurysm therapies and in neurocritical care since the turn of the century, the outcomes after CA rupture remain dismal. The mortality associated with aneurysmal SAH is approximately 50%, and of the survivors, approximately one-third have long-term neurocognitive deficits and one-half require permanent assistance.^[2,3] Since post-SAH management outcomes have seemingly plateaued in the past decade, the ideal window for improving overall outcomes in CA patients is prior to rupture.^[4]

However, interventions for unruptured CAs are not without risk, so their risk to benefit profiles must be compared to the natural history of unruptured CAs.^[5,6] Naggara *et al.*^[7] performed a systematic review of endovascular treatment outcomes for unruptured CAs and found a 5% rate of unfavorable outcomes. Similarly, Kotowski *et al.*^[8] performed a systematic review of surgical outcomes for unruptured CAs and reported a 7% rate of unfavorable outcomes. Even as advances in neurointerventional techniques and endovascular technologies, including newer generation flow-diverting stents (i.e. Surpass, flow redirection endoluminal device), intermediate coverage stents (i.e. LVIS), aneurysm neck and bifurcation reconstruction devices (i.e. PulseRider, Barrel, Eclips), and intrasaccular flow disruptors (i.e. WEB, Luna),

continue to improve interventional outcomes for CAs, treatment of unruptured CAs continues to expose patients to potential morbidity and mortality.^[9,10] Therefore, a medical therapy that effectively reduces the hemorrhage risk of an unruptured CA with a reasonable safety profile may improve the long-term outcomes for patients harboring these lesions. Unfortunately, such a therapy does not currently exist, although the efficacies of novel and existing pharmacologic agents have been investigated.^[11]

A crucial component to the development of an effective drug to stabilize or induce regression of CAs is acquiring an understanding of their pathogenesis. Two common pathogenic features shared by CAs and extracranial aneurysms are (1) chronic inflammation, with an accompanying increase in the expression of pro-inflammatory cytokines and matrix metalloproteinases, initiates and exacerbates CA development, and (2) progressive loss of smooth muscle cells (SMCs) in an artery's tunica media, which are critical for providing contractility and mechanical stability of the vessel wall. One of the difficulties in evaluating CA pathophysiology is the lack of animal models that accurately recapitulate the human disease. A mouse CA model is advantageous over models in other animals, due to the plethora of different genetic knockouts that are available in mice. An increasingly popular mouse CA model was initially devised by Nuki *et al.*,^[12] in which CAs are generated by a combination of induced systemic hypertension and intracranial elastase injection into the basal cisterns. A number of recent studies have used this mouse model to investigate the role of various endogenous factors in CA pathogenesis.^[13-16]

Inflammation has been shown to be a central contributor to the pathogenesis of CAs. Hasan *et al.*^[17] showed that ferumoxytol-enhanced magnetic resonance

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10.4103/2347-8659.153968

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imaging (MRI) can be used to evaluate inflammation and destabilization of the CA wall by utilizing ferumoxytol uptake as a surrogate indicator of macrophage turnover. More recently, Edjlali *et al.*^[18] found that circumferential enhancement of the CA wall on MRI can identify CAs prone to rupture. Although our knowledge of CA pathobiology has improved significantly over the past decade, there remains much to be learned and tested. One challenge in determining the role of the immune system in CA pathogenesis is the phenotypic plasticity exhibited by SMCs, a unique phenomenon not observed in cardiac and skeletal muscle cells. In the setting of vascular injury or inflammation, SMCs undergo phenotypic modulation, a process by which markers of mature SMCs are downregulated and markers of inflammatory cells, such as macrophages, are upregulated.^[19] Given the inability of conventional immunohistochemical staining methods to identify transdifferentiated SMCs, evaluation of SMC epigenetic signatures and SMC lineage tracing studies are necessary to accurately assess the contribution of SMCs to vascular lesions, such as CAs.^[20] Thus, the respective roles of cells of SMC and myeloid lineage in CA formation, progression, and rupture remains incompletely defined.^[21]

Despite the current limitations in our understanding of CA pathogenesis, the future of CA translational and clinical research is promising. In this special issue of *Neuroimmunology and Neuroinflammation* on the topic of “The Role of Inflammation in Cerebral Aneurysms”, we have assembled a collection of articles from renowned experts in the field of cerebrovascular disease, and attempt to lift the veil on the pathobiology of intracranial aneurysms.

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Cite this article as: Ding D. Inflammation of the cerebral arteries: lifting the veil on the pathobiology of intracranial aneurysms. *Neuroimmunol Neuroinflammation* 2015;2(2):49-50.

Source of Support: Nil. **Conflict of Interest:** No.

Received: 19-01-2015; **Accepted:** 25-01-2015

Advances in the imaging of cerebral aneurysm inflammation

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ABSTRACT

Cerebral aneurysm formation, growth and rupture are thought to be the result of a complex interaction between cerebrovascular hemodynamics and pathobiology. Recently, new evidence has emerged regarding the role of inflammation in the walls of cerebral aneurysms. Noninvasive methods to characterize the degree of inflammation in aneurysms could enable clinicians to estimate the risk of future aneurysm growth and rupture, influencing treatment. This review examines emerging techniques of imaging inflammatory biomarkers in cerebral aneurysms.

Key words: Ferrosoferric oxide, inflammation, intracranial aneurysm, magnetic resonance angiography, subarachnoid hemorrhage

INTRODUCTION

Intracranial aneurysms are a substantial source of intracranial hemorrhage worldwide. Many aneurysms are detected incidentally, and the treatment calculus regarding unruptured aneurysms remains debatable. Early studies relied on aneurysm diameter, positing that small aneurysms nearly never ruptured;^[1] however, recent data suggest that some small aneurysms confer a significant rupture risk.^[2] More recently, complex morphologic and hemodynamic characteristics have been suggested to risk-stratify unruptured aneurysms for treatment.^[3-5] Inflammation is related to hemodynamic stress,^[6] but relying on only morphologic and hemodynamic factors alone does not account for the role of inflammation in the pathobiology of cerebral aneurysms.

Several studies have demonstrated that inflammation plays a key role in cerebral aneurysm formation and rupture.^[7-9] Specifically, the role of macrophages in the response to inflammatory mediators has been proposed as a mechanism for aneurysm rupture.^[10,11] However,

these studies rely on histological analysis of aneurysm tissue.

Recently, the development of noninvasive imaging of inflammatory markers has been developed and applied to the study of cerebral aneurysms. Preliminary results are promising that the link between aneurysm rupture risk and inflammation is strong, and that such inflammation can be imaged in a clinical setting.

IMAGING OF MYELOPEROXIDASE

Myeloperoxidase, a potent bactericidal substance primarily housed in the granules of neutrophils, is present in the inflammatory environment. It is present in noninfectious inflammatory reactions such as those accompanying atherosclerosis^[9] and vasculopathy.^[12]

Gounis *et al.*^[13] observed that increased myeloperoxidase expression in aneurysm tissue harvested during surgery was associated with all ruptured aneurysms, as well as those unruptured aneurysms that were considered “high-risk” for rupture based on demographic and anatomic characteristics. The same group has studied a paramagnetic agent (di-5-hydroxytryptamide of gadopentetate dimeglumine) that highlights the presence of myeloperoxidase in animal models of general vascular disease.^[14] While no human studies of this magnetic resonance contrast agent have been performed, this represents a promising agent in the noninvasive detection of aneurysmal inflammation.

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DOI:
10.4103/2347-8659.153970

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IMAGING OF MACROPHAGE ACTIVITY

A recent sub-analysis of a large population-based prospective study of unruptured aneurysms demonstrated the protective effect of aspirin on the rupture risk of cerebral aneurysms.^[15] The authors posit that the antiinflammatory effects of aspirin confer this risk reduction. In the presence of inflammation, the upregulation of cyclooxygenase-2 (COX-2) and microsomal prostaglandin E2 synthase-1 (mPGES-1) increases prostaglandin production, which in turn increases matrix metalloproteinase-9 (MMP-9) production by macrophages. This causes the degradation of proteins in the extracellular environment. This activity may lead to the weakening of the aneurysm wall, which when exposed to increased hemodynamic stress, leads to aneurysmal rupture.^[5,6] Aspirin has been shown to attenuate the expression of COX-2 and mPGES-1, thus reducing the production of MMP-9 by macrophages.^[16]

Histological studies of ruptured versus unruptured aneurysms have demonstrated an early (< 12 h) macrophage infiltrate, which some authors postulate may be responsible for an acute inflammatory reaction that precipitates aneurysm rupture.^[8] The imaging of macrophage activity, therefore, could aid in the detection of a prerule inflammatory state signaling aneurysm wall instability and urgent need for treatment.

Macrophage imaging has recently been piloted in humans in part due to the development of ferumoxytol. Ferumoxytol is a superparamagnetic form of iron oxide originally developed for the treatment of iron deficiency anemia.^[17] Because ferumoxytol is detectable using conventional magnetic resonance imaging (MRI), and cleared by macrophagocytosis,^[18] imaging after ferumoxytol infusion can highlight macrophage activity and hence inflammation.

The differential uptake of ferumoxytol infusion can be used to determine the degree of inflammatory activity of macrophages in cerebral vessels [Figure 1]. A histological study of unruptured aneurysms imaged using this modality prior to surgical resection detected iron particles as well as macrophage infiltration in the aneurysm wall, while only macrophages were detected in the tissue of a control group without ferumoxytol infusion.^[19]

A further study of ferumoxytol correlated its early uptake on serial MRIs with impending aneurysm rupture.^[20] Twenty-two patients with thirty unruptured aneurysms underwent MRI after ferumoxytol infusion at 3 time points: immediately, after 24 h, and after 72 h. The presence of ferumoxytol uptake was defined as a reduction in aneurysmal T2 signal when compared to baseline MRI. Ferumoxytol activity was defined as “early” if this change was detected in 24 h, and “late” if detected only 72 h after infusion. Fourteen aneurysms underwent subsequent surgical clipping, while sixteen were observed based on small aneurysm size, patient age or co-morbidity, or patient preference. Histological analysis was performed on all surgically treated aneurysms, as well as five ruptured aneurysms not imaged using ferumoxytol.

Among surgically repaired aneurysms with early uptake ($n = 4$), immunohistochemical analysis revealed COX-2, mPGES-1 and M1 macrophage activity similar to that of ruptured aneurysms. Those unruptured aneurysms with late uptake ($n = 5$) had significantly less COX-2 and mPGES-1 activity compared to both ruptured aneurysms and unruptured aneurysms with early ferumoxytol uptake.

Of the fourteen aneurysms not surgically treated, three demonstrated early signal changes, eight late

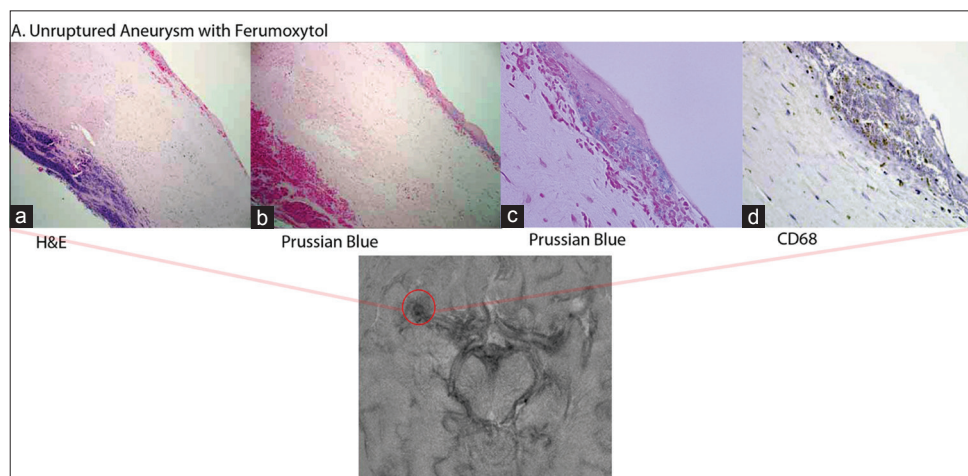


Figure 1: Histology and ferumoxytol-enhanced magnetic resonance imaging (MRI) of an unruptured middle cerebral artery (MCA) aneurysm: (a) HE $\times 100$; (b) Prussian Blue stain showing iron oxide nanoparticles seen mostly in the adventitia; (c) higher magnification of Prussian blue stain demonstrating iron oxide nanoparticles; (d) CD68 showing positive staining for macrophages MRI illustrates right MCA aneurysm with ferumoxytol uptake. Figure adapted with permission from Hasan *et al.*^[19]

changes, and five no changes. Importantly, all three aneurysms with early signal change ruptured within 6 months of imaging, while none of the remaining aneurysms ruptured or changed in size on follow-up imaging. The authors concluded that aneurysms with early ferumoxytol uptake have a greater degree of inflammation, suggesting an unstable state prone to aneurysm rupture.

The same group then examined the effect of aspirin on ferumoxytol uptake in intracranial aneurysms. A preliminary study demonstrated that administering aspirin 81 mg daily for 3 months to patients with cerebral aneurysms reduced the uptake of ferumoxytol.^[21] Subsequently, a small prospective study of eleven patients with twelve unruptured aneurysms compared patients started on daily aspirin ($n = 6$) and a control group ($n = 5$).^[22] After a baseline ferumoxytol MRI, patients were imaged at 3 months and then underwent surgical aneurysm repair. Patients on aspirin demonstrated an obvious reduction in ferumoxytol uptake compared to baseline, while uptake in the control group did not change. Histological analysis of COX-2, mPGES-1 and macrophages showed significantly lower activity in aneurysms of patients in the aspirin group compared to the control group.

Three conclusions can be drawn from the above ferumoxytol studies. First, ferumoxytol-enhanced MRI can be used to measure aneurysmal inflammation from macrophage activity. Second, early uptake of ferumoxytol is associated with a proinflammatory state that may herald impending aneurysm rupture (though with such a small patient population, definitive statistical conclusions are not possible). Finally, the antiinflammatory effects of aspirin seem to correlate (both radiographically and histologically) with macrophage activity, possibly accounting for the protective effect of aspirin in unruptured aneurysms.

Currently, the role for imaging of aneurysm inflammation is limited to research trials. Future trials of large-scale aneurysm imaging using ferumoxytol are likely the next step in the incorporation of this technique into aneurysm treatment. The limitations of current technology include difficulty interpreting images for evidence of differential uptake of ferumoxytol, which requires experienced neuroradiologists, as well as standardization of the dosage and timing of administration of contrast. In addition, the intriguing finding of reduced inflammation after aspirin administration (and its possible protective effect against aneurysm rupture) is based on a very small number of patients, and further research of a large cohort is required for definitive statistical analysis. Such results could substantially alter the treatment of unruptured

aneurysms for which there is treatment equipoise or patient co-morbidity.

CONCLUSION

Preliminary advances in the imaging of cerebral aneurysm inflammation have highlighted the importance of pathobiology in aneurysm rupture. Future studies of aneurysm surveillance using such imaging, especially ferumoxytol-enhanced MRI, promise to improve clinicians' understanding of individual rupture risk beyond simple anatomical metrics such as aneurysm diameter. Finally, antiinflammatory therapy may play a role in the treatment of unruptured aneurysms.

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Cite this article as: Levitt MR, Kalani MY, Moon K, McDougall CG, Albuquerque FC. Advances in the imaging of cerebral aneurysm inflammation. *Neuroimmunol Neuroinflammation* 2015;2(2):51-4.

Source of Support: Nil. **Conflict of Interest:** No.

Received: 13-08-2014; **Accepted:** 01-09-2014

Cerebral aneurysms and inflammation

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ABSTRACT

Multiple inflammatory factors, playing a crucial role in cerebral aneurysm formation, have been identified. Tumor necrosis factor- α (TNF- α) has been revealed to have a close connection with several risk factors that affect aneurysm formation. Remarkable expression in aneurysm walls of mRNA for TNF- α has been observed in humans. Possible therapeutic interventions to reduce the formation of cerebral aneurysms may include the inhibition of mediators of inflammation.

Key words: Cerebral aneurysm, inflammation, molecular biology

INTRODUCTION

Most unruptured aneurysms, which are detected incidentally show stable clinical courses, exhibit fewer inflammatory or degenerative changes in the walls of affected blood vessels, and have a low risk of rupture. However, some unruptured aneurysms show significant changes in size and shape, and the rupture risk seems to be high. These aneurysms may rupture in the early phase of development or enlarge in a short time due to thinning of vessel walls resulting from the advancement of degenerative changes. It is of clinical relevance to accurately estimate the rupture risk of cerebral aneurysms, but no definitive methods exist to distinguish rupture-prone aneurysms from rupture-resistant ones. Recently, the rupture risk of unruptured cerebral aneurysms was reported. The 5-year cumulative rupture rates for aneurysms located in the internal carotid artery, anterior communicating artery, anterior cerebral artery, or middle cerebral artery in patients without a history of subarachnoid hemorrhage were 0%, 2.6%, 14.5%, and 40% for aneurysms < 7 mm, 7-12 mm, 13-24 mm, and 25 mm or greater, respectively.^[1] By comparison, the rupture rates of aneurysms involving the posterior circulation and the posterior communicating artery were 2.5%, 14.5%, 18.4%, and 50%, respectively, for the same

size categories. In one study, the annual rupture rate of unruptured cerebral aneurysms in a Japanese cohort was 0.95%.^[2] In another, the average annual risk of rupture associated with small unruptured aneurysms was 0.54% overall, 0.34% for single aneurysms and 0.95% for multiple aneurysms.^[3] The molecular mechanisms leading to the occurrence, development, and rupture of cerebral aneurysms have been experimentally investigated. Ruptured aneurysms manifest significant endothelial damage, structural changes in vessel walls, and inflammatory cell invasion compared to unruptured aneurysms.^[4] The walls of ruptured aneurysms are fragile, possibly because macrophage infiltration into the aneurysm wall results in the loss of smooth muscle cells and degeneration of matrix proteins. In this manuscript, we discuss the molecular mechanisms of cerebral aneurysm development, focusing on inflammatory processes.

INFLAMMATION AND ABDOMINAL AORTIC ANEURYSMS

The crucial role of inflammatory reactions can be seen in the formation of abdominal aortic aneurysms (AAAs). Important histological features of vessel walls with AAAs include chronic inflammatory cell infiltration of the adventitia and media, elastin fragmentation, degeneration, and attenuation of the media. Collagen in the media and adventitia provides tensile strength to the aortic wall. Collagen synthesis increases during the early stages of aneurysm formation, suggesting a repair process.^[5] Inflammation-related mediators in aneurysm growth include matrix-degrading proteinases, proinflammatory cytokines, and chemokines.^[6] In later

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10.4103/2347-8659.153977

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stages, degeneration of collagen exceeds its synthesis, and when accompanied by excessive degradation of other extracellular matrix macromolecules such as elastin, ultimately favors AAA rupture. Indeed, AAAs exhibit increased local production of enzymes capable of degrading the extracellular matrix proteins collagen and elastin.^[7-9] Oxidative stress and elevation of hemodynamic stress lead to degeneration of elastin or collagen. The activation of Th1 cytokines via interferon gamma and interleukin-6 polymorphism is accelerated.^[10,11] The combination of inflammatory reactions and inherited vascular fragileness, along with environmental factors like advanced age or smoking, contributes to the formation and augmentation of AAAs.

MOLECULAR BIOLOGY OF CEREBRAL ANEURYSM FORMATION

Multiple inflammatory factors have been identified that play a crucial role in cerebral aneurysm formation.^[12] Inflammatory cells such as macrophages, monocytes, and T lymphocytes have been found in aneurysm walls.^[4,13] The infiltration of leukocytes is related to the impairment or elimination of collagen fibers. The plasma levels of cytokines, collagenase, and elastase are elevated in patients with cerebral aneurysms.^[14,15] Recently, nuclear factor-kappa B (NF- κ B) and tumor necrosis factor-alpha (TNF- α) have been widely investigated as potentially key molecules in the inflammatory process. NF- κ B is a transcription factor that is known to be closely related to inflammation. NF- κ B is activated in endothelial cells at the site of arterial bifurcation in the early stages of aneurysm formation, which induces hemodynamic stress.^[16] This activation is attributable to hemodynamic stress in the affected endothelial cells. It is thought that activated NF- κ B incites several downstream inflammation-related genes at the transcriptional level. Monocyte chemoattractant protein-1 (MCP-1) is one target of NF- κ B and is an indispensable factor for the migration of macrophages to the lesion site. The transcription of MCP-1 is controlled by NF- κ B at the cerebral aneurysmal wall. MCP-1 is secreted from the endothelial cell layer in the early stages of aneurysm formation and from all layers of the arterial wall in later stages. Macrophage infiltration can be suppressed by the use of MCP-1 knockout mice or MCP-1 inhibitor, and leads to inhibition of aneurysm formation.^[17] This evidence highlights the significance of MCP-1 and macrophage infiltration. Other factors controlled by NF- κ B are inducible nitric oxide synthase (iNOS) and interleukin-1 beta (IL-1 β), which are known as apoptosis-inducing factors. An investigation of experimentally induced cerebral aneurysms in rats revealed that apoptosis occurs in smooth muscle cells

located within the medial layer of affected vessels, and is associated with inflammation.^[18] iNOS activity results in the production of nitric oxide, which is an important factor involved in inflammatory reactions and the preservation of arterial regulation. iNOS is principally expressed in inflammatory cells such as macrophages, and may impair arterial wall integrity or induce apoptosis. The expression of iNOS is facilitated in the media and adventitia during the early phase of aneurysm formation. The incidence of experimentally induced cerebral aneurysms in iNOS knockout mice is the same as that in control mice, however the aneurysm size is significantly smaller.^[19] This suggests that iNOS contribute to aneurysmal augmentation by promoting apoptosis in medial smooth muscle cells. IL-1 β is an inflammatory cytokine which is activated by cleaved caspase 1. IL-1 β is also produced in the early phase of aneurysm formation, mainly by medial smooth muscle cells. In IL-1 β knockout mice, the progression of aneurysm development is significantly impaired.^[20] This means that inflammatory reactions in the arterial wall contribute to aneurysm enlargement and that IL-1 β is a significant mediator of this process.

TUMOR NECROSIS FACTOR-ALPHA

Tumor necrosis factor-alpha has been revealed to have a close connection with several risk factors that affect aneurysm formation. Remarkable expression in aneurysm walls of mRNA for TNF- α has been observed in humans.^[21,22] In addition, therapeutic administration of a TNF- α inhibitor significantly reduced aneurysm formation in rats.^[23] There has been some investigation into the relationship between TNF- α expression and aneurysm formation or rupture. Inflammation induced by expression of TNF- α leads to the degeneration of endothelial cells, the internal elastic lamina, and medial smooth muscle. Cerebral aneurysms are stabilized when the expression of TNF- α is reduced, or expression of anti-inflammatory cytokines increases, however continuous expression of TNF- α induces aneurysmal rupture.^[24] TNF- α also increases the permeability of the aneurysm wall via cytokine cascades and induces the migration of macrophages or neutrophils to inflamed endothelial cells. In addition, TNF- α plays a role in other pathological manifestations such as modulation of the blood-brain barrier, fluid accumulation, and regulation of intracranial blood flow in aneurysmal subarachnoid hemorrhage.^[25] In transgenic mice that are deficient for TNF- α or TNF receptors, susceptibility to nitric oxide is notably increased.^[26,27] In mice with congenital TNF- α receptor deficiency, the deleterious effects of oxidative stress are increased by traumatic or ischemic loading, which indicates that stimulation of antioxidant pathways by TNF- α may provide protection against oxidative damage.^[28]

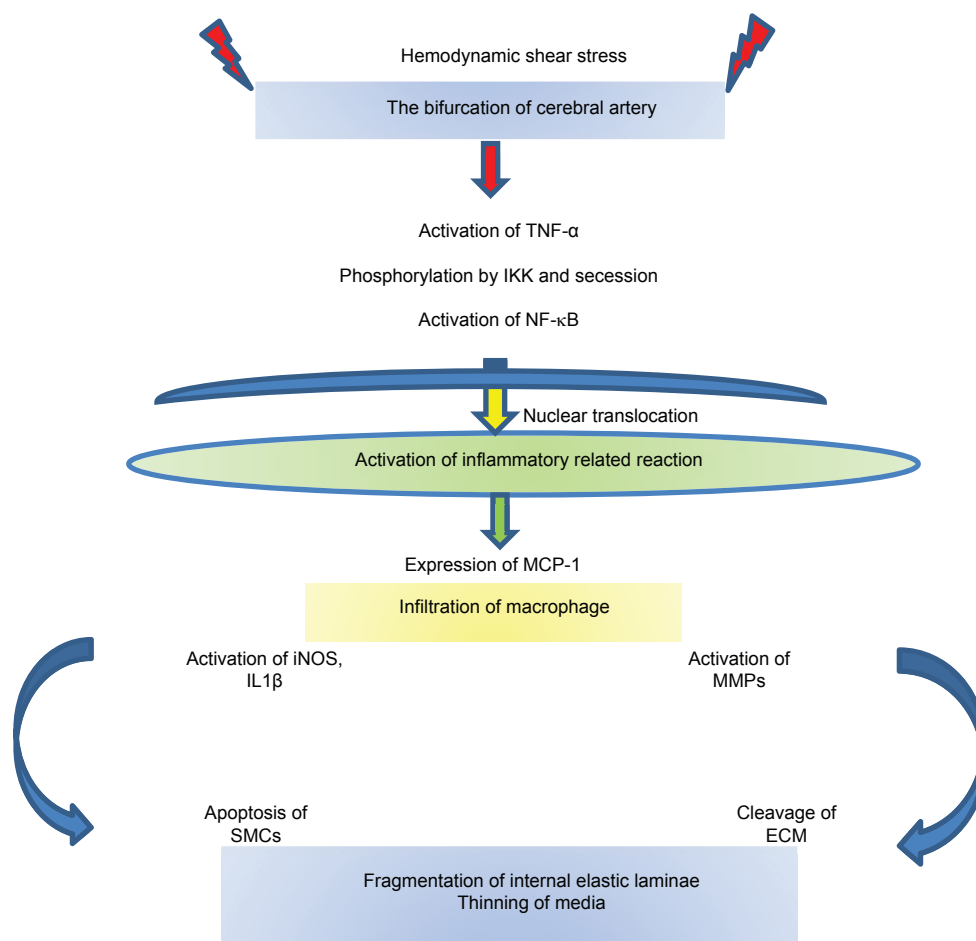


Figure 1: Factors associated with the formation of cerebral aneurysms. TNF- α : tumor necrosis factor-alpha; IKK: inhibitor of kappa kinase; NF- κ B: nuclear factor-kappa B; MCP-1: monocyte chemoattractant protein-1; iNOS: inducible nitric oxide synthase; IL-1 β : interleukin-1 β ; MMPs: matrix metalloproteinases; SMCs: smooth muscle cells; ECM: extracellular matrix

MECHANISMS OF CEREBRAL ANEURYSM FORMATION IN HUMANS

Aneurysm formation has been shown to occur at sites of constant hemodynamic stress both in humans and in experimentally induced models of cerebral aneurysm. This means that hemodynamic stress initiates early stage aneurysm formation. Aneurysm formation progresses when degenerative changes exceed vessel repair due to vascular remodeling. In addition to inflammation and apoptosis, extracellular matrix decomposition and endothelial dysfunction play critical roles in aneurysm formation. It has been found that gene expression in cerebral aneurysms may be linked to specific genetic regions. Furthermore, genetic regions that promote cerebral aneurysm formation are also linked to AAAs.^[29] The factor analysis which affected to cerebral aneurysmal formation in human would progress in the future.

HISTOPATHOLOGY OF RUPTURED ANEURYSMS

Loss of the internal elastic lamina and degeneration of medial vascular smooth muscle are common histopathological features of cerebral aneurysms. These degenerative changes occur in the aneurysm wall and can lead to aneurysmal rupture. Following rupture, the

wall around the ruptured site is immensely thin and covered by a thrombosed fibrin plug. Infiltration of inflammatory cells such as neutrophils, lymphocytes, and macrophages into the adventitia also occurs. In addition, complement and immunoglobulin deposits form between the medial vascular smooth muscle and adventitia.^[13] Loss of vascular smooth muscle cells, thinning of collagen fibers, and inflammation are more prominent in the walls of ruptured aneurysms compared to unruptured ones.^[30] Therefore, these factors seem to play an important role in the weakening of aneurysmal walls [Figure 1].

CONCLUSION

Inflammatory cells have been found in the walls of cerebral aneurysms, and several inflammatory factors are reported to play crucial roles in cerebral aneurysm formation. Possible therapeutic interventions to reduce the formation of cerebral aneurysms may include the inhibition of mediators of inflammation.

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Cite this article as: Yokoi T, Saito M, Yoshimura Y, Tsuji K, Nozaki K. Cerebral aneurysms and inflammation. *Neuroimmunol Neuroinflammation* 2015;2(2):55-8.

Source of Support: Nil. **Conflict of Interest:** No.

Received: 10-09-2014; **Accepted:** 30-09-2014

Hemodynamics, inflammation, vascular remodeling, and the development and rupture of intracranial aneurysms: a review

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ABSTRACT

The central nervous system is an immunologically active environment where several components of the immune and inflammatory response interact among them and with the constituents of nervous tissue and vasculature in a critically orchestrated manner, influencing physiologic and pathologic processes. In particular, inflammation takes a central role in the pathogenesis of intracranial aneurysms (IAs). The common pathway for aneurysm formation involves endothelial dysfunction and injury, a mounting inflammatory response, vascular smooth muscle cells (VSMCs) phenotypic modulation, extracellular matrix remodeling, and subsequent cell death and vessel wall degeneration. We conducted a literature review (1980-2014) by Medline and EMBASE databases using the searching terms "IA" and "cerebral aneurysm" and further search was performed to link the search terms with the following key words: inflammation, hemodynamic(s), remodeling, macrophages, neutrophils, lymphocytes, complement, VSMCs, mast cells, cytokines, and inflammatory biomarkers. The aim of this review was to summarize the most recent and pertinent evidences regarding the articulated processes of aneurysms formation, growth, and rupture. Knowledge of these processes may guide the diagnosis and treatment of these vascular malformations, the most common cause of subarachnoid hemorrhage, which prognosis remains dismal.

Key words: Inflammation, hemodynamics, vascular remodeling, intracranial aneurysms

INTRODUCTION

Saccular intracranial aneurysms (IAs) are the most frequent cause of subarachnoid hemorrhage (SAH), the stroke type with the higher morbidity and mortality.^[1,2] A precise evaluation of their rupture risk is crucial to orient treatment of unruptured IAs relatively to the risk of endovascular or surgical treatment.^[2-7] Statistic studies involved patients suffering from aneurysm rupture found that cigarette smoking, arterial hypertension, ethnic origin, age, previous SAH, size ≥ 7 mm, localization of IAs at the posterior circulation and aneurysm's shape are the most important variables regarding the rupture risk.^[2-7]

However, the majority of IAs diagnosed following their rupture is small and located on the anterior circulation,^[8,9] which indicates that the statistical approach does not allow individualizing the risk of rupture. Elucidating the pathogenic pathways inherent to the development and rupture of IAs may allow identifying more reliable markers of rupture-prone IAs. A growing body of evidence supports the correlation between modification of hemodynamic factors and arterial wall alteration leading to IAs development and rupture.^[10-13] Particularly, wall shear stress (WSS) gradient might be an important factor of vascular remodeling through multiple mechanisms involving endothelial cells (ECs) and vascular smooth muscle cells (VSMCs) modification of gene expression triggered by local inflammatory reaction and leading to degenerative changes of arterial wall.^[14] In this article, we summarize the existing data, extracted from a review of the pertinent literature, regarding inflammation and hemodynamic stress in the pathogenesis of IAs. Our endeavor is to explore the causative relationships that may link hemodynamics, inflammation, vascular

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DOI:
10.4103/2347-8659.154885

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remodeling and the development and rupture of IAs. This approach may provide effective tools to predict the individual risk of aneurysmal rupture more reliably than statistical methodology.

METHODS OF THE REVIEW

The literature review was conducted using Medline and EMBASE searches that included works published between 1980 and 2014. The terms “IA” and “cerebral aneurysm” were used as text words and MESH headings with appropriate subheadings. A further search was performed to link cerebral and IAs and the following key words: inflammation, hemodynamic(s), remodeling, macrophages, neutrophils, lymphocytes, complement, VSMCs, mast cells, cytokines, and inflammatory biomarkers. Textbooks, journal bibliographies, and conference proceedings were also included. Language restrictions were not used.

INFLAMMATION AND INTRACRANIAL ANEURYSMS REMODELING

The central nervous system is an immunologically active environment where a complex set of interactions links the various constituents of the immune and inflammatory system with the constituents of nervous tissue and vasculature.^[15] Aneurysm formation begins with a hemodynamically triggered endothelial dysfunction where inflammation initiated by nuclear factor kappa-light-chain-enhancer of activated B cells (NF- κ B) activation and imbalance between nitric oxide (NO) and peroxynitrite anion (ONOO⁻) in favor of ONOO⁻ seem to hold a key role.^[16] The following mounting inflammatory response implicates several inflammatory cells and mediators and phenotypic modulation of VSMCs from a contractile to a pro-inflammatory/pro-matrix remodeling phenotype with myointimal hyperplasia, inflammation and wall degeneration.

Several *in vitro* and *in vivo* studies found evidences that infiltration of inflammatory cells in the arterial wall initiates aneurysm formation and promotes its rupture through production of inflammatory cytokines, adhesion molecules, immunoglobulins, reactive oxygen species (ROS), complement and inflammatory cell-induced upregulation of matrix-degrading proteinases, among which macrophages, neutrophils, and lymphocytes hold a central role.^[17,18]

Macrophages

Monocytes originally produced in bone marrow enter circulation and infiltrate endothelium at the site of the hemodynamic injury, where they differentiate into macrophages.^[18] Macrophage is one of the first cell

types to respond to injury, but it also regulates the later immune response.^[18] The role of these inflammatory cells in IAs formation is demonstrated by the fact that macrophage depleted mouse displays a lower risk of IAs.^[19] Macrophage action on the arterial wall is mediated by secretion of cytokines and proteinases.

Cytokines are peptides, proteins, and glycoproteins that mediate inflammatory and immune response.^[20-22] Several cytokines secreted by macrophages have been found involved in the pathogenesis of IA especially monocyte chemoattractant protein-1 (MCP-1), tumor necrosis factor alpha (TNF- α), stromal cells derived factor-1 (SDF-1/CXCL12). MCP-1 is a chemotactic cytokine, also known as chemokine, for monocytes, lymphocytes, and some granulocytes secreted by macrophages.^[23] Its implication in the development of IAs is demonstrated by a decrease of IAs and arterial wall inflammation in MCP-1 knockout mice.^[24] TNF- α is another proinflammatory cytokine secreted by macrophages.^[18] Experimental data suggest a critical role of TNF- α in the formation and rupture of aneurysms in a murine model of cerebral aneurysm formation induced by hypertension and a single stereotactic injection of elastase into the basal cistern.^[25] TNF- α knockout mice and those pre-treated with 3,6'-dithiothalidomide (DTH), a synthesized TNF- α inhibitor, had significantly decreased the incidence of aneurysm formation and rupture as compared to sham mice. Protein and mRNA expression of TNF- α in the cerebral vasculature were not significantly different in TNF- α knockout mice and in those pre-treated with DTH. However, TNF- α expression was higher in unruptured and the highest in ruptured aneurysms when compared to other conditions of aneurysms, where it co-localized to both smooth muscle cells and macrophages. SAH occurred between 7 and 21 days following aneurysm induction. Initiation of DTH treatment 6 days after aneurysm induction did not alter the incidence of aneurysm formation but resulted in aneurysmal stabilization and a significant decrease in rupture. Therefore, it can be inferred that inhibitors of TNF- α could be beneficial in preventing aneurysmal progression and rupture.^[25] TNF- α upregulates the adhesion molecules such as intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1) and E-selectin in ECs, fibroblasts, and SMCs.^[26] These adhesion molecules attract and facilitate migration of leucocytes through the arterial wall, predisposing to atherosclerosis and IAs development.^[26] Macrophages secrete also another potent chemoattractant cytokine, such as SDF-1/CXCL12.^[27-29] Besides promoting angiogenesis by recruiting endothelial progenitor cells from the bone marrow through a CXCR4 dependent mechanism,^[30] SDF-1 is associated with angiogenesis and migration

and proliferation of macrophages in the walls of human and murine aneurysms, possibly playing a role in the development of IAs.^[31] Macrophages also directly promote degradation of ECM by secreting a zinc and calcium-dependent family of endopeptidases known as matrix metalloproteinases (MMPs) and modulate their activity by producing tissue inhibitors of metalloproteinases (TIMPs), MCP-1 and TNF- α .^[32-34] Beside from degrading all kinds of ECM proteins, these proteinases, particularly MMP-9 secreted also by VSMCs,^[35,36] induce macrophage migration and infiltration across ECM.^[37] Overexpression of MMP-9 in the wall of excised IA was first documented by Kim *et al.*,^[38] followed by Takemura *et al.*^[39] who later showed by immunohistochemical analysis of the overexpression of MMP-1, -2, and -9 in aneurysm walls. Moreover, the levels of MMP-2 and -9 were found to be higher in ruptured compared with unruptured aneurysms in a series of 30 patients, suggesting that MMP-mediated excessive breakdown of vessel ECM eventually leads to aneurysmal rupture.^[40] The central role of MMPs in the pathogenesis of IAs is further demonstrated by the fact that inhibition of TIMPs promotes aneurysm formation in a rodent model.^[41] Under physiological conditions, MMPs production is regulated at the level of transcription. An imbalance between the active MMPs and TIMPs leads to the accelerated destruction of connective tissue associated with several vascular diseases including IAs.^[42]

Neutrophils

Other important inflammatory cells migrating to the site of arterial injury are neutrophils. As for macrophages, they secrete cytokines and MMPs. In addition, they produce peroxidases.^[18] The array of neutrophil-derived cytokines is similar to that of macrophages, including TNF- α and cxc-chemokines such as interleukin (IL)-1 β . However, unlike macrophages, they do not secrete cc-chemokines such as MCP-1.^[39] IL-1 β and cxc-chemokines are involved in various inflammatory cellular activities such as cell proliferation, differentiation, and apoptosis. Upregulation of IL-1 β in animal models of IAs is associated with aneurysm formation and progression by reduced collagen biosynthesis in the aneurysm wall both at the transcriptional and post-transcriptional levels.^[43,44] In addition, neutrophils secrete macrophage inflammatory protein 1- α , which reinforces cytokines' action to promote inflammatory cell recruitment, migration, activation, and differentiation.^[45]

Lymphocytes

Other first-responding inflammatory cells found in IAs wall are lymphocytes. They infiltrate arterial wall in early phases of aneurysm formation and rupture.^[46] They are involved in the production of

proinflammatory cytokines such as TNF- α , interferon- γ (IFN- γ) and IL-6.^[47]

Complement

The role of complement in the mechanism of IA formation is not fully elucidated. In one study by a Helsinki group that compared ruptured with unruptured IA, the expression and activation of complement membrane attack complex were greater in ruptured samples and was associated significantly with aneurysm wall degeneration and inflammatory cell infiltration.^[48] These authors showed in another study that complement activation occurs via the classical pathway as evidenced by the presence of classical pathway activators (IgG, IgM, C reactive protein, oxidized low-density lipoprotein) in the IA wall.^[49]

Vascular smooth muscle cells

As mentioned above, VSMCs are crucial in the process of IAs formation, and rupture. They are mostly found in the medial layer and synthesize the matrix for the structural integrity to the arterial wall. Thinning of this layer contributes to aneurysm formation and rupture.^[50] In response to endothelial injury VSMCs first migrate into the intima where they multiply and give rise to myointimal hyperplasia.^[51] Successively, VSMCs from a differentiated phenotype whose primary characteristic is contraction, dedifferentiation, losing capacity of expressing contractile genes, like myocardin, acquiring the capacity to express genes that may affect the rigidity or elasticity of the vascular wall such as collagen $\alpha 2(I)$ gene (*COL1A2*) and upregulating proinflammatory genes, such as MMPs, MCP-1, VCAM 1, and IL.^[52,53] This phenotypic modulation of VSMCs is induced by TNF- α and mediated by Kruppel-like transcription factor 4.^[54] Phenotypically modulated VSMCs are no longer in spindle shape forming tightly compacted bands, but spider-like cells dissociated from each other, nonproliferating and noncontractile.^[55] Likewise, aneurysmal rupture is associated with degeneration and caspase-mediated apoptotic loss of VSMCs of the aneurysm wall.^[17,56,57]

Mast cells

Although best known for their role in allergy and anaphylaxis, mast cells play an important role in the inflammatory reaction leading to IAs formation and rupture mainly via cytokines release and expression and activation of MMPs.^[58] Indeed, IAs formation is associated with the proliferation and degranulation of mastocytes, and ruptured aneurysms wall is richer in mast cells than unruptured IAs.^[59] The finding that inhibitors of mast cell degranulation decrease the inflammatory reaction in aneurysm walls and block the progression of IAs in mice further support the

participation of mast cells in the pathogenesis and rupture of IAs.^[58]

Inflammatory cells interaction and arterial wall degeneration

Inflammation of arterial wall leading to formation of IAs is initiated by the infiltration of inflammatory cells (macrophages, neutrophils and lymphocytes), which release proinflammatory cytokines and proteinases as well as chemokines and chemoattractant cytokines, for the upregulating recruitment of inflammatory cells into the aneurysm wall.^[60,61] In particular, levels of MCP-1, chemokine (C-C motif) ligand-5 (CCL5), monokine-induced-by-[gamma]-interferon, interferon-[gamma]-induced protein-10, Eotaxin, 2 other chemokines, IL-8 and IL-17 have been found to be higher in blood samples taken from the lumen of human IAs than blood samples from femoral arteries of the same patients.^[61] Inflammatory cytokines lead to degradation and apoptosis of ECs and VSMCs through activation and upregulation of immune cells migration and activity.^[21] Immune cells target not just cells but also ECM, the scaffolding structure that provides the arterial wall with tensile strength, elasticity, compressibility, adhesiveness as well as communicability between cells constituting the vessel wall.^[17,18] In particular, macrophages secrete MMPs resulting in excessive proteolytic activity against connective tissue proteins, including collagens, elastin, and proteoglycans, which causes focal degradation of the vascular ECM and may contribute to aneurysm formation and growth.^[31] Macrophages, in conjunction with lymphocytes, also act on VSMCs, for vessel wall remodeling.^[21] Cytokines and growth factors secreted by macrophages and T-lymphocytes affect VSMCs phenotype changes^[62] and promote their apoptosis.^[48,63,64] One of the key initiators of apoptosis is interaction between the Fas receptor, which is expressed on the surface of both inflammatory cells and VSMCs, and its ligand (Fas-ligand, FasL), which is expressed on the surface of macrophages and T-lymphocytes.^[65] Their interaction induces VSMCs apoptosis through upregulation of cytokines such as TNF- α and interferons expressed by inflammatory cells.^[17,54,56,57] These cytokines promote also the synthesis of NO, another factor inducing apoptosis.^[66] Apoptotic loss of VSMCs induces arterial wall weakening by reducing matrix synthesis.^[63,64]

HEMODYNAMIC FACTORS INDUCE ARTERIAL WALL INJURY AND INITIATE WALL INFLAMMATION

Endothelial dysfunction initially and vascular remodeling subsequently are triggered by shear stress.^[67] This explains why IA is commonly found at arterial junctions, bifurcations or abrupt vascular angles where excessive hemodynamic stresses are exerted on

arterial walls.^[68] There is a close relation between WSS, endothelial dysfunction, and the downstream inflammatory reaction.^[69,70]

Computational flow dynamic studies coupled with histological studies of the aneurysm wall demonstrated a correlation between hemodynamic conditions and inflammatory changes of intracranial arterial wall leading to aneurysm formation and rupture.^[10-13,70] The most highlighted, even though, controversial factor studied is WSS,^[12,71,72] which is the component of the forces coplanar with the cross-section of the artery, originating from blood circulation and acting on arterial walls.^[73] WSS is related to dynamic viscosity of blood, flow velocity parallel to the arterial wall and distance of the vector to the wall.^[73] Whether high or low WSS is involved in the arterial wall inflammatory damage, and development and rupture of IA are still matter of debate.^[74] Hemodynamics in IAs is complex and includes areas of low and high WSS.^[71,72] Several studies show that exposure to abnormal WSS drives endothelium-mediated proinflammatory reactions,^[75] MMPs activation,^[76-79] apoptosis of ECs and VSMCs,^[80] ECM degradation, and arterial wall remodeling.^[36,81] Spatial gradients and changes in WSS magnitude regulate ECs gene expression through the upregulation of transcription factors such as NF- κ B under the conditions responding to cytokines, free radicals and other stimuli implicated in cell survival.^[82-84] Oxidative stress in the arterial wall promotes IAs formation inducing direct endothelial injury, VSMCs phenotypic modulation and apoptosis, recruitment and invasion of inflammatory cells through upregulation of chemotactic cytokines and adhesion molecules, and MMPs activation.^[85] Oxidative stress reflects an imbalance between the production of ROS and the arterial wall's ability to readily detoxify the reactive intermediates or to repair the resulting damages. The ability of the arterial wall to counteract oxidative stress effects largely repose on NO action.^[16] NO is an endothelium-derived relaxing factor that has several actions translating in anti-atherosclerotic properties: it modulates vasomotor tone, inhibits expression of MCP-1 and VCAM-1, prevents propagation of lipid oxidation, inhibits VSMC proliferation, decreases platelet aggregation^[86] and inhibits expression and activity of MMPs.^[86] Practically, all risk factors for arterial wall damage (hypercholesterolemia, diabetes, insulin resistance, arterial hypertension, cigarette smoking) reduce production of endothelial NO through increased production of superoxide and other ROS.^[16,86-90] These strong oxidants both disrupt NO-mediated arterial wall protection decreasing availability of NO and promote arterial wall inflammation increasing ONOO⁻ production.^[16,86-90] Under physiological conditions, unidirectional laminar

shear stress increases NO availability through a biphasic action: within seconds after stress the endothelial nitric oxide synthase (eNOS) enzyme expressed in ECs is activated by a calcium-independent mechanisms.^[91] Subsequently, eNOS expression is upregulated through NF- κ B activation by a transient, one hour lasting, increase in eNOS mRNA transcription and a sustained increase in eNOS mRNA stability.^[92] However, NF- κ B activation leads also to an increased expression of pro-inflammatory genes encoding cytokines, VCAM-1, ICAM-1, tissue factor and MCP-1.^[16,86-90] At a first glance, the NF- κ B mediated protective and damaging effect appears to be contradictory. Nonetheless, the evidence of a negative feedback pathway on NF- κ B activation mediated by NO production resolves this apparent paradox. If eNOS levels are relatively high, sufficient NO is produced to shut down NF- κ B activation soon after shear is applied to endothelium through a classical negative feedback pathway.^[16] Conversely, if eNOS levels are relatively low, NF- κ B activation persists enough to restore eNOS to normal levels in order to ensure a long-lasting protection to ECs. The double effect of NF- κ B activation also explains why shear stress may reveal both harmful and protective to endothelium. As a matter of fact, in case of alteration of the inhibitory limb of the NO-mediated negative feedback, the proinflammatory action of shear stress prevails, leading to endothelium damage, with elongation, migration, change in density, and loss of ECs and to VSMCs phenotype change with acquisition of pro-inflammatory/pro-matrix remodeling properties.^[17,50,61] ROS may also play a role in the pathogenesis of IAs.^[93,94] Aoki *et al.*^[95] demonstrated the upregulation of genes producing ROS and the downregulation of ROS-eliminating genes in a murine model of IAs. Moreover, the same study showed a similar effect of edaravone, a free radical scavenger, and of the deletion of ROS-producing gene: in two separate groups of animals both effectively inhibited IAs formation by suppressing inflammation in aneurysmal walls.^[95] Moreover, low WSS may provide upregulation of proinflammatory cytokines and their receptors, such as IL-1 α , IL-1 receptor, IL-6, and MCP-1.^[96] In addition, an *in vitro* study showed that WSS on ECs causes a differential modulation of TNF- α -induced expression of adhesion molecules such as ICAM-1, VCAM-1, and E-selectin by reducing intracellular ECs ROS levels.^[97] This may cause inhibition of TNF- α -induced VCAM-1 and E-selectin expression in ECs through inhibition of NF- κ B activation.^[97] However, the same authors showed that WSS-induced production of TNF- α stimulates the expression of another adhesion molecule, ICAM-1.^[97] These apparently discordant findings indicate that a more thorough study of the cross-talk between these signaling molecules may shed further light onto the biological end-points produced

by the WSS in modulating cytokine-induced adhesion molecule expression in ECs. Although the processes by which hemodynamic factors affect inflammation of the artery wall is incompletely known, vascular remodeling in response to abnormal WSS correlates with increased ECs and VSMCs apoptosis,^[98,99] with upregulation of MMPs activity in both ECs^[33] and VSMCs^[36] and with upregulation of several transcription factors and inflammatory cytokines by inflammatory cells.^[18] Along these lines, Wang *et al.*^[82] showed in canine models that in areas of high shear stress (arterial bifurcations), aneurysm wall remodeling is associated with IL-1 β and MMPs expression along with a loss of eNOS expression. In line with these findings, EC injury was found by Jamous *et al.*^[100,101] to be the earliest change in aneurysm wall, followed by the formation of an inflammatory zone that leads to proteolytic destruction of the vascular ECM by MMPs and ultimately to aneurysm formation.

In summary, under physiologic condition shear stress promotes both endothelial NF- κ B upregulation and immediate eNOS activation. In turn, NF- κ B triggers both transient upregulation of eNOS and increased eNOS stability. This results in increased NO synthesis. NO protects arterial wall through a “direct” action on it as well as “indirectly” through a negative feedback on NF- κ B activation. When the chain of events in red prevails, shear stress sustains arterial wall protection. Failure of NO-mediated direct and/or indirect arterial wall protection shifts the balance towards inflammation.

When the degeneration in the arterial wall, including loss of endothelial and smooth muscle cells and degradation of ECM are not healed, chronic remodeling of tissue takes place to alter the biomechanical properties of arterial wall and aneurysm formation, which eventually rupture [Figure 1].^[63,64]

INFLAMMATORY BIOMARKERS AND ESTIMATION OF RISK OF RUPTURE

Giving that considerable evidence suggest the involvement of inflammation in development and rupture of IAs,^[19-25,31,35-41,48-64,70,76-81,93,94,99-101] preoperative noninvasive assessment of inflammatory status of the aneurysm wall may guide management of unruptured IAs.^[102-105] One possible tool to identify rupture-prone IAs is ferumoxytol-enhanced magnetic resonance imaging (MRI).^[102-104] Ferumoxytol is an ultrasmall superparamagnetic particle of iron oxide that reveals phagocytic activity of inflammatory cells because it is cleared by macrophages.^[102-104] Ferumoxytol is hypointense on MRI T2*-weighted gradient echo sequences and hyperintense on T1-weighted spin echo sequences. It is detected inside blood vessels for ≤ 72 h and begins to clear within 24 h from injection. Its early

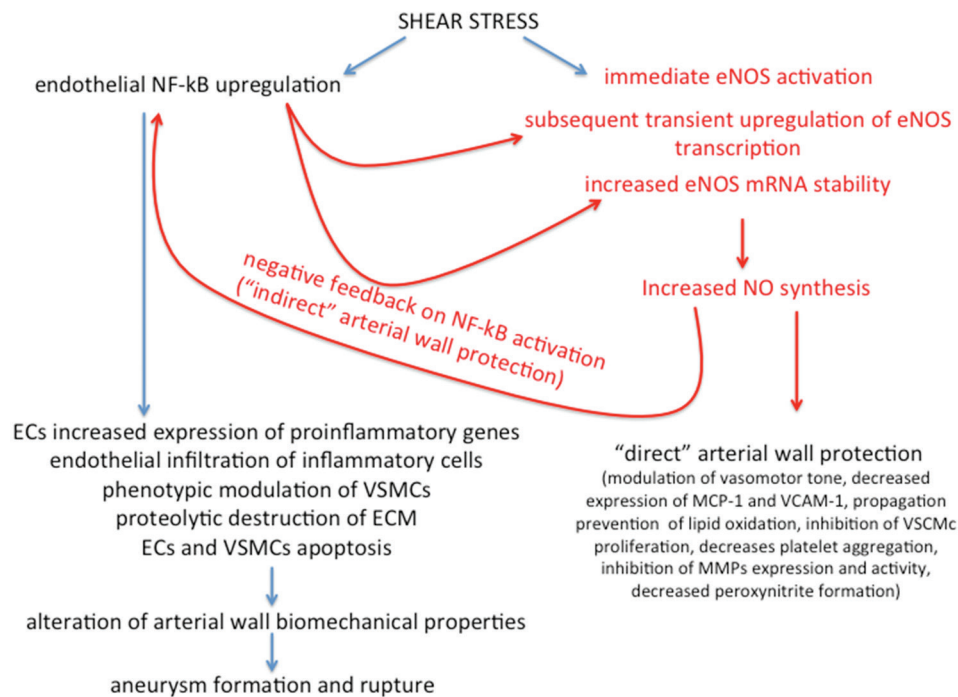


Figure 1: Impact on arterial wall condition of balance between shear stress-mediated endothelial nuclear factor kappa B-cell upregulation and nitric oxide synthesis

uptake, within 24 h, is secondary to macrophage uptake and considering the crucial role of such inflammatory cells in development and rupture of IAs, it indicates active inflammation in aneurysm walls of unstable aneurysm, as suggested by one recent study.^[102] These authors found that early ferumoxytol uptake was significantly higher in aneurysms with marked upregulation of inflammatory molecules such as cyclooxygenase-2 and microsomal prostaglandin E synthase-1 and macrophages, independently from site and size of the aneurysms.^[101] Moreover, all three unruptured IAs of their series with early ferumoxytol uptake that were managed conservatively ruptured less than 6 months after diagnosis, which supports the hypothesis that inflammation is the cause but not the consequence of the rupture.^[102] Another line of research aiming at differentiating ruptured and unruptured IAs on the basis of distinctive patterns of expression of inflammatory markers is the analysis of gene expression profile.^[103] A recent study compared the gene expression arrays of ruptured and unruptured aneurysms and found a significant difference of expression of genes encoding macrophage-mediated inflammatory molecules according to the age of patients.^[105] In particular, genes involved in vascular remodeling, inflammation, and atherosclerosis such as S100/calgranulin genes (*S100A8*, *S100A9*, and *S100A12*), cluster of differentiation 163, myeloperoxidase (MPO), were upregulated, while genes for Krüppel-like family of transcription factors (*KLF2*, *KLF12*, and *KLF15*) and *CDKN2*, which are respectively anti-inflammatory regulators and inhibitors of cellular proliferation, were downregulated, together with cell adhesion molecules and cytoskeletal proteins of arterial

wall.^[105] Therefore, the authors conclude that some of these identified genes may help identifying IAs at risk of rupture, which warrant early treatment.^[105]

CONCLUSION

Aneurysm formation begins with a hemodynamically triggered endothelial inflammatory dysfunction, which is the cause rather than the consequence of aneurysms' development and rupture. The proinflammatory action of shear stress prevails over its endothelium protective action when the balance between NF-κB-mediated production of NO and proinflammatory mediators (ROS, cytokines, adhesion molecules) shifts in favor of inflammation because of alteration of the inhibitory limb of the NO-mediated negative feedback on NF-κB activation. Targets of the inflammatory reaction are, besides ECs, ECM, and VSMCs. Endothelial injury, VSMCs phenotypic modulation with acquisition of pro-inflammatory/pro-matrix remodeling properties and subsequent Fas-mediated apoptotic cell death lead to the arterial wall weakening and aneurysm formation and rupture. Clarifying the causative relationships that link hemodynamics, inflammation, vascular remodeling, and the development and rupture of IAs may provide effective tools to predict the individual risk of aneurysmal rupture and aid the treatment decision-making process.

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Cite this article as: Signorelli F, Gory B, Riva R, Labeyrie PE, Pelissou-Guyotat I, Turjman F. Hemodynamics, inflammation, vascular remodeling, and the development and rupture of intracranial aneurysms: a review. *Neuroimmunol Neuroinflammation* 2015;2(2):59-67.

Source of Support: Nil. **Conflict of Interest:** No.

Received: 01-02-2015; **Accepted:** 05-03-2015

Inflammation and intracranial aneurysms: mechanisms of initiation, growth, and rupture

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ABSTRACT

Outcomes following aneurysmal subarachnoid hemorrhage remain poor in many patients, despite advances in microsurgical and endovascular management. Consequently, considerable effort has been placed in determining the mechanisms of aneurysm formation, growth, and rupture. Various environmental and genetic factors are implicated as key components in the aneurysm pathogenesis. Currently, sufficient evidence exists to incriminate the inflammatory response as the common pathway leading to aneurysm generation and rupture. Central to this model is the interaction between the vessel wall and inflammatory cells. Dysfunction of the endothelium and vascular smooth muscle cells (VSMCs) promotes a chronic pathological inflammatory response that progressively weakens the vessel wall. We review the literature pertaining to the cellular and chemical mechanisms of inflammation that contribute to aneurysm development. Hemodynamic stress and alterations in blood flow are discussed regarding their role in promoting chronic inflammation. Endothelial cell and VSMC dysfunction are examined concerning vascular remodeling. The contribution of inflammatory cytokines, especially tumor necrosis factor- α is illustrated. Inflammatory cell infiltration, particularly macrophage-mediated deterioration of vascular integrity, is reviewed. We discuss the inflammation as a means to determine aneurysms at greatest risk of rupture. Finally, future therapeutic implications of pharmacologic modulation of the inflammation are discussed.

Key words: Aneurysm, endothelium, inflammation, subarachnoid hemorrhage, vascular smooth muscle cells

INTRODUCTION

Intracranial aneurysms and subarachnoid hemorrhage (SAH) represent significant disease entities, with ruptured aneurysms accounting for severe disability and death in approximately 27,000 Americans each year.^[1] Among the general population, approximately 4-5% of individuals harbor an unruptured aneurysm.^[2] However, with the evolution of sophisticated imaging modalities, such as magnetic resonance imaging (MRI), magnetic resonance angiography, and computed tomographic angiography, unruptured incidental aneurysms are continually discovered with increasing frequency. Microsurgical and endovascular obliteration remains the mainstays of treatment, yet both are associated with a significant risk of morbidity and mortality.^[3,4] In addition, a significant number of unruptured aneurysms are treated preemptively

to avoid the catastrophic sequelae associated with SAH. Despite the prevalence of these lesions, the often-devastating nature of the disease, and the risks associated with treatment, relatively little is known about the aneurysm pathogenesis and natural history. As a result, there has been a significant effort to define the mechanisms underlying aneurysm formation and growth. Although the evolution of aneurysms from initiation to rupture is undoubtedly multifactorial in nature, the inflammatory response appears to play a critical role in the pathogenesis of these lesions.

Further understanding of the relationship between the inflammatory response and aneurysm evolution may have important clinical implications in the future. Identification of patients prone to pathologic inflammatory states could allow detection of a population more likely to suffer aneurysm rupture. Additionally, the genetic and cellular processes mediating inflammation represent attractive targets for possible pharmacologic intervention. We review the current literature pertaining to the role of inflammation in the generation of aneurysm formation and rupture. The various vascular inflammatory stimuli are discussed, with special attention paid to hemodynamic stress and alterations in blood flow. Endothelial cell

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10.4103/2347-8659.153975

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and vascular smooth muscle cell (VSMC) dysfunction are detailed and examined in relation to vascular remodeling. The contribution of multiple cytokines to a sustained pathologic inflammatory state and their influence on aneurysm formation are outlined. Special emphasis is placed on a key inflammatory mediator, tumor necrosis factor- α (TNF- α). The contribution of inflammatory cell infiltration, particularly the potential of macrophage-mediated rupture, is detailed. Finally, future therapeutic implications of pharmacologic modulation of the inflammatory response are discussed.

INTRACRANIAL ANEURYSMS AND THE INFLAMMATORY RESPONSE

Multiple studies have identified various risk factors for aneurysmal expansion and rupture.^[5] Genetics plays an important role, with approximately 10% of SAH patients having two or more family members also affected by unruptured or ruptured aneurysms.^[6,7] The propensity for SAH within specific ethnic groups, particularly the Finish and Japanese populations, further highlights the contribution of genetics.^[8,9] Gender also appears to play a role, as women appear to more frequently develop intracranial aneurysms and perhaps suffer from ruptured aneurysms more often than men.^[10,11] Environmental factors, particularly smoking, have been clearly linked to a higher incidence of SAH.^[11] Additional studies have linked binge drinking to aneurysm rupture.^[12,13] Chronically uncontrolled hypertension clearly correlates with aneurysm formation in animal models and clinical studies have identified hypertension as a risk factor for aneurysmal SAH.^[10,11,14-16]

Due to the variability in contributing risk factors, attempts have been made to identify a unifying underlying pathophysiologic mechanism that promotes aneurysm formation and rupture.^[5] There is a tremendous mounting body of evidence that the inflammatory response represents a common endpoint that drives aneurysm evolution, which is succinctly summarized as initiation of development, growth, and potential rupture.^[17] Hemodynamic stress and disruption of blood flow, oxidative stress, injurious environmental elements (i.e. cigarette smoking and cocaine), and pro-inflammatory genetic alterations all initiate a sustained and pathologic inflammatory response.^[18,19] As a result, the intracranial vasculature is subjected to endothelial cell dysfunction, elevated inflammatory cell infiltration, detrimental changes within the tunica media, and exposure to increased concentrations of proteases. These processes lead to weakening, dilation, and remodeling of the vessel walls, which are key components in aneurysm formation and rupture.

ENDOTHELIAL CELL DYSFUNCTION, VASCULAR REMODELING, AND INFLAMMATION

Vascular remodeling is a complex process that is driven, in large part, by hemodynamic stresses along vessel walls.^[18] The propensity for aneurysms to form at vessel branch points and the association of aneurysms with environmental stimuli known to disrupt vascular integrity (smoking, hypertension) highlights the role of abnormal blood flow and shear stress in aneurysm formation.^[17] High wall shear stress has been shown to initiate activation of the inflammatory response.^[20-22] Central to this process is the endothelial cells, which act as an interface between blood flow and the vessel wall.^[17] Through the process of mechanotransduction, these cells respond to the mechanical stimuli of shear, stretch, and flow by altering their physical structure and initiating biologic signaling.^[23-25] Multiple mechanical sensors have been identified, including, ion channels, integrins, cell adhesion molecules, and G protein-coupled receptors at the apical and basal surfaces of endothelial cells.^[26-29] Activation of these sensors initiates intracellular cascades that result in a sustained inflammatory response [Table 1].

Aoki *et al.*^[30] demonstrated a direct link between shear stress and activation of the inflammatory cascade in a rat model. Cyclooxygenase-2 (COX-2) activity, which is induced by hemodynamic force, generates prostaglandin E2 (PGE2), leading to activation of the pro-inflammatory mediator nuclear factor- κ B (NF- κ B).^[30] Inflammation is then sustained through a positive feedback loop containing PGE2 and NF- κ B, creating an environment in which vessel wall degradation can occur. Inhibition of COX-2 or loss of the PGE2 R ρ suppressed NF- κ B-mediated chronic inflammation and was associated with a decreased incidence of cerebral aneurysms. Additional support for the role of NF- κ B-mediated pathways in inflammation-induced aneurysm formation is found in diminished aneurysm formation and growth in rats treated with statins, which inhibit NF- κ B activity.^[31-33]

Evidence also exists that implicates angiogenesis secondary to inflammation-driven endothelial cell dysfunction as a significant contributor to aneurysm formation. The presence of angiogenic growth factors, including, vascular endothelial growth factor (VEGF) and basic fibroblast growth factor, within aneurysm walls has been well documented within the literature.^[34] In pathologic states VEGF induces changes in the endothelium leading to increased permeability at intercellular junctions and activation of pathways resulting in the breakdown of the tunica media and extracellular matrix.^[35,36] Importantly, VEGF may also

Table 1: Summary of the role of inflammatory mediators in aneurysm growth and rupture

	Endothelial cells	Vascular smooth muscle cells	Macrophages
Role in vascular homeostasis	Interface between blood flow and vessel wall Mechanotransduction Respond to shear stress and flow	Contractile state maintains vessel wall integrity	M2 subset is antiinflammatory Play a role in vascular repair
Associated cell signaling molecules (secreted by or possess receptors for)	VEGF bFGF SDF-1 COX-2 PGE2 NF-κB TNF-α	NF-κB MMPs IL-1β MCP-1 TNF-α	MMPs Elastases MCP-1 NF-κB TNF-α
Role in aneurysm formation	Migration grants inflammatory cells increased access to vessel wall Proliferation/neovascularization leads to increased inflammatory cell access to vessel wall Complex cell signaling leading to chronic inflammatory state	Secretory phenotype leads to inflammatory state Erratic migration Apoptosis Weakening of vessel wall	Propagate inflammatory response May play significant role in progression to rupture Release elastases and MMPs involved in vessel wall degradation

VEGF: vascular endothelial growth factor; bFGF: basic fibroblast growth factor; SDF-1: stromal cell-derived factor-1; COX-2: cyclooxygenase-2; PGE2: prostaglandin E2; NF-κB: nuclear factor-κB; TNF-α: tumor necrosis factor-α; MMP: matrix metalloproteinases; IL-1β: interleukin-1β; MCP-1: monocyte chemoattractant protein-1

initiate the genesis of new capillary tubes, microvascular sprouting, and maturation of proliferating vessels.^[37-39] bFGF targets endothelial cells, fibrocytes, and myocytes and mediates vascular wall maturation during angiogenesis.^[40-43] Hoh *et al.*^[44] recently reported on the expression of stromal cell-derived factor-1 (SDF-1), a chemokine with pro-angiogenic and pro-inflammatory properties, in the walls of human and murine intracranial aneurysms. SDF-1 promoted endothelial cell migration and proliferation, as well as capillary tube formation in *in vitro* studies.

Vessel proliferation within aneurysm walls is a proposed mechanism by which inflammatory cells gain increased access to the underlying tunica media, thereby accelerating degradation of this layer. The vaso vasorum is typically not present in the intracranial vasculature, with the exception of the proximal intracranial carotid and vertebral arteries.^[45] However, multiple case reports have described the presence of an extensive vaso vasorum with the walls of intracranial aneurysms.^[44,46,47] Neovascularization in these cases was also associated with inflammatory cell invasion on histopathologic examination.

INFLAMMATION-DRIVEN DEGRADATION OF VASCULAR SMOOTH MUSCLE AND THE EXTRACELLULAR MATRIX

Under normal physiologic conditions VSMCs, the primary cellular component of the tunica media, remain in a contractile state, maintaining the integrity of the vessel wall. In pathologic conditions, such as those that arise in the setting of increased hemodynamic stress, endothelial dysfunction, as well as direct VSMC injury, leads to disruption of the tunica media and extracellular matrix.^[48,49] Central to this process is the VSMC transition from a contractile

to a secretory phenotype defined by a loss of markers of contractility and expression of pro-inflammatory cytokines and matrix metalloproteinases (MMPs).^[17,50-54] Alterations in VSMC phenotype have been reported in the setting of atherosclerotic lesions, in which these cells upregulate the production of NF-κB, secrete cytokines, release MMPs, and migrate into the intima, where proliferation results in vessel stenosis.^[55,56] Interestingly, in atherosclerotic lesions where inflammation leads to VSMC migration and proliferation, intracranial aneurysm walls are defined by VSMC erratic migration and apoptosis.^[48] As aneurysm formation progresses, substantial thinning of the tunica media and cellular loss is observed.^[48,57,58] Ruptured aneurysms are more frequently found to have hypocellular and hyalinized walls when compared to unruptured aneurysms, highlighting the progressive nature of wall destruction.^[59,60]

Multiple inflammatory cascades appear to be involved in VSMC dysfunction and death [Table 1]. Interleukin-1β (IL-1β) is a pro-inflammatory cytokine that initiates a number of deleterious effects within the VSMCs and extracellular matrix.^[48] IL-1β plays an important role in recruiting inflammatory cells to atherosclerotic lesions and areas of vessel injury.^[61,62] This cytokine also activates NF-κB, which is responsible for inducing inflammatory cascades, promoting pro-inflammatory gene expression, and mediating downregulation of procollagen synthesis within the tunica media.^[48,63] IL-1β also directly induces apoptosis of VSMCs, thereby promoting thinning of the aneurysm wall.^[48] Further supporting the role of IL-1β in VSMC degradation is the impairment of aneurysm growth in IL-1β deficient mice.^[64]

Ets-1 is a transcription factor primarily activated in VSMCs residing in the tunica media.^[65] Multiple

studies have shown Ets-1 to play a role in the regulation of vascular inflammation, pathologic remodeling, and angiogenesis.^[66-69] Aoki *et al.*^[65] demonstrated upregulation and activation of Ets-1 in intracerebral aneurysm VSMCs, strongly implicating this inflammatory transcription factor in aneurysm evolution.

Vascular smooth muscle cells in a secretory state generate increased monocyte chemoattractant protein-1 (MCP-1), a chemokine involved in the attraction and migration of monocytes and macrophages to areas of damaged tunica media.^[70] MCP-1 upregulation has been demonstrated in the early stages of cerebral aneurysm formation in rat models, and MCP-1 blockade resulted in decreased macrophage infiltration and aneurysm progression.^[71,72] Chalouhi *et al.*^[73] reported high levels of MCP-1 in the lumen of unruptured cerebral aneurysms, implicating this chemokine in early aneurysm formation.

Normal maintenance of the extracellular matrix is dependent on the balance between MMPs and their inhibitors, tissue inhibitors of matrix metalloproteinases (TIMPs).^[52] Imbalance in this system results in excessive breakdown of the collagen and elastin of the extracellular matrix and resultant vessel wall weakening, a key contributor to aneurysm development and rupture. MMPs and TIMPs have been linked to the development of atherosclerotic lesions and the genesis of abdominal aortic aneurysms.^[12,74-80] Immunohistochemistry and western blotting have demonstrated the presence of MMPs within the walls of intracranial aneurysms.^[81,82] In SAH patients, elevated serum MMP-9 levels have been documented, with normalization occurring by postbleed day 12.^[83] Cigarette smoke, a well-established, inflammatory stimulus for aneurysm initiation and growth, has been demonstrated to induce macrophage differentiation and increased release of MMP-2/9.^[84] In a comparison of smokers and nonsmokers, the carotids of smokers demonstrated elevated levels of MMPs and a decreased concentration of TIMPs and elastin compared to nonsmokers.^[85]

Laboratory investigations also support derangement of MMP and TIMP interactions as a key component in the aneurysm pathogenesis. In a rat model, Aoki *et al.*^[51] demonstrated increased levels of MMP-2 and MMP-9 in aneurysm walls, with increasing expression as aneurysms progressed. Ali *et al.*^[86] reported MMP stimulation by cigarette smoke extract in rat cerebral VSMCs *in vitro* and in carotid VSMCs *in vivo*.

TUMOR NECROSIS FACTOR- α

Tumor necrosis factor- α has emerged as a potential key contributor in the generation, growth, and eventual

rupture of intracranial aneurysms. Multiple studies have demonstrated increased levels of TNF- α within the cerebral circulation in response to injury or ischemia.^[87,88] Elevated levels of TNF- α mRNA have been demonstrated in intracranial aneurysms with reverse transcription-polymerase chain reaction.^[89] Plasma TNF- α is also increased in aneurysm patients while additional authors have reported an association between single-nucleotide polymorphisms in the TNF- α gene and an increased risk of aneurysm incidence and rupture.^[90-94]

Tumor necrosis factor- α is a well-established mediator of inflammation and apoptosis, working through multiple receptors to modulate various cellular responses to injury. Tumor necrosis factor- α receptor 1 (TNFR1) primarily binds the soluble form of TNF- α and plays a central role in TNF- α -induced cellular signaling.^[95,96] Importantly, TNFR1 contains a death domain that plays a role in TNF- α -mediated apoptosis.^[97,98] A second receptor, TNFR2, is activated by membrane-bound TNF- α and primarily found on endothelial and immunomodulatory cells. Activation of TNFR1 signals apoptosis through the activation of the Fas-associated death domain (FADD), which in turn, binds and activates pro-caspase 8. Ultimately, this pathway leads to the activation of multiple proteases that result in apoptosis. Jayaraman *et al.*^[2] postulate that a complex interaction between TNF- α and both receptors induces and promotes aneurysm growth. Initially, endothelial activation is driven by TNFR2 and membrane-bound TNF- α . Activated endothelial cells generate high concentrations of soluble TNF- α , which interacts with the TNFR1, leading to endothelial cell dysfunction and death. Endothelial cell death, in turn, creates increased vascular permeability, thereby creating multiple pathways for macrophage infiltration, MMP generation, and loss of the VSMC layer. An additional receptor, tTNF-Rp55, initiates apoptosis through the induction of caspases.^[89] The TNF-Rp55 also induces the recruitment and activation of caspases through interaction with the FADD protein.^[99] Documented increased expression of the FADD protein in human aneurysms also supports TNF- α -mediated apoptosis as a causative factor in aneurysm formation.^[89]

Tumor necrosis factor- α also appears to play an important role in the pathologic VSMC changes observed in aneurysm formation and rupture, driving these cells to change from a contractile to a synthetic and pro-inflammatory phenotype. Furthermore, the secretory phenotype of VSMCs participates in the secretion of TNF- α when exposed to inflammatory stimuli.^[100,101] Ali *et al.*^[102] demonstrated TNF- α to suppress expression of contractile genes and induce the expression of pro-inflammatory genes in VSMCs

in vivo and *in vitro*. In the same study, TNF- α induced increased expression of Kruppel-like transcription factor 4, a known regulator of VSMC differentiation.^[102] Additional studies have identified cigarette smoke, a known potent pro-inflammatory stimulus, to induce similar phenotypic changes in VSMCs, including increased secretion of TNF- α .^[100] Clearly the data shows TNF- α secretion to be involved in a positive feedback loop with VSMCs, with TNF- α stimulating phenotypic change and thus driving its own secretion from these cells. The fact that VSMCs appear to first exhibit pathologic migration, followed by disappearance prior to rupture suggests TNF- α first induces phenotypic change that is followed by apoptosis in the presence of chronically sustained levels of the cytokine.^[103]

INFLAMMATORY CELL MIGRATION AND INFILTRATION

Endothelial cell dysfunction and apoptosis increase the permeability of vessel walls, allowing for enhanced binding and transmigration of inflammatory cells into the underlying VSMC layer. T-cells have been demonstrated within aneurysm walls where they respond to antigen presentation by monocytes and macrophages.^[104] In a rat model, mast cells were significantly increased within the walls of forming cerebral aneurysms.^[105] Inhibition of mast cell degranulation diminished aneurysm size, prevented thinning of the tunica media, blocked NF- κ B activation, and decreased expression of MCP-1, MMP, and IL-1 β . An evaluation of human intracerebral aneurysms found mast cells to be significantly increased in ruptured compared to unruptured aneurysms.^[106] These findings suggest mast cells play an important role in aneurysm growth and rupture.

Monocytes and macrophages appear to be essential to aneurysm formation and rupture, a finding that has been repeatedly demonstrated in animal and clinical studies. These cells secrete MMPs and elastases which are responsible for degradation of the extracellular matrix and the internal elastic lamina.^[107] Kanematsu *et al.*^[108] observed macrophage depletion and inhibition of MCP-1 to be associated with a reduced incidence of intracranial aneurysms. Aoki *et al.*^[72] also observed a significant decrease in cerebral aneurysm formation, macrophage accumulation, and expression of MMP-2 and MMP-9 in MCP-1 deficient mice. In a murine model, Ruzevick *et al.*^[109] observed the haptoglobin 2-2 (Hp2-2) genotype, which is linked to a pro-inflammatory state, to be associated with significantly larger aneurysms and a greater number of macrophages within the aneurysm walls. Histopathological examination of ruptured and unruptured human intracranial

aneurysms has demonstrated macrophage infiltration within aneurysmal walls.^[59,60,110] Chalouhi *et al.*^[73] found high plasma concentrations of MCP-1 within the lumens of intracranial aneurysms, suggesting active recruitment of macrophages and other inflammatory cells.

There is mounting evidence that macrophage invasion may be a causal factor in rupture. Frosen *et al.*^[60] found more prominent macrophage infiltration in ruptured aneurysms compared to that found in unruptured aneurysms. Of particular interest was the infiltration of macrophages observed within the first 12 h after rupture, suggesting that macrophages may induce the rupture. MRI investigations of aneurysms with ferumoxytol (AMAG Pharmaceuticals, Lexington, Massachusetts, USA), a superparamagnetic iron oxide particle cleared by macrophages, have also linked macrophage infiltration with rupture.^[111-113] In a study of 48 unruptured aneurysms, all aneurysms that showed early uptake of ferumoxytol on MRI ruptured within 6 months.^[114] Among aneurysms demonstrating late uptake, there were no ruptures or increase in size during the follow-up period. Immunohistochemical analysis found greater levels of inflammation in aneurysms with early uptake of ferumoxytol. The authors propose that early uptake of ferumoxytol is associated with more prominent inflammation, increased macrophage infiltration, and thus, a greater risk of rupture. Follow-up investigations utilizing aspirin as an anti-inflammatory agent found a decrease in aneurysm wall signal intensity on ferumoxytol MRI and a diminished number of macrophages on immunostaining.^[111] Hasan *et al.*^[106] examined the M1 (pro-inflammatory) and M2 (anti-inflammatory) subsets of macrophages in a population of ruptured and unruptured clipped aneurysms. While M1 and M2 macrophages were observed in equal proportions in unruptured aneurysms, M1 macrophages were found in a significantly greater proportion in ruptured aneurysms.

Therapeutic implications

Current treatment options for ruptured and unruptured cerebral aneurysms include microsurgical and endovascular obliteration. These interventions are associated with a significant morbidity and mortality, the risk that is magnified in cases of asymptomatic unruptured aneurysms. Thus, study of the mechanisms underlying aneurysm evolution is critical to establish a better understanding of which aneurysms are more likely to rupture. Additionally, this data may provide insight into the biological pathways best-suited for pharmacological intervention, stabilization of the aneurysm wall, and prevention of rupture. The

inflammatory process appears to possess multiple targets ideally suited for such pharmacological treatment.

The wide range of pro-inflammatory pathways induced by TNF- α makes this cytokine an attractive target for blockade.^[2] Preservation of endothelial function, maintenance of the VSMC contractile phenotype, and inhibition of inflammatory cell migration are all potential benefits of TNF- α antagonism. MMP-2 and MMP-9 blockade has proven to limit aneurysm development in animal models, possibly identifying an additional mechanism with clinical relevance.^[51] Modification of VSMC phenotype continues to be an area of interest, with both cytokine and transcription factor-mediated changes representing possible therapeutic targets.^[102] Macrophages represent an additional cellular target for pharmacologic intervention. Hasan *et al.*^[113] has already shown aspirin to potentially decrease the risk of aneurysm rupture, a clinical benefit possibly derived from diminished macrophage infiltration of the aneurysm wall. Nakakoa *et al.*^[115] has demonstrated gene expression profiles in ruptured aneurysms that indicate the macrophage-mediated inflammation as a key contributor to aneurysm rupture. Future studies may determine these identified genes as diagnostic markers for aneurysms prone to rupture or potential therapeutic targets. Importantly, in all of these areas the therapeutic benefit must be weighed against the risks of altering what, in many instances, is a natural and protective biological response.^[2]

CONCLUSION

Cerebral aneurysms remain a significant health and socioeconomic problem, with the majority of ruptures leading to death or severe disability. Microsurgical and endovascular interventions represent the two modalities of treatment; however, both are associated with risk. An understanding of the mechanisms leading to aneurysm development is necessary to identify markers predictive of growth and rupture, as well as targets for pharmacologic intervention. Through multiple pathways, the inflammatory response appears to drive the initiation of aneurysm formation, potentiate continued growth, and contribute to eventual rupture. Animal and human studies highlight the complex interaction between the endothelium, VSMCs, and inflammatory cells. Phenotypic change, cytokine production, apoptosis, and cellular migration all partake in aneurysm evolution. Delineation of those at greatest risk for rupture would afford improved patient selection with regards to aneurysm obliteration. Furthermore, the definition of these various processes may hold promising pharmacological therapeutic implications in the future.

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Cite this article as: Amenta PS, Valle E, Dumont AS, Medel R. Inflammation and intracranial aneurysms: mechanisms of initiation, growth, and rupture. *Neuroimmunol Neuroinflammation* 2015;2(2):68-76.

Source of Support: Nil. **Conflict of Interest:** No.

Received: 01-09-2014; **Accepted:** 11-11-2014

Inflammation in human cerebral aneurysms: pathogenesis, diagnostic imaging, genetics, and therapeutics

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ABSTRACT

Intracranial aneurysms are a life-threatening cerebrovascular pathology with a probability of spontaneous rupture. Current intervention techniques carry inherent risk. Recent investigation has reinforced inflammation's role in the pathophysiological process of cerebral aneurysms. These data suggest alternative diagnostic and noninvasive therapeutic strategies. Furthermore, novel characteristics of the underlying disease have been elucidated through distinct bioinformatic and gene expression profile analyses. This article will emphasize the most recent investigation, highlighting findings of clinical significance and etiological relevance.

Key words: Diagnostic imaging, genetics, intracranial aneurysms, pathogenesis, therapeutics

INTRODUCTION

Intracranial aneurysms (IAs) are a common multifactorial cerebrovascular disease. While having a 3.2% prevalence rate in the general population and 1.1% annual rupture risk, aneurysms remain lethal upon rupture in 40% of cases.^[1-3] Risk for aneurysmal subarachnoid hemorrhage (SAH) is higher in hypertensive patients, smokers, heavy drinkers, and females.^[2,4] The current interventional techniques, surgical clippings and endovascular occlusions, remain invasive despite advancements in technology. Although SAH risk factors are known, a more complete understanding of the complex pathophysiology underlying IA formation, progression, and rupture is needed. The majority of evidence from intensive investigation has implicated a mounting inflammatory response during the aneurysm pathogenesis.^[5,6] This data ultimately provides promising targets for *in vivo* molecular imaging and noninvasive IA therapeutics. This article will discuss inflammation

as it pertains to IA pathogenesis, with a focus on the most recent investigation. Furthermore, it will offer a review of recent genetic and bioinformatic analyses, highlighting findings of pathological significance and methodological diversity. The final sections will further illuminate both innovations in diagnostic imaging of aneurysmal inflammation and experimentally efficacious noninvasive attempts at IA prevention and regression.

PATHOGENESIS

Hemodynamic insult is considered to be one of the first steps in activating the cerebral vessel wall's inflammatory response.^[7] There is a continuous balance between hemodynamic stress and the integrity of the vessel wall.^[5] Upon the hemodynamic insult, this balance is perturbed, leading to vessel wall weakening. Dilation results, as extracellular matrix is degraded by increased levels of matrix metalloproteinases (MMP) compounded by concomitant apoptotic death of vascular smooth muscle cells (VSMCs).^[7] Initially, the vessel wall is highly organized. Integral disturbances lead to less organization within the aneurysm wall and fewer distinct layers.^[5] Simultaneously, MMP activation has been found to facilitate flow-induced internal elastic lamina (IEL) fragmentation.^[8] Similar IEL fragmentation is a feature commonly observed in atherosclerotic lesions.^[9]

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10.4103/2347-8659.154433

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Vascular smooth muscles cells (VSMCs), mainly found in the media layer, are recognized as major producers of matrix in the vessel wall. Upon endothelial injury, intimal thickening occurs as VSMCs migrate into the intima and proliferate.^[7,10] Phenotypic transformation is seen in these migrated VSMCs as environmental change induces a switch from a contractile phenotype to a synthetic pro-inflammatory matrix remodeling phenotype in these cells.^[11] Nakajima *et al.*^[12] showed phenotypically modulated VSMCs have lower expression of contractile smooth muscle myosin heavy chain isoforms when immunohistochemically stained. Furthermore, the group observed decreases in contractile protein staining intensity in ruptured IA, suggesting VSMC phenotypic modulation is related to a rupture mechanism.

Endothelial cells are also affected by the hemodynamic insult. As blood flows, mechanical stimulus has notable effects on the cells of the vascular system.^[13] Shear stress influences arterial physiology, in particular, the cellular function of the vessel wall.^[13] Consequently, IAs develop in vessel regions exposed to high hemodynamic stress such as arterial bifurcations and sharp angles.^[7] Endothelial cells under laminar flow become aligned with the flow but endothelial cells under turbulent flow become cuboidal due to F-actin reorganization.^[14] Experimentally, endothelial cells respond to hemodynamic stress with the up-regulation of the inflammatory mediator, prostaglandin E receptor 2 (EP2), during the formation of cerebral aneurysms.^[15] When shear stress was applied to primary endothelial cells, EP2 was found to be up-regulated.^[15] The stimulation of EP2 in primary endothelial cells also led to the activation of the transcription factor NF- κ B, a well-studied transcriptional mediator of inflammation in IA.^[15]

As cerebral vessel walls undergo change during aneurysm development, the formation of new vessels, angiogenesis, also contributes to aneurysmal progression.^[16] Angiogenesis indirectly advances the inflammatory process of aneurysm progression by aiding in the delivery of inflammatory cells to vessel walls. Hoh *et al.*^[16] recently showed that stromal cell-derived factor-1 (SDF-1) is expressed in murine carotid IAs, murine circle of Willis IAs, and human cerebral aneurysms. The study found increased levels of progenitor cells expressing the SDF-1 receptor, CXCR4, in mice with carotid and cerebral aneurysms. Study findings also showed SDF-1 to promote endothelial cell migration, endothelial cell tube formation (angiogenesis), and macrophage migration.^[16] Inhibition of SDF-1 in the murine model significantly decreased the amount of endothelial cells and capillaries in the aneurysm wall, and the overall rate of aneurysm formation was reduced.^[16]

MEDIATORS OF INFLAMMATION

Human and animal studies have both shown that inflammatory cells and mediators are involved in IA pathogenesis [Figure 1]. A number of these inflammatory cells and mediators are highlighted in this section, with a special focus on the most recent investigation. Mast cells infiltration into the aneurysm vessel wall has been illustrated throughout the pathophysiological chronology of IA development.^[17] Ishibashi *et al.*^[17] analyzed mast cells levels at different time intervals following aneurysm induction in a rat model and discovered that the number of mast cells in the IA walls significantly increases over time. To further understand mast cells' role in the aneurysm pathogenesis, mast cell degranulation inhibitors, tranilast and emedastine, were administered into an aneurysm-induced rat model. The inhibitors had an impact on aneurysm progression by decreasing aneurysm size, as well as the extent of media thinning in the induced IAs. Mast cells' contribution to the inflammatory process was simultaneously studied through the analysis of mast cell degranulation's effect on cultured arterial smooth muscle cells of rat IAs. The group found that mast cell degranulation promoted MMP-2, MMP-9, and iNOS expression.^[17] Based on previous findings that iNOS deficiency leads to a decrease in apoptotic smooth muscle cell death, Ishibashi *et al.*^[17] and Sadamasa *et al.*^[18] suggested that the inhibition of mast cell degranulation impacts the process of media thinning through its induction of iNOS. The authors also concluded that the decrease in media thinning results from mast cells' modulation of MMP-2 and MMP-9 expression.^[17,19] Overall, these data suggest that the degranulation of mast cells plays a role in IA formation.

T cells and macrophage infiltration have found to be associated with human cerebral aneurysm rupture.^[20] Kanematsu *et al.*^[21] studied leukocyte infiltration into the aneurysm wall of an aneurysm-induced mouse model and found macrophages to be one of the dominant leukocytes present. Cerebral aneurysm incidence was found to be significantly attenuated in mice with lower macrophage levels as well as in mice with inhibited monocyte chemoattractant protein-1 (MCP-1), a macrophage chemoattractant, when compared with wildtype mice.^[21] Certain proteinases secreted by macrophages have been found to be involved in the vascular remodeling of aneurysm formation. Aoki *et al.*^[19] demonstrated that in aneurysm-induced rats, the expression of MMP-2 and MMP-9, was present in the arterial wall undergoing the beginning stages of aneurysm formation. This expression was found to increase alongside the progression of the studied cerebral aneurysms. Macrophages were determined to be the main secretors of the MMPs in the study.^[19] Further connection has been found between

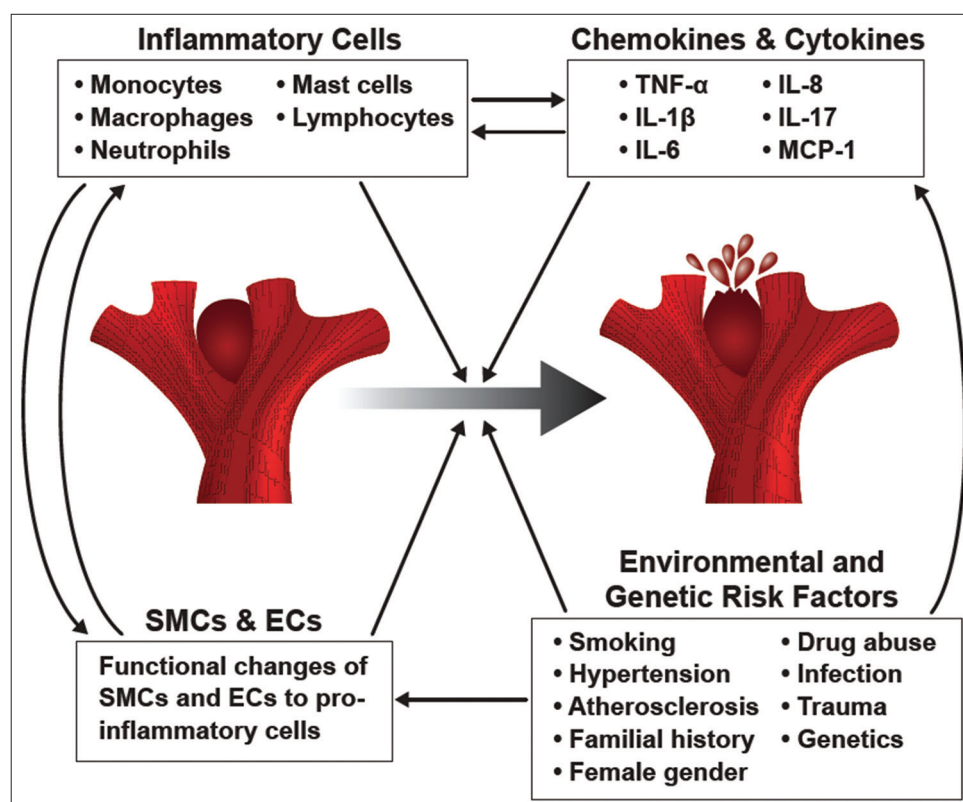


Figure 1: Summary of the factors contributing to cerebral aneurysm pathogenesis. Inflammatory cells, cytokines, chemokines, changes to the vascular smooth muscle cells and endothelial cells, and environmental as well as genetic risk factors all play a role in the development of cerebral aneurysms and their progression to rupture

macrophage presence and aneurysm rupture. Hasan *et al.*^[22] showed that in human cerebral aneurysms, M1 and M2 macrophages were found to be in equal proportions; however, M1 macrophages presented in higher levels than M2 macrophages in ruptured human cerebral aneurysms. This means that there were more macrophages promoting inflammation, M1 macrophages, than macrophages working to repair the vessel tissue and decrease inflammation, M2 macrophages. This imbalance was also correlated with an increase in mast cell presence in ruptured aneurysms.^[22]

The role of the inflammatory mediators, chemokines, has been studied in aneurysm formation. Chemokines promote chemotaxis in particular, the migration of inflammatory cells during the inflammatory response. In recent years, Chalouhi *et al.*^[23] demonstrated that plasma concentrations of RANTES, MIG, IP-10, eotaxin, interleukin 8 (IL-8), IL-17, and the monocyte chemoattractant protein-1 were significantly increased in the lumen of unruptured human cerebral aneurysms when compared to femoral arterial plasma of the same 16 patients. In addition, the study found increased plasma concentrations of MCP-1 in unruptured aneurysms.^[23] This reaffirms the work of Aoki *et al.*^[24] who observed increased MCP-1 expression in rat arterial walls. These data indicate that inflammatory cells are being actively recruited to the aneurysm wall as a result of high chemokine levels, further contributing to IA formation and eventual rupture.^[23]

Aoki *et al.*^[25] confirmed in rodent models that tumor necrosis factor- α (TNF- α) levels are higher in cerebral aneurysms, particularly in the endothelial cells of these lesions, during their formation and progression when compared to the TNF- α levels of control cerebral arteries. Aoki *et al.*^[25] further investigated the TNF- α -TNFR1 signaling pathway and found that IA formation was suppressed in rodents deficient in TNFR1. The authors concluded that TNFR1 deficiency blocks NF- κ B activation and MCP-1 expression, ultimately inhibiting macrophage infiltration. Intervening with this signaling pathway may serve as a future therapeutic strategy.^[25] TNF- α has been previously identified as a contributor to the phenotypic modulation of VSMCs *in vivo* following hemodynamic stress.^[26] The transcription factor, NF- κ B, has also been found to be essential in the activation and recruitment of macrophages as it influences the expression of a number of pro-inflammatory genes.^[5,6] Aoki *et al.*^[27] demonstrated the activation of NF- κ B and expression of downstream genes; these changes seen in the vessel wall of the early stages of aneurysm development in a rat model. The study specifically showed that mice deficient in NF- κ B p50 subunit had a lower incidence of aneurysm formation and macrophage infiltration into the aneurysm wall. Furthermore, Aoki *et al.*^[28] showed that the up-regulation of interleukin-1 β (IL-1 β) and activation of NF- κ B significantly reduced collagen biosynthesis, a process linked to IA progression and rupture.

A GENETIC APPROACH

Progress in understanding IA pathogenesis has been slowed by its multifactorial nature. Heritable genetic variance has long been recognized as an IA risk factor. Indeed familial genetic predisposition leads to increases in IA prevalence.^[29,30] The establishment of gene-prevalence and pathway-prevalence correlations may illuminate novel facets of IA pathophysiology worthy of noninvasive therapeutic intervention. This section will discuss variety recent genetic methods and their significance with respect to the inflammatory pathophysiology of IA.

The aforementioned considerations motivated the use of genome-wide association study (GWAS) to identify common genetic variants in patients who harbor IAs. Recent GWAS have identified several novel IA susceptibility loci, many unsurprisingly associated with structural genes.^[31,32] Comprehensive meta-analysis of all genetic association studies (including GWASs) of sporadic IA found 19 single nucleotide polymorphism associations across the expansive international discovery cohort.^[33] The strongest three of these associations were robust to sensitivity analyses for statistical heterogeneity and ethnicity.^[33] Critically, this meta-analysis confirmed that the previously studied proinflammatory cytokine IL-6 G572C single nucleotide polymorphism was also statistically associated with sporadic IA.^[6] However, initial sensitivity analysis revealed statistical heterogeneity among sample studies. Specifically, when one Chinese study was excluded, the association no longer held.^[33] These variable results suggest more work is needed to precisely define this genetic association and role of IL-6 in IA biology.

In the genomic era, many candidate gene association studies and GWAS have identified common genetic variants that predispose individuals to IA. These methods have recently been bolstered by pathway and network-based analysis (PANOGA) of GWAS data, which ultimately highlights uncommon genetic variants in common pathologically relevant biological pathways. These approaches make use of protein-protein interaction networks. For example, such PANOGA analysis of GWAS data recently implicated pathways conserved across aneurysmal population cohorts, including (named by KEGG term) T-cell receptor signaling and chemokine signaling.^[34] Such methods reinforce inflammations importance in sporadic IA formation and will play an important role in elucidating the complex genetic etiology of IA formation and rupture.

As noted above, common genetic variance only explains a fraction of the heritability in cases of complex

disease. Furthermore, low-frequency predisposing genetic abnormalities are typically found in isolated populations with small founder populations, subsequent bottleneck affects, and genetic drift. One such population, with a higher rate of IA incidence, is the thoroughly characterized genetic isolate of Finland.^[35] A recent study revealed four novel low-frequency risk loci utilizing an alternative genetic approach.^[36] The study focused on the Finnish population, enriched the percentage of familial IA patients in the discovery sample, and increased genome-wide coverage through imputing genotyped variants against the 1000 genomes project reference panel (v3, March 2012 release).^[36] The first novel variant rs74972714 at 2q23.3 is located 40 Kb downstream of LYPD6.^[36] LYPD6 can inhibit transcriptional activity of the AP-1 transcription factor complex, a key activated mediator of inflammation in endothelial cells subject to atherogenic blood flow conditions.^[37,38] The variant rs113816216 at 5q31.3 is located in the intron of FSTL4, a poorly characterized gene.^[36] However, the FSTL4 paralog FSTL1 encodes a protein that induces innate immunity by acting as a toll-like receptor-4 (TLR-4) agonist.^[39] The variant rs150927513 at 7p22.1 is located in the intron of Radil.^[36] Radil has been implicated in the control of neutrophil adhesion and chemotaxis.^[40] Finally, two IA associated common variants, rs12472355 and rs919433 at 2q33.1 were located 30 Kb upstream and intronic of ANKRD44, respectively.^[36] ANKRD44 is a likely subunit of protein phosphatase-6 whose functions include inhibition of NF- κ B activation.^[41,42] Ultimately, this study suggests varied investigative methodologies may yield novel associations in diseases with complex inheritance patterns and illustrates the use of population isolates as genetic tools. Collectively the genetic analyses presented in this section further highlight inflammation's role in IA biology.

INFLAMMATORY GENE EXPRESSION PROFILING

Many studies have attempted to quantify characteristic gene expression patterns in IA. These potentially powerful and informative studies (as reviewed extensively by Chalouhi *et al.*^[51]) are limited by inherent differences in cohort size, quantification techniques, status of sample aneurysms examined (onset vs. rupture), and control tissue used. As such, the results have been unsurprisingly heterogeneous. This section will discuss recent novel approaches, including a discussion of peripheral blood cell transcriptome analysis in patients with aneurysmal SAH.

To tackle the problem of biological heterogeneity among sample aneurysms, Nakaoka *et al.*^[43] determined the gene expression levels in 8 ruptured cerebral arteries (within 24 h post SAH), 5 unruptured cerebral aneurysms,

and 10 control aneurysms (superficial temporal arteries) utilizing agilent microarrays. Critically, utilizing hierarchical clustering and nonnegative matrix factorization, the samples were classified into homogeneous subgroups showing similar gene expression patterns. Focusing on ruptured IA in an early onset age (average age 46.6) sub group, and unruptured IA allowed the investigators to note expressional differences. They observed increased levels of markers for both macrophage infiltration (CD163) and oxidative stress (myeloperoxidase). Significant increases in S100/calgranulin expression, which acts through TLR-2 to recruit macrophages/monocytes and neutrophils, were similarly reported as selectively overexpressed in early ruptured IA. TLR-6 that forms heterodimers with TLR-2, was also significantly overexpressed, ultimately suggesting this pro-inflammatory signaling pathway is involved in IA wall inflammation and rupture.^[43] Finally, Krüppel-like transcription factors (KLF) KLF-2, KLF-12, and KLF-15 were all down-regulated in the early ruptured IA subgroup. KLF-2 is known to possess anti-inflammatory functionality.^[44] This study was the first to show distinct gene expression differences between early and late ruptured IA. In addition, these data further implicate macrophage-mediated inflammation in IA rupture.

Differential gene expression in peripheral blood has been observed in response to both brain arteriovenous malformations and abdominal aortic aneurysms.^[45,46] The first peripheral blood transcriptome analysis in patients with SAH from ruptured IA was recently carried out by Pera *et al.*^[47] The group found that the gene expression profile of venous blood obtained prior to neurosurgical intervention differed significantly from control patients who did not harbor IA. The Illumina HumanHT-12 v4 microarrays revealed T-lymphocyte subpopulation specific transcripts were down-regulated, whereas transcripts related to neutrophils and monocytes were up-regulated. Based on these data, the authors developed a L/MN index, defined as the mean folds of standardized peripheral blood expression levels of lymphocyte related genes against expression of neutrophil and monocyte related genes. This ratio was statistically associated with clinical prognosis; the L/MN value was lower in patients who died during hospitalization compared with RA patients who survived. When blood gene expression profiles are compared with transcriptomic analyses of IA tissue, inflammation is the common denominator.^[47] However, these inflammatory changes likely reflect a systemic response to ruptured IA bleeding. These data further suggest novel molecular biomarkers, which may prove beneficial for optimum management of aneurysmal SAH patients.

DIRECT IN VIVO IMAGING OF INFLAMMATION

The recent development of novel *in vivo* imaging techniques directly targeting specific immune cell subsets and inflammatory enzymatic biomarkers allows clinicians to quantitatively access the inflammatory status of pathologically relevant tissue.^[48] Given the role of macrophages in IA pathogenesis, several studies conducted by Hasan *et al.*^[49-52] investigated the possibility of *in vivo* aneurysm wall macrophage quantification through infusion of a carbohydrate coated superparamagnetic iron oxide nanoparticle, ferumoxytol (AMAG Pharmaceuticals, Lexington, Massachusetts, USA). Ferumoxytol is cleared by macrophages in either the arterial lumen or subendothelium of IAs, thus allowing for delayed visualization of macrophage activity as a surrogate biomarker for inflammatory status of a particular lesion.^[53] The optimal imaging chronology for this novel ferumoxytol enhanced MRI was found to be 5 mg/kg ferumoxytol with imaging at 72 h postinjection.^[49] A critical observation was made in a subsequent follow-up study. The authors found that the time of ferumoxytol uptake may be indicative of aneurysmal stability and rupture propensity. Those exhibiting early uptake (24 h postinjection) ruptured within 6 months.^[51] Furthermore, immunostaining of surgically resected aneurysm dome tissue revealed increased expression of COX-2, mPGES, and the number of M1 variety macrophages in aneurysms with early uptake. It should be noted that this suggests imaging beyond 24 h may prove unnecessary with respect to rupture risk stratification; however, 72 h remains the optimal timing for imaging macrophages in the walls of IAs. This time interval may still be used to access the efficacy of anti-inflammatory therapeutics.^[51] Additionally, previous studies that provided evidence of inflammatory changes in ruptured aneurysms had fallen short of providing evidence of its involvement prior to rupture, rather than as a response to rupture.^[5] These data, therefore, provide the first direct evidence that inflammation is a causal factor in IA rupture progression. Finally, the group was able to use this novel noninvasive technique to monitor the therapeutic effects of aspirin as it curtailed the inflammatory progression of cerebral aneurysms in human patients.^[54] In this study, the findings of ferumoxytol enhanced MRI correlated well with a subsequent immunohistochemical assessment.^[54] Taken together, these studies suggest that ferumoxytol enhanced MRI may indicate active inflammation in aneurysm walls and allow neurosurgeons to optimize decisions regarding intervention or observation of cerebral aneurysms.

Recent histological evidence suggests that the myeloperoxidase, a secretable oxidoreductase of azurophilic granules of polymorphonuclear

cells (primarily neutrophil granulocytes), may potentially be used as a tissue-specific biomarker of inflammation and cerebral aneurysm instability.^[55] Previously, Deleo *et al.*^[56] developed a method to access MPO enzymatic activity as an inflammatory biomarker in a rabbit model of IA. The group used clinical field strength MRI and an MPO specific paramagnetic substrate, di-5-hydroxytryptamide of gadopentetate dimeglumine, as an MR contrast agent. Following endovascular injection of *Escherichia coli* lipopolysaccharide, which resulted in inflammatory cell infiltration into the aneurysm wall and increased active MPO expression, the investigators found the MR enhancement ratios were consistent with the inflammatory changes.^[56] Ultimately, these studies suggest enzymatically specific MR imaging may help identify aneurysms with a significant rupture propensity.

These studies further highlight inflammation's role in the progression and rupture of cerebral aneurysms. *In vivo* targeted molecular imaging may ultimately provide the needed noninvasive metric required for optimal management of IA. Given the strong association of inflammation and macrophage infiltration with IA rupture, IA experts have agreed on the importance of these findings and suggested that larger scale studies are needed.^[53]

THERAPEUTICS TARGETING INFLAMMATION

Studies focused on developing noninvasive IA therapeutics reaffirm inflammation's pathophysiological role in IA formation and progression. Hasan *et al.*^[57] investigated the anti-inflammatory effect of acetylsalicylic acid (aspirin) on the progression of aneurysm to rupture. A secondary analysis of the ISUIA study revealed that the aspirin decreased patients' risk of aneurysm rupture by 60%. Furthermore, the group found ruptured aneurysms had higher levels of cyclooxygenase-2 (COX-2) and microsomal prostaglandin E2 synthase 1 (mPGES-1) expression.^[58] An exploration of acetylsalicylic acid's effect on inflammatory mediators through ferumoxytol enhanced MRI and immunostaining found aspirin-treated patients to have both decreased macrophage infiltration and COX-2 and mPGES-1 expression.^[52] These pro-inflammatory enzymes were found to be overexpressed in ruptured IA tissue. Taken together, these findings suggest that low doses of aspirin (81 mg daily for 3 months) may effectively attenuate inflammation in IA, preventing acute SAH.^[57]

Angiotensin 1-7 has also been explored as a potential therapeutic option as Ang 1-7 is an antagonist to Ang 2. Ang 2 has been shown to increase the expression of various pro-inflammatory cytokines as well as promote blood vessel extracellular matrix remodeling.^[59] Peña

Silva *et al.*^[60] explored the therapeutic effect of Ang 1-7 in aneurysm-induced mice and found that Ang 1-7 decreased the frequency of mortality and IA rupture. The authors believe that Ang 1-7 acts through a Mas receptor-dependent pathway as Ang 1-7 administration did not decrease the frequency of aneurysm rupture in Mas KO mice.^[60] To investigate the applicability of Ang 1-7 as a therapeutic option, immunostaining was performed on human cerebral aneurysms to confirm Mas receptor presence. Immunostaining for Mas receptors was found to be positive in unruptured and ruptured aneurysms. The expression of Mas receptors was also identified in the intima and media layers of control human cerebral arteries.^[60] These data suggest Angiotensin 1-7 mediated targeting of the Mas receptor pathway may be an efficacious noninvasive treatment modality.

Aoki *et al.*^[27] found that in aneurysm-induced rats, the activation of NF- κ B in the arterial wall of earlier stages of aneurysmal development corresponded with the expression of the downstream pro-inflammatory genes, vascular cell adhesion molecule-1 (VCAM-1) and MCP-1. The group explored the inhibitory effects of NF- κ B through the use of a synthesized decoy oligodeoxynucleotide (ODN) in a rat model. Investigators found that the facilitation of ODN 1-week following aneurysmal induction inhibited VCAM-1 and MCP-1 expression and overall, reduced aneurysm size and IEL disruption.^[27] In a follow-up study, the authors used chimeric decoy ODNs against both NF- κ B and proinflammatory transcription factor Ets-1.^[61] Aoki *et al.*^[61] found that chimeric decoy ODNs reduced IA size and thickened the walls of existing IAs in a rat model. Rats treated with chimeric decoy ODNs also showed a reduction in MCP-1 expression and macrophage infiltration into the aneurysm wall. If nuclease resistant ODNs can be administered transorally or transvenously, these findings suggest that NF- κ B and Ets-1 are both potential therapeutic targets in human IAs.^[61]

Additionally, Aoki *et al.*^[19] tested the effects of tolylsam, a selective inhibitor of the gelatinases MMP-2, MMP-9, and MMP-12, in a rat model. Facilitation of tolylsam did inhibit aneurysm progression, as the rate of advanced aneurysms in the tolylsam group was lower; however, the incidence of aneurysm development in the tolylsam group and control group was not different. The authors concluded that the tolylsam may delay aneurysm progression rather than formation.^[19]

Granulocytes were found to be present in the cerebral aneurysm wall.^[17] Specifically, Ishibashi *et al.*^[17] reaffirmed mast cells' role in aneurysm pathogenesis by administering in a rat model the mast cell inhibitor, tranilast (N-(3,4-dimethoxycinnamoyl) anthranilic acid; Kissei Pharmaceutical, Nagano, Japan) and emedastine

difumarate, (1-(2-ethoxyethyl)-2-(hexahydro-4-methyl-1H-1,4-diazepin-1-yl)-benzimidazole difumarate; Kowa, Tokyo, Japan). The facilitation of these inhibitors proved mast cells' contribution to IEL and media degeneration as both of these processes were suppressed.^[17] Ultimately, the facilitation of these inhibitors reduced the size of the induced aneurysms and the thinning of the vessel's media layer. Inhibitors of mast cell degranulation have shown to be useful in the treatment of other inflammatory processes such as allergies.^[17] As a result, the authors believe mast cell inhibitors may prove to be a practical and safe future anti-inflammatory treatment for IAs.^[17]

TNF- α -TNFR1 cascade inhibition is a strategy that has been recently explored by Aoki *et al.*^[25] Anti-TNF- α antibody or soluble TNF receptor has been successfully used in the treatment of an inflammatory disease, such as rheumatoid arthritis.^[25] The authors note that the use of these inhibitors in the treatment of aneurysms would be likely efficacious; however, the expense of these drugs makes an alternative inhibitor of the TNF- α -TNFR1 signaling cascade desirable.^[25] In addition, increased levels of TNF- α in unruptured and ruptured IAs in an *in vivo* model were reconfirmed by Starke *et al.*^[62] This follow-up study focused on the therapeutic strategy of the TNF- α -TNFR1 signaling cascade by analyzing TNF- α knockout mice and mice administered an inhibitor of TNF- α synthesis, 3,6'dithiothalidomide (DTH). Both groups showed a lower incidence of aneurysm formation and rupture when compared with control mice.^[62] The study also found that the inhibitor DTH led to an increase in aneurysm stabilization and consequently, a decrease in aneurysm rupture upon formation.^[62] Although DTH is an inhibitor of TNF- α synthesis, the authors noted that the inhibitor's actions are not fully understood as other properties of the inhibitor may explain aneurysm stabilization and prevention of rupture.^[62]

CONCLUSION

Intensive investigation has implicated the inflammation in the complex pathophysiological processes that underlie IA development, progression, and rupture. Ongoing research shows how these inflammatory mechanisms can be clinically accessed and therapeutically modulated. Although advances in microsurgical and endovascular management of IA will inevitably lead to lower procedural complication rates, the need for a safe and effective noninvasive therapeutic strategy to prevent aneurysmal SAH will remain. Overall, these data suggest potential alternative medical treatment strategies for patients with human cerebral aneurysms.

ACKNOWLEDGMENTS

We would like to thank Ms. Teresa Ruggle for her assistance with the preparation of the figure.

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Cite this article as: Dooley SA, Hudson JS, Hasan DM. Inflammation in human cerebral aneurysms: pathogenesis, diagnostic imaging, genetics, and therapeutics. *Neuroimmunol Neuroinflammation* 2015;2(2):77-85.

Source of Support: Nil. **Conflict of Interest:** No.

Received: 14-09-2014; **Accepted:** 30-09-2014

Inflammation mediates the pathogenesis of cerebral aneurysm and becomes therapeutic target

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ABSTRACT

The treatment of cerebral aneurysms (CAs) is of social importance, because poor outcomes result in subarachnoid hemorrhages after rupture. However, there is currently no medical treatment available to prevent the progression and rupture of CAs, which results in a large number of patients without receiving treatment. Recent studies using human samples have revealed the presence of inflammatory responses in lesions and also the possible correlation of inflammation with CA progression or rupture. Furthermore, experimental studies using animal models of CAs have supported the notion from human studies and have clarified the crucial contribution of inflammation to the pathogenesis. In this process, a vicious cycle/positive feedback loop includes the nuclear factor-kappa B (NF- κ B) activation, which plays a role in amplifying inflammatory responses to the point of chronicity. In addition, the infiltration of macrophages via NF- κ B-mediated monocyte chemoattractant protein 1 induction expands inflammation in whole arterial walls and contributes to the degeneration of media by producing various cytokines and tissue-destructive proteases. These series of studies have provided an important insight - antiinflammatory drugs can be therapeutically significant in the treatment of CAs. Indeed, in animal models, some drugs with an antiinflammatory effect effectively suppressed CA formation and progression, which supports this hypothesis. In addition, in human cases, some case-control studies have reported the preventive effect of statins and nonsteroidal antiinflammatory drugs on CA rupture. Therefore, the development of novel medical treatment for preventing the progression and rupture of CAs is needed in the near future. In this literature review, articles were selected by performing a PubMed search using the key words "cerebral aneurysm" and "inflammation".

Key words: Cerebral aneurysm, inflammation, macrophage, nuclear factor-kappa B

INTRODUCTION

Cerebral aneurysms (CAs) have a great impact on society because of their high incidence and subsequent subarachnoid hemorrhages after rupture.^[1,2] In recent times, a large cohort study in Japan reported that the annual rate of CA rupture was 0.95%, and the risk was increased according to the size of CA.^[3] Since subarachnoid hemorrhages have a high mortality rate of up to 50%, the prevention of CA rupture and enlargement are of considerable significance to society.^[4] Many CAs are detected through brain examinations before the rupture, so there is a chance for preventative treatment. Among these incidentally detected CAs, only

those with a higher probability of rupture (e.g. CAs with a large size or irregular shape) are selected for surgical treatment.^[1-3,5] Importantly, the remaining portion of CAs, more than half,^[3] receive no treatment, except for the treatment of risk factors related with rupture, and are only followed-up with monitoring rupture and enlargement. Considering that there is a high incidence of CAs in the general population and poor outcomes resulting from subarachnoid hemorrhages despite intensive treatment, the development of a new drug therapy for unruptured CAs is indispensable. Therefore, the mechanisms underlying the formation and progression of CAs need to be clarified.

INFLAMMATION AND CEREBRAL ANEURYSM FORMATION, PROGRESSION AND RUPTURE

Over the past couple of decades, a large number of studies have examined the underlying mechanism of CA formation, progression and rupture by investigating human CA specimens. These series of studies have identified the presence of the inflammatory

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10.4103/2347-8659.154381

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responses in CA lesions and have suggested the role of the inflammatory processes in the pathogenesis of CAs. For example, the expression and induction of pro-inflammatory factors such as tumor necrosis factor alpha (TNF- α), infiltration of inflammatory cells in the CA lesions (mainly macrophages), and the change in cell population during CA progression or rupture were identified.^[6-13] Furthermore, comprehensive gene expression analyses have revealed the induction of pro-inflammatory genes in lesions such as TNF- α and the up-regulation of the inflammation-related pathways through bioinformatics analyses such as antigen processing, immune responses, and responses to outward stimuli, which indicated a significant contribution to the pathogenesis of CAs.^[14-16] Comprehensive gene expression analyses have also identified an increase in extracellular matrix turnover.^[17,18] By linkage analyses, pro-inflammatory genes or extracellular matrix-related genes positively correlate with CAs.^[19-22] However, a considerable limitation is present in studies using human samples because of the variety of background characteristics such as the genetics and clinical history. Furthermore, we cannot examine the exact association of each inflammation-related factor with CA progression through pharmacological inhibition or genetic modification. However, the establishment and use of animal CA models^[23-27] has overcome these intrinsic limitations associated with human samples and has greatly advanced our understanding of the mechanisms that regulate CA formation, progression, and rupture.

In a rodent model, CAs are induced at the bifurcation sites of intracranial arteries through an increase in hemodynamic stress, which is also a trigger of CA formation in human,^[28-30] and is achieved by performing one-sided carotid ligation and inducing systemic hypertension through salt over-loading.^[23-25] Because CAs induced in models share common pathological features with human cases (e.g. disrupted internal elastic lamina and degenerative changes of the media, including the loss of medial smooth muscle cells) and also spontaneous rupture, they highly mimic human CAs and are presumably suitable for examining the mechanisms underlying CA formation and progression.^[23,24] In some models, elastase is injected into the basal cistern to degenerate internal elastic lamina in intracranial arteries and to facilitate CA formation and progression in combination with induced systemic hypertension by angiotensin II infusion.^[26,27] In this model, induced CAs in mice can rupture at a higher rate than that in former models; therefore, they can be used to examine the mechanisms regulating CA rupture.^[27]

Recent experimental studies mainly using animal models of CAs^[23-26] have clarified the involvement of

inflammatory responses in the pathogenesis of CA formation and progression^[22,31-41] and have supported the notion that inflammation in arterial walls contributes to the pathogenesis in human cases. Nuclear factor-kappa B (NF- κ B) is a master transcription factor regulating the induction of various pro-inflammatory genes through the activation of responses to nociceptive stimuli.^[42] Experimental studies have revealed the crucial role of NF- κ B in the pathogenesis of CAs by triggering and regulating the inflammatory processes in lesions^[43] [Figure 1]. During CA formation, many NF- κ B-activated cytokines/mediators such as interleukin (IL)-1 β ,^[38] prostaglandinE₂,^[34] TNF- α ,^[37,40,41] and reactive oxygen species^[44] are induced, and they significantly contribute to CA formation and progression. Furthermore, NF- κ B-targeted pro-inflammatory genes, including matrix metalloproteinase-9,^[26,32] cyclooxygenase-2 (COX-2),^[34] inducible nitric oxide synthase,^[36,39] monocyte chemoattractant protein-1 (MCP-1),^[31,35] TNF- α ,^[37,40,41] and IL-1 β ^[38] are induced and likewise contribute to CA formation and progression. In addition, the critical contribution of NF- κ B to the pathogenesis of CA is demonstrated in the deficiency of the NF- κ B p50 subunit in mouse or the inhibition of the NF- κ B transcriptional activity by decoy oligonucleotides treatment in the rat, which both significantly suppress CA formation and progression by inhibiting NF- κ B-mediated inflammatory responses in lesions.^[33] Importantly, vicious cycles are formed around NF- κ B activation in lesions; for example, TNF- α activates NF- κ B and then NF- κ B transcriptionally induces TNF- α . Similarly, NF- κ B forms the positive feedback loop of the COX-2-prostaglandinE₂-EP2-NF- κ B pathway^[34,45] [Figure 1], and COX-2 is induced by hemodynamic stress loaded on the arterial walls at bifurcation sites of the intracranial arteries producing prostaglandinE₂.^[34,45] Then synthesized prostaglandinE₂ acts on one of its specific receptor subtypes, EP2, and further activates NF- κ B. Because NF- κ B transcriptionally induces the COX-2 expression, once hemodynamic stress activates COX-2, another positive feedback loop between the prostaglandin system and NF- κ B is formed^[34,45] [Figure 1]. The presence of this vicious cycle and positive feedback loops amplifies and prolongs the triggered inflammatory responses. Along with the amplified inflammation, the infiltration of macrophages and other major inflammatory cells in the CA walls, which are recruited via the NF- κ B-induced MCP-1 expression, contributes to the further expansion of inflammation in the whole arterial walls^[31,35,43] [Figure 1]. MCP-1 is first induced in the endothelial cells during CA formation and recruits macrophages in the arterial walls.^[31] Then recruited macrophages produce various cytokines and tissue-destructive proteinases such as MMP-9 that contribute to the expansion of inflammation and

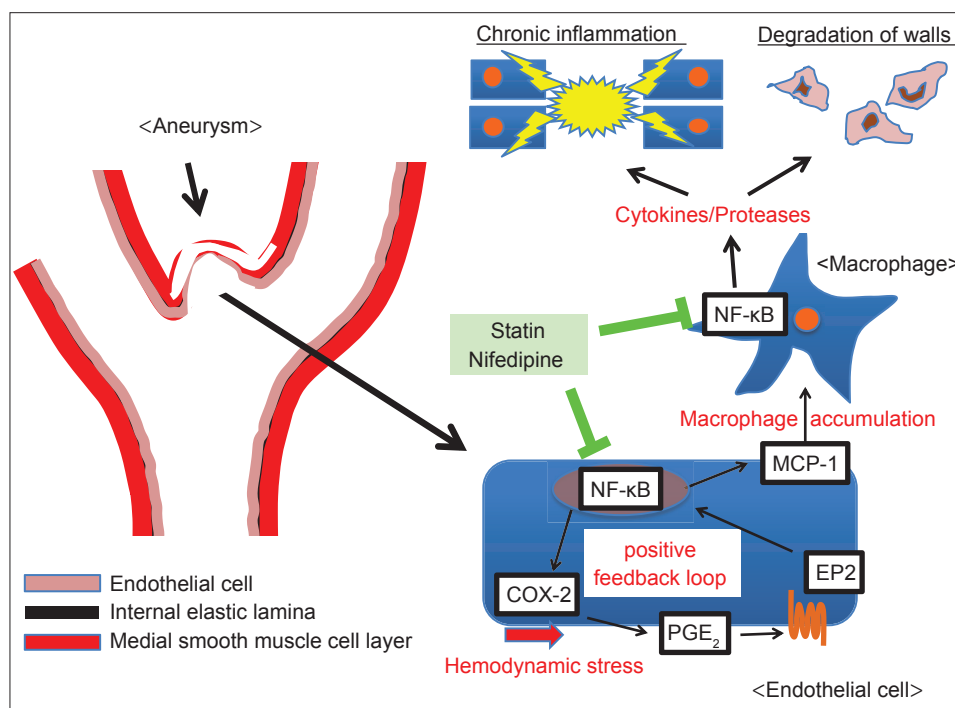


Figure 1: Schematic drawing of the mechanisms that regulate aneurysmal formation and progression. PGE₂: prostaglandin E₂; COX-2: cyclooxygenase-2; MCP-1: monocyte chemoattractant protein-1; NF-κB: nuclear factor-kappa B

tissue degeneration observed in CAs^[31,35] [Figure 1]. The critical contribution of MCP-1 mediated macrophages recruitment/infiltration in the pathogenesis is clearly shown by recent experimental reports in which the deficiency of MCP-1, administration of the dominant negative form of MCP-1 (7-ND), or depletion of macrophages by clodronate liposome all significantly suppressed CA formation and progression.^[31,35]

The remaining question to be solved is whether the processes are regulating the initiation and progression of CAs are different. This important issue remains to be elucidated. As an initiation, as well as the progression of CAs, can be suppressed by inhibiting the inflammatory processes in lesions, these two steps of the pathogenesis presumably share the same underlying mechanisms in terms of inflammation. However, because the hemodynamic status surrounding CA lesions is completely different (e.g. a high hemodynamic status at the prospective site of the initiation^[46,47] but a low hemodynamic status in the dome of the enlarging CAs^[48,49]), there must be some differences in the processes that regulate the initiation and progression of CAs, and this is worthy of investigation.

In summary, based on the recent studies on CAs, long-lasting inflammatory responses in arterial walls play a crucial role in CA formation and progression, and NF-κB mediates this inflammation as a major transcription factor that regulates inflammation. In addition, the presence of a vicious cycle/positive feedback loop (i.e. NF-κB activation

and macrophage infiltration via the NF-κB-induced MCP-1 expression) seems to be two major mechanisms that contribute to the amplification, expansion, and chronicity of inflammatory responses.

This recent experimental evidence on the role of inflammation in CAs may be useful in developing of therapeutic drugs for CA treatment. Recent experimental studies in human and rodent models have greatly advanced our understanding of the pathogenesis of CAs, making it more likely that the current treatment of CAs will be improved.

POTENTIAL OF ANTIINFLAMMATORY DRUGS FOR TREATING CEREBRAL ANEURYSMS IN ANIMAL MODELS

As discussed, a long-lasting inflammatory response is detected in CA lesions, which plays a crucial role in the pathogenesis of CAs. Recent findings in rodent models have amassed evidence indicating the therapeutic effect of antiinflammatory drugs on the further enlargement or rupture of CAs and have proposed the potential of these drugs for treating CA.^[31,33-36,50-58] Among these drugs that have a suppressive effect on CAs in animal models, statin,^[50,51,55] nifedipine,^[52] and emedastine difumarate^[53] are already used in humans with clinical indications. Therefore, these drugs are good candidates for treating CAs in humans to prevent rupture or enlargement. We summarize the effect of these drugs on CAs in animal models.

Statin, a hydroxyl-3-methylglutaryl coenzyme A reductase inhibitor, is widely used as a cholesterol-lowering drug. In addition, it has an antiinflammatory and an anti-NF- κ B effect, which are well known as “pleiotropic effects of statins”, although the precise mechanisms underlying its antiinflammatory effect remain to be elucidated.^[59-61] Different kinds of statins orally administered to rat all effectively suppress inflammatory responses in CA lesions, which is evident by the activation of NF- κ B and the expression of pro-inflammatory factors.^[50,51] Furthermore, simvastatin orally given to rats exerts a protective effect on the endothelial cells in CA lesions.^[51] Thereby, statins significantly suppress CA formation and enlargement of preexisting CAs in the rat model through their pleiotropic antiinflammatory effect.^[50,51,55] Statins are prescribed to many patients with hypercholesterolemia, and since its safety is well established, statins may be promising drugs for treating patients with CAs and hypercholesterolemia [Figure 1].

Nifedipine is a Ca^{2+} channel blocker and a widely used antihypertensive drug. In addition to its antihypertensive effect, recently published *in vitro* experiments demonstrated its suppressive effect on the activation/induction of pro-inflammatory factors such as NF- κ B and MCP-1 in cultured cells.^[62-64] Consistently, nifedipine subcutaneously injected into rat with CAs inhibits the activation of NF- κ B and suppresses the infiltration of macrophages via the NF- κ B-mediated MCP-1 induction.^[52] The CA formation and the enlargement of preexisting CAs are inhibited due to the reduction of the inflammatory response.^[52] Since hypertension is a major risk factor for rupture of preexisting CAs, nifedipine may be a promising drug candidate for preventing CA rupture through its synergistic preventive effects on hypertension and inflammation [Figure 1]. Similarly, imidapril, a widely used angiotensin-converting enzyme inhibitor, exerts the potent suppressive effect on CAs induced in rat models through its anti-MMP-9 effect, which is independent of its antihypertensive effect.^[54] Therefore, imidapril may also be a good drug candidate for treating CAs.

The mast cell is a major cell type that regulates allergic inflammation through the release of histamines from its granules.^[65] Since it is a tissue-dwelling cell and contains a variety of cytokines and proteinases in its granules, it can rapidly respond to outward inflammatory stimuli and trigger inflammation.^[65] Indeed, in inflammation-related diseases, such as vascular disease, atherosclerosis and aortic aneurysm, mast cells contribute to their pathogenesis by triggering and regulating inflammation through the release of cytokines and proteinases such as IL-6 and

chymase.^[66-70] In human CAs, the presence of mast cells has been identified,^[11] and the increasing number of infiltrated mast cells in ruptured CAs indicates this cell's role in the pathogenesis of CAs.^[11] The significant contribution of mast cells to CAs was shown by a recent study^[53] in which there was an increase in mast cells during CA formation and progression. Further, the pharmacological inhibition of the degranulation in mast cells by emedastine difumarate or tranilast, which are widely used antiallergy drugs in humans, effectively suppresses CA formation and progression by inhibiting the inflammatory responses of lesions.^[53] These findings suggest the potential of the degranulation inhibitor of mast cells as drugs for treating CAs in humans.

POTENTIAL OF ANTIINFLAMMATORY DRUGS FOR TREATING CEREBRAL ANEURYSMS IN HUMANS

In human cases, the beneficial effect of statins and nonsteroidal antiinflammatory drugs (NSAIDs) on CA rupture has been demonstrated, especially through prospective intervention trials.

A recent hospital-based case-control study implicated the potential of statins for preventing CA rupture in humans.^[71] They enrolled 117 cases (patients with subarachnoid hemorrhage due to CA rupture) and 304 controls (patients with unruptured CAs) from 15 institutions in Japan, and the use of statins in each group was statistically compared. The patients' background characteristics including age were similar.^[71] As expected from previous reports, the size of the CAs and the patients' current smoking status were properly selected as factors that correlated with rupture;^[3,5,72,73] therefore, this study's results seemed reliable. Statins were used in 9.4% of cases (11/117 cases) and 26.0% of the controls (79/304 controls), and the ratio of statins used between the groups was statistically different ($P < 0.001$).^[71] Furthermore, after stratifying the data by the serum cholesterol level, administration of a statin was still inversely correlated with the risk of CA rupture in patients with serum cholesterol levels > 130 mg/dL.^[71] According to logistic regression analysis, the use of any statins, independent of the type of statins, was inversely correlated with CA rupture with an adjusted odds ratio of 0.30.^[71] Therefore, statins can be promising drugs for preventing CA rupture in humans with hypercholesterolemia. However, statins have a potent cholesterol-lowering effect and can sometimes decrease the serum cholesterol level below the normal limit even in patients without hypercholesterolemia. Although hypercholesterolemia was not a risk factor for CAs, and about 74% patients were without hypercholesterolemia in this study, the safety of statins on patients without hypercholesterolemia should be

considered, and further randomized placebo-controlled study is warranted.

Nonsteroidal antiinflammatory drugs are broad COX inhibitors that involute symptoms of acute inflammation such as fever, swelling *etc.* A recent experimental study revealed the involvement of COX-2, the inducible form of COX, in the pathway of CA formation and progression by triggering and maintaining inflammation in lesions, suggesting the therapeutic effect of NSAIDs on CA rupture and progression. Conversely, NSAIDs inhibit the production of thromboxane A₂ (a prostaglandin formed by the sequential actions of COX and thromboxane synthase from arachidonic acid) and thereby exert an antiplatelet effect, creating the potential for an increase in CA rupture and exacerbation of a subarachnoid hemorrhage after rupture. Consistent with these conflicting findings on NSAID treatment, results from recently published case-control studies were controversial in terms of the preventive effect of NSAIDs (i.e. the anti-inflammatory and antiplatelet effects) on CA rupture.^[74-76] In a nested case-control study that enrolled patients from the International Study of Unruptured Intracranial Aneurysms (58 cases and 213 controls), frequent aspirin usage, 3 times/week, suppressed CA rupture with an adjusted odds ratio of 0.27 ($P = 0.03$) according to multivariable risk factor analyses.^[74] Careful attention is necessary to interpret the data, because enrolled patients had relatively large aneurysms located at the posterior circulation, which are not representative of unruptured CAs.^[74] Another study also demonstrated the suppressive effect of aspirin on CA rupture.^[75] This study enrolled 717 consecutive patients with CAs (30 patients were excluded due to clopidogrel and/or warfarin use) and 897 CAs. During the follow-up, 274 patients presented with aneurysmal subarachnoid hemorrhage. The rate of CA rupture (subarachnoid hemorrhage) was significantly different between the groups ($P = 0.016$) with 40% of patients not using aspirin and 28% using aspirin,^[75] suggesting the preventive effect of aspirin on CA rupture. Notably, aspirin did not influence the overall outcome.^[75] Conversely, a recently published large nested case-control study enrolled 2,065 patients with subarachnoid hemorrhages and 20,649 controls, and it showed a significant increase in the risk of subarachnoid hemorrhage with an odd ratio of 1.5.^[76] In addition, since NSAIDs have a considerable side effect (i.e. gastrointestinal hemorrhage), especially in elder patients, the administration of NSAIDs should be accompanied continuous attention and careful follow-up. However, the selective COX-2 inhibitor can eliminate the side effect derived from the nonselective inhibition of endogenous prostaglandin synthesis via COX-1 activity that is seen in NSAID treatment; therefore, the selective COX-2 inhibitor is another

and more promising drug candidate for treating CAs. Indeed, in animal models, celecoxib, a selective COX-2 inhibitor, effectively suppressed CA progression.^[34,45] However, a recent clinical trial for colon cancer reported an increase of cardiac failure and myocardial infarction with selective COX-2 inhibitor use^[77] presumably due to the impairing balance between thromboxane A₂ and prostacyclin, suggesting that COX-2 inhibitors may not be therapeutic drugs for preventing CA rupture. As previously discussed, currently published case-control studies have shown the controversy over the effect of NSAIDs on CA rupture. Although NSAIDs can be drug candidates for preventing the rupture of preexisting CAs, future randomized-control studies are warranted.

CONCLUSION

Cerebral aneurysms are of social importance, because of the resultant subarachnoid hemorrhage after rupture. The current problem with treating CAs is the lack of medical treatment to prevent their enlargement or rupture. Recent studies on human samples and experimental models have revealed the crucial role that chronic inflammatory responses play in the pathogenesis of CAs. Some drug candidates for treating CAs have been identified through experimental and case-control studies in humans. Therefore, the development of medical treatment for CAs is more likely in the near future.

ACKNOWLEDGMENTS

I would like to express my gratitude to all the researchers, collaborators, technical assistants and secretaries contributing to our studies cited in the present manuscript. I also express my sincere gratitude to grants supporting our research works.

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Cite this article as: Aoki T. Inflammation mediates the pathogenesis of cerebral aneurysm and becomes therapeutic target. *Neuroimmunol Neuroinflammation* 2015;2(2):86-92.

Source of Support: Nil. **Conflict of Interest:** No.

Received: 26-06-2014; **Accepted:** 01-09-2014

Role of the complement cascade in cerebral aneurysm formation, growth, and rupture

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ABSTRACT

Rupture of intracranial aneurysms is the most common cause of nontraumatic subarachnoid hemorrhage, but the intricate neuroinflammatory processes which contribute to aneurysm pathophysiology are not well-understood. Mounting evidence has implicated the complement cascade in the progression of aneurysms from their formation to rupture. In this article, we identify and review studies that have sought to determine the role of the complement system in the aneurysm pathogenesis. The studies were generally conducted by immunohistological analyses on aneurysm tissue collected intraoperatively, and multiple components of the complement cascade and its modulators were identified in specific regions of the aneurysm wall. The results of the studies suggest that the complement cascade is locally upregulated and disinhibited in the perianeurysmal environment, and that it contributes to chronic as well as acute immunological damage to the aneurysm wall. In the future, understanding the mechanisms at work in complement-mediated damage is necessary to leading the development of novel therapies.

Key words: Aneurysm, complement, neuroinflammation, rupture, subarachnoid hemorrhage

INTRODUCTION

Saccular or “berry” aneurysms, which are characterized by an outpouching from one side of an affected artery, have a prevalence of 3.2% in the general population^[1,2] and account for 90% of intracranial aneurysms.^[3,4] Ruptured saccular aneurysms are responsible for 85% of cases of nontraumatic subarachnoid hemorrhage (SAH),^[5] which carries a high case-fatality rate of 27-44%^[6] and often leaves survivors with significant functional and cognitive deficits.^[2,7] Clinically, aneurysms have mainly been characterized by their location and morphological features (size, shape, *etc.*),^[8] but less attention has been paid to the underlying immune processes, which contribute to their formation, growth, or rupture. Understanding these mechanisms is important to facilitate the development

of novel diagnostic and therapeutic strategies, and histopathological findings may contribute to conventional clinical and radiological factors.^[9,10] Recently, there has been growing evidence that the complement cascade, a major effector arm of the innate immune system,^[11] plays a role in the pathophysiology of intracranial aneurysms, and more broadly, in cerebrovascular conditions. In this article, we review the complement system as it relates to the pathogenesis of intracranial saccular aneurysms. A thorough review of the literature was conducted on PubMed, MEDLINE, EMBASE, and Cochrane library databases using the search terms: “complement”, “aneurysm”, “SAH”, “hemorrhagic stroke”, “neuroinflammation”, and “saccular” in varying combinations. Only original research articles that, at least in part, investigated the role of the complement cascade in intracranial aneurysms were selected.

HISTOPATHOLOGICAL MECHANISMS OF SACCULAR ANEURYSM FORMATION, GROWTH, AND RUPTURE

The progression of saccular aneurysms from formation to rupture involves a complex interplay of hemodynamic

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10.4103/2347-8659.154888

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forces and changes in aneurysm morphology (luminal factors), vessel wall integrity (extra-luminal factors), immunological pathways, and patient-specific factors.^[8,12-14] Clinical management and risk stratification has generally relied on luminal characteristics (i.e. size, location), and patient-specific risk factors, mainly smoking, hypertension, heavy alcohol consumption, positive family history,^[15-18] but this does not differentiate among aneurysms that have similar morphological features yet heterogeneous natural histories and pathological features.

Strong hemodynamic forces are thought to play a role in initiating aneurysm formation as a result of high shear stress on vessel walls, which is greatest at the bifurcations of cerebral arteries, where aneurysms occur most often.^[19] Shear stress has been shown to lead to loss and damage of endothelial cells, loss of the internal elastic lamina (IEL), migration of vascular smooth muscle cells (SMCs), and induction of intracellular pathways in endothelial cells that induce pro-inflammatory cytokine release.^[8,20-24]

Endothelial injury is considered to be a necessary first step in the aneurysm pathogenesis.^[25,26] In response to increased wall stress, endothelial cells undergo not only morphological, but also functional changes, in which they upregulate production of pro-inflammatory signals (cytokines, interleukins such as interleukin-1 β [IL-1 β], leukocyte chemoattractants) as well as matrix metalloproteinases (MMPs),^[27] resulting in recruitment of inflammatory cells and MMP-mediated enzymatic remodeling of the vessel wall. In addition, disruption of the IEL, which may initially be in the form of shear stress-induced tears, is a characteristic histopathological feature of saccular aneurysms.^[8,24] As the IEL is damaged, the hemodynamic environment becomes turbulent within the lumen of the nascent aneurysm, leading to further endothelial damage and eventually de-endothelialization within the luminal surface of the aneurysm.^[22,28] This hemodynamic environment, in concert with the exposed subendothelial matrix, promotes formation of intraluminal thrombi which consist of layers of platelets, red blood cells, lipid-laden macrophages, and other leukocytes leading to increased oxidative stress, inflammation, and further cell death.^[14,29-30] The subsequent inflammatory response within the damaged vessel wall consists of leukocytic infiltration with macrophages, T-cells, and mast cells, and is found in both ruptured and unruptured aneurysms.^[8,15,21,31-33] This inflammatory response results in progressive disorganization and loss of SMC and extracellular matrix (ECM) from the media and open and stretched collagen in the adventitia.^[8]

Hemodynamic and environmental factors both contribute to phenotypic modulation and eventually damage of and death to SMCs.^[25] Initially, SMCs adapt to hemodynamic stress by migrating from the tunica media into the intima to form myointimal hyperplasia. However, in response to local signals, they also become a secretory, pro-inflammatory cell type characterized by upregulation of necrosis factor- κ B signaling, increased production of MMP, IL-1 β , tumor necrosis factor- α , and initiation of pathways leading to SMC apoptosis.^[34-40] As SMCs are largely responsible for the production of the ECM,^[41] their apoptosis results in decreased synthesis of the new matrix, which exacerbates the effects of the overabundant MMPs and further weakens the vessel wall. MMPs are also produced by macrophages,^[42,43] which play a large role in neuroinflammation, and in intracranial aneurysms there is downregulation of tissue inhibitors of MMPs^[42,44] and up-regulation of proteolytic cathepsins.^[45]

In effect, the key processes believed to be responsible for weakening of the vessel wall, and potential subsequent rupture, include enzymatic degradation of the ECM, progressive loss of SMCs, and also the decreased synthesis of new collagen fibers.^[34] The result is a chronic state of remodeling and inflammation that ultimately results in critical weakening of the vessel wall that is progressively less capable of withstanding hemodynamic stress, thus leading to growth and potentially rupture of the aneurysm.^[25] Inflammation causing enzymatic remodeling of the vascular ECM is a shared pathological mechanism also seen in several other vascular diseases including atherosclerosis, abdominal aortic aneurysms, and arteritides.^[46-48] Atherosclerosis is itself a common feature in saccular intracranial aneurysms and as in abdominal aortic aneurysms, is associated with aneurysmal progression and likelihood of rupture.^[25] The contribution of atherosclerosis and patient risk factors, including hypertension and smoking are similar to its contribution to abdominal aortic aneurysms.^[46]

Although the acute mechanism of aneurysm rupture has yet to be fully elucidated, there are several key factors that play a role. Clinically, a major predictor of rupture is increased diameter > 7 mm, above which the 5-year risk of rupture increases from 2.6% for 12 mm to 40% for 25 mm.^[49] In addition, using patient-specific computation flow dynamics analyses, it has been shown that small size of the impingement region within the aneurysm, unsteady flow dynamics, and concentrated inflow jet are all associated with rupture of the aneurysm.^[14,50,51] However, repeated studies have shown that a higher degree of inflammatory infiltration is associated with aneurysm wall degradation and subsequent higher risk of rupture.^[13,31,52,53] Owing to

the highly effective and quickly-amplified nature of the activated complement cascade, the acute pathogenesis of aneurysm rupture is of particular interest. Understanding the role of complement in this mechanism as well as the chronic processes responsible for aneurysm development is invaluable for future clinical endeavors.

FUNCTION OF THE COMPLEMENT SYSTEM

The complement system, a network of approximately 30 plasma and membrane-associated proteins, is a major mediator of innate immunity, functioning in cell lysis (e.g. lysis of microbes, virus-infected cells, tumor cells), inflammation, cell signaling, chemotaxis, opsonization, and vascular effects.^[54-57] In addition, complement facilitates the adaptive immune response by functioning in antigen presentation, immunologic memory, and costimulation of B-cells via antigen receptors. The presentation of “nonself” or damaged cells leads to a cascade of events that result in the destruction of the microbes or targeted cells and subsequent inflammation. The cascade is catalyzed by complement components (many of which are proteases) that circulate in inactive forms (zymogens) until they are activated by several mechanisms.^[10] Excessive complement activation, however, damages healthy tissue, and is implicated in a variety of central nervous system conditions (SAH, intracerebral hemorrhage, ischemic stroke, ischemia-reperfusion injury, and multiple sclerosis^[58-60]) as well as myocardial infarctions and asthma.^[61-64] In SAH in particular, complement activation has been associated with poorer functional outcomes and even vasospasm.^[60,65-67]

Dysregulation of any of the above processes, deficiencies in the complement proteins, and activation by various molecules can lead to a pathological over-or under-activation of the complement system. These complement disorders [Table 1] include paroxysmal nocturnal hemoglobinuria (PNH), hereditary angioedema, and atypical hemolytic uremic syndrome.

In PNH, for example, decreased expression of the complement regulators CD55 and CD59 allows for complement-mediated lysis of red blood cells in a predominantly intravascular hemolysis.^[10,68] Anticomplement therapy already exists to treat many of these conditions that directly result from complement dysregulation. These include a complement component-1 (C1)-inhibitor concentrate (which inactivates C1r and C1s and mannose-binding lectin (MBL)-associated protein 2 (MASP 2) and is approved to treat hereditary angioedema^[69,70]), as well as eculizumab (a monoclonal antibody approved for PNH and atypical hemolytic uremic syndrome^[71]). Anticomplement therapy may also attenuate the damage from ischemia-reperfusion injury.^[72-74]

CLASSICAL, LECTIN, AND ALTERNATIVE PATHWAYS

There are three recognized pathways of complement system activation: the classical, lectin, and alternative pathways [Figure 1]. The common point of each pathway is the formation of a C3 convertase, which activates C3 by cleaving it into C3b and C3a.^[75] C3 activation serves as a nidus for amplification of the complement response. All three pathways eventually form C5 convertases that cleave C5 into C5a and C5b, after which the C5b fragment initiates assembly of C6, C7, C8, and C9 into the membrane attack complex (MAC; also known as the terminal complement cascade, or C5b-9) which lyses the cell by forming a pore in the lipid bilayer.^[57]

The classical pathway is primarily activated by antigen-antibody complexes. After binding to an antigen, the Fc region of the antibody (typically IgM or IgG) undergoes a conformational change that allows it to bind to the C1q subunit of C1, a multimer that also contains C1r and C1s subunits. The C1s subunit then cleaves C4 and C2, and then two of the products, C4b and C2a, associate to form the C3-convertase, C4bC2a. C4bC2a also serves as the C3 convertase in

Table 1: Complement pathway disorders

Classic pathway	Membrane attack complex	Alternative pathway	Control proteins	Others
C1q deficiency C1r/C1s deficiency	C5-9 deficiency	Factor B deficiency Factor D deficiency	Factor I deficiency Factor H deficiency	Serosal protease deficiency Mannose binding lectin deficiency
C4 deficiency C2 deficiency C3 deficiency Scleroderma Immunoglobulin A nephropathy Henoch-Schonlein purpura Membranous nephropathy Systemic lupus erythematosus			C4 binding protein deficiency C1 inhibitor protein deficiency Complement receptor 1-3 deficiency Paroxysmal nocturnal hemoglobinuria Leukocyte adhesion deficiency syndrome Hereditary angioedema Age-related macular degeneration	

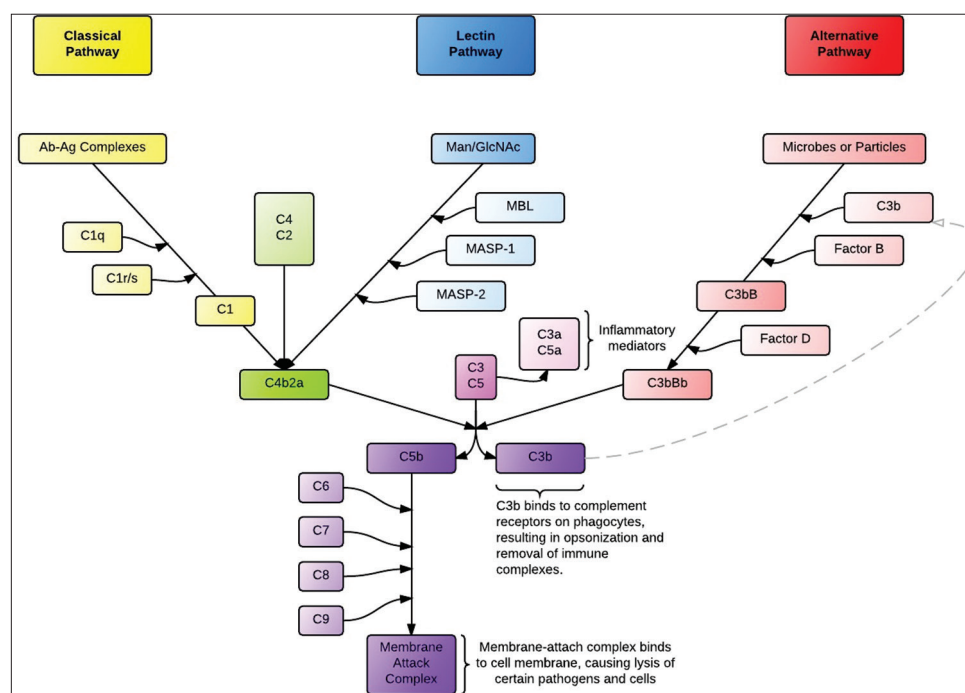


Figure 1: The three pathways of the complement cascade

the lectin (mannose-binding) pathway, a complement pathway triggered when MBL or Ficolin bind to terminal mannose groups on the surfaces of microbes. This binding allows MBL to associate with circulating MASPs, which then cleave C4 and C2 to form the C4bC2a C3 convertase.^[76,77] In both the classical and lectin pathways, C3b may then bind to C4b2a to form the C5 convertase, C4b2a3b. This is different from the C5 convertase in the alternative pathway, where C2 and C4 do not play a major role.

The alternative pathway is triggered by carbohydrates or proteins found on self and nonself surfaces.^[10] In the plasma, C3 is spontaneously hydrolyzed (but not cleaved) at a low rate to form iC3, which is amplified in the environment near pathogens due to these carbohydrates or proteins. iC3 binds to Factor B, which Factor D then cleaves into to Bb and Ba, producing the fluid-phase C3 convertase (iC3Bb). The fluid-phase C3 convertase cleaves C3 to C3a and C3b, creating sufficient C3b to bind with Bb and form C3bBb, which, when stabilized by Properdin on a microbial surface, acts as the principal C3 convertase of the alternative complement pathway. The “alternative” C3 convertase C3bBb may then associate with an additional C3b to form the C5 convertase, C3bBbC3b.^[75] The C5 convertases from any of the three pathways may then facilitate the assembly of the MAC. In addition, several of the cleavage byproducts, chiefly, C3a and C5a, known as anaphylatoxins, initiate a cascade of signals which induces leukocyte chemotaxis,^[57] the effects of which include degranulation and resulting smooth muscle contraction and increased vascular permeability.^[55,56] Cleavage products of C3 also cause

a parallel pathway of direct cerebral injury via iron toxicity.^[78,79]

A link exists between the complement and coagulation cascades, as kallikrein and thrombin may also cleave C3 and C5.^[80,81] In addition, C-reactive protein (CRP) may either activate complement by recruiting C1q to the surface of damaged cells or regulate the cascade by recruiting complement inhibitors.^[82,83] Cholesterol-containing lipids and enzymatically-modified or oxidized LDLs in atherosclerosis can activate complement via C1,^[84-87] and these also play a role in the pathogenesis of coronary atherosclerotic plaques.^[88]

MODULATORS OF THE COMPLEMENT SYSTEM

The complement system is highly regulated by activators and inhibitors so as to confine the destructive mechanisms of the complement system to nonhost surfaces while protecting the healthy tissue, and alteration of these modulators plays a role in a variety of disease processes.^[57] As noted the alternative pathway C3 convertase (C3bBb) is stabilized on the microbial cell surface by Properdin,^[89] which is released by a variety of leukocytes,^[90-92] some of which, as mentioned, are present in aneurysm walls. In addition to microbial surfaces, Properdin can also bind to apoptotic and necrotic cells and facilitate a complement response.^[89] Regulators of complement activation inhibit the complement system at two predominant steps: either at the level of convertases, or in the assembly of the MAC.^[57,93] Factor I acts at the convertase level by cleaving C3b into its inactive form, iC3b, which is unable to form an active C3 convertase

with Bb. This process achieves specificity to protecting host cells from complement activation because it requires the host membrane-bound cofactors including complement receptor-1 (CR-1) and membrane-cofactor protein (MCP).^[57,75,94-96] CR-1 in particular is a potent inhibitor of the classical and alternative pathways, and also plays a role in clearing immune complexes and antigen presentation to B-cells.^[10,57] Other inhibitors at the convertase level include decay accelerating factor (DAF), which inhibits assembly of C3 convertases, and Factor H, which serves as a cofactor for factor I and DAF. Inhibitors of assembly of the MAC include CD59, vitronectin, and S-protein.^[10,95,97]

ROLE OF COMPLEMENT IN ANEURYSMS

There has been a growing interest in the role that complement plays in the pathogenesis of cerebral aneurysms, which has paralleled similar work done with aortic aneurysms.^[47,98,99] Much of the esteemed work has been done by the Neurosurgery Research Group at the Helsinki University Central Hospital, Helsinki, Finland, and has taken the research model of previous studies which have examined ruptured versus unruptured aneurysms.^[32] The studies have largely been done by standard immunohistological localization of complement in aneurysm tissue samples taken intraoperatively immediately after aneurysm clipping.

The first study to demonstrate complement deposition in aneurysm walls was by Chyatte *et al.*,^[48] who compared 25 aneurysm samples taken during microsurgical repair to 11 control samples from basilar arteries taken at autopsy. Compared to the basilar samples, they found significantly more deposition of immunoreactive C9 and C3c (a breakdown product of C3), and both C3c and C9 were deposited throughout the aneurysm wall, often diffusely. They also found increased presence of immunoglobulins (IgG and IgM) and leukocytes (CD68 macrophages and T-lymphocytes). Due to the deposition of earlier (C3c) as well as terminal complement products (C9), the authors concluded that complement activation, in concert with other inflammatory mediators, played a role in the pathogenesis of aneurysms.

In a 2006 study, Tulamo *et al.*^[100] investigated the role of the MAC in aneurysm rupture by comparing samples from 26 unruptured to 32 ruptured samples. Using a monoclonal mouse antibody to stain the C5b-9 complex (MAC), the authors found that the immunostaining for the MAC was approximately twice as dense in ruptured versus unruptured samples (median: 39% vs. 20%, $P < 0.001$, respectively). A greater concentration of MAC was also significantly associated with structural pathology of the aneurysmal wall

including wall degeneration, de-endothelialization, and degenerative change of the outer wall, as well as infiltrates of CD163+ macrophages and T-lymphocytes. Together, these results suggested a role for activated complement in saccular aneurysm degeneration and rupture.

Using a similar experimental model as their first study, Tulamo *et al.*^[84] then sought to determine, which complement pathway was involved in aneurysm rupture by staining for complement components specific to the alternative and classical pathways, as well as their potential activators. It was found that components of the classical pathway, C1q and C4b/iC4b, as well as the MAC and C3b/iC3b and C3d, were present in significantly greater concentrations and were more widely distributed in ruptured versus unruptured aneurysms, and specifically the staining tended to localize along the ECM in a band-like pattern in the outer aneurysm wall. In a smaller, separate sample of unruptured aneurysms, the authors reported heavy immunostaining for CRP, MAC, oxidized LDL, and IgG, which was increased in concentration from the lumen-to-adventitia direction. Of note, however, although the authors report that tissue from human tonsils was used as a positive control (presumably due to the dense immune elements), they did not have separate, nonaneurysmal tissue that served as a negative control. Tulamo *et al.*^[84] concluded that these findings most likely represented activation of the classical pathway, due to the presence of its potential activators (including IgG, oxidized LDL, and CRP) and C1q deposition, which is specific to the classical pathway. The alternative pathway was less likely to play a role because there was little staining of the specific marker, Properdin. They suggested these immunoglobulins and complement components leak out and accumulated in the aneurysmal wall due to endothelial dysfunction and impaired clearance mechanisms.

A third similar study by Tulamo *et al.*^[101] found that deposition of the MAC was greater in the outer wall than in the lumen of ruptured compared with unruptured aneurysms. This was associated with increased deposition of the complement inhibitors, including the Factor H polymorphic variant Y402H (associated with age-related macular degeneration^[102]), C4b binding protein, and protectin (CD59, a MAC inhibitor); however, the outer wall lacked inhibitors, especially protectin. Other inhibitors, such as MCP and DAF, were only sparsely expressed by adventitial mural cells. The authors suggested that the increased MAC activity in the outer wall may be the result of decreased complement inhibitors in that region, and that the outer wall's decreased ability to inhibit the complement cascade may facilitate eventual rupture. However,

judging from the finding of their second study, in which IgG was more densely deposited towards the outer aneurysm wall, it is unclear whether this graded IgG concentration could be the primary factor in the greater MAC activity in the outer wall.

Studies on genetic expression profiles have also identified the role of complement-related genes in aneurysmal tissue. In a small study,^[103] which compared aneurysm samples to control superficial temporal artery tissue, there was upregulated expression of three genes for C1q, the deposition of which was found in the aforementioned study by Tulamo *et al.*,^[84] as well as those for complement Factor D, Factor H, Factor B, and C3a. The authors pointed out that these alterations in the expression profile of these genes represent a change in equilibrium of the complement system in the perianeurysmal environment. There has also been an animal study by Aoki *et al.*,^[104] in which the investigators used a DNA microarray to compare intimal and medial gene expression in cerebral aneurysms versus normal cerebral arteries. It was found that in the media there was upregulation of Factor H and C4 expression, although downregulation of C3 and C6. By contrast, C3 and C6 were upregulated in the intima. The authors argue that their differing results from the initial study by Tulamo *et al.*,^[100] may be explained by different regulation of complement mRNA expression between the endothelial cells and the SMCs in cerebral aneurysms.

Although the genetic and histopathological studies mentioned were no doubt pioneering, there are certain points of discussion. Among the studies assessing the role of the complement in aneurysms, the experimental design often differed significantly. In general, the study sizes were small, although this is what would be expected given the limits of intraoperative sample collection. In addition, one study was an animal model.^[104] Whereas some of the studies compared aneurysmal tissue to control samples (such as the superficial temporal artery),^[48,103,104] the three studies by Tulamo *et al.*^[84,100,101] only compared ruptured to unruptured aneurysms. In effect, although several studies provide compelling evidence that there is more complement deposition and activation in aneurysms than in healthy cerebrovascular tissue, those by Tulamo *et al.*^[84,100,101] generally cannot make this comparison since these studies lacked controls from nonaneurysmal vascular tissue.

In general, the method of specimen collection involved taking a sample of aneurysm tissue, intraoperatively, that was distal to the placement of a surgical clip and then running immunohistological analyses. There is a possibility, therefore, that the pathology of the sample and/or its immunological characteristics may have changed between the time that the sample was collected and analyzed. This

time period varied widely among and within studies, including one of the studies in which samples varied from 4 h to 47 days.^[101] However, although this is a consideration for judging the quality of the immunologically analyzed specimens, they were generally flash-frozen in liquid nitrogen so as to minimize degradation and preserve their immunohistological staining characteristics. In addition, the aneurysm samples represent patients requiring surgical, but not endovascular intervention, which imparts a selection bias to patients with certain clinical and aneurysmal morphological characteristics. The results of the Tulamo studies from Finland may also have limitations in their external validity because Finland represents a relatively homogenous population, and the prevalence of intracranial aneurysms is twice as high as other comparable countries.^[1]

A larger issue with interpreting the results of the analyses, in which authors have attributed the aneurysm pathogenesis to complement activation, are confounding causes of the observed immunohistological properties of aneurysm tissue. In several of the human studies there were statistically significant differences in the proportions of patients with characteristics known to affect aneurysm pathology (including smoking history, hypertension, and family history of aneurysms) as well as differences in the gross aneurysm morphology. Furthermore, it is unclear whether the increased complement activation in ruptured compared with unruptured samples (as was reported in the studies by Tulamo *et al.*^[84,100,101]) contributed to the acute mechanism of rupture, or whether the resulting rupture and SAH hemorrhage occurred for other reasons (i.e. hemodynamic factors, *etc.*) and then initiated a secondary complement and inflammatory response as a result of the injury. Indeed, as mentioned, there is evidence that the complement system is upregulated in patients with SAH.^[60,65,66] In response to this concern, Tulamo *et al.*^[84,100,101] have argued that the increased density of macrophage infiltration in ruptured versus unruptured aneurysms argues for a more chronic inflammatory process, as dense accumulation of macrophages typically occurs over days to weeks following an acute injury. In addition, they point out that although less concentrated, complement deposits were found in unruptured aneurysms. Their argument is also supported by the numerous animal and human studies that have shown inflammatory infiltrate in aneurysms that have not ruptured.^[22,32,36,37] Tulamo *et al.*^[84,100,101] suggest that the hemorrhagic insult and physical factors may have contributed to complement activation but that more studies will be needed.

CONCLUSION

There is mounting evidence that the complement cascade plays a role in the chronic as well as acute inflammation

that has been associated with aneurysm formation, growth, and rupture. In effect, the physicochemical stress that occurs in intracranial arteries may lead to exposure and deposition of complement-activating factors in the aneurysm wall, which later predispose it to rupture. Specifically, assembly of MACs in the outer aneurysmal wall may be involved in the acute mechanism of rupture. Future, multicenter studies will need to compare a large number of aneurysmal samples to appropriate controls so that the aneurysm pathophysiology may be better understood. Furthermore, the complement cascade may provide a target for future therapies in the treatment of aneurysms and SAH.

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Cite this article as: Taylor BE, Appelboom G, Zilinyi R, Goodman A, Chapel D, LoPresti M, Connolly ES. Role of the complement cascade in cerebral aneurysm formation, growth, and rupture. *Neuroimmunol Neuroinflammation* 2015;2(2):93-101.

Source of Support: Nil. **Conflict of Interest:** No.

Received: 10-09-2014; **Accepted:** 26-09-2014

The role of inflammation in cerebral aneurysms

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ABSTRACT

The natural history of unruptured intracranial aneurysms (IAs) is poorly understood. At present, risk factors for aneurysm rupture are limited to demographics and rudimentary anatomic features of the aneurysm. The first sign of aneurysm destabilization and rupture may be subarachnoid hemorrhage, a potentially devastating brain injury with high morbidity and mortality. An emerging body of literature suggests a complex inflammatory cascade likely promotes aneurysm wall remodeling and progressive ballooning of the arterial wall, ultimately terminating in aneurysm rupture. These events likely begin with hemodynamic, flow-related endothelial injury; the injured endothelium stimulates inflammation, including the recruitment and transmigration of inflammatory cells, particularly macrophages. Various proteases are secreted by the inflammatory infiltrate, resulting in degradation of the extracellular matrix and the structural changes unique to IAs. Detailed understanding of these inflammatory processes may result in (1) early identification of patients at high risk for aneurysm rupture, perhaps via arterial wall imaging, and (2) targeted, noninvasive therapies to treat or even prevent cerebral aneurysms.

Key words: Aneurysms, atherosclerosis, inflammation, intracranial

INTRODUCTION

Subarachnoid hemorrhage due to intracranial aneurysm (IA) rupture is a devastating disease. Initial mortality may be as high as 40-50%, and of those who survive, one-third to one-half are left with permanent neurologic deficits.^[1] When an unruptured aneurysm is discovered in a patient, current therapeutic options to prevent aneurysm rupture include invasive endovascular occlusion versus surgical therapy, or close radiologic follow-up with intervention when the risk of rupture is deemed high enough. That being said, risk stratification of patients with an unruptured IA is based on a limited understanding of natural history, size appears to contribute to aneurysm destabilization,^[2] but there are likely other, poorly understood factors at play. And as demonstrated in the International Study of Unruptured Intracranial Aneurysms (ISUIA), invasive endovascular or surgical treatments are associated with an overall 1-year morbidity/mortality of 10%.^[3]

In order to appropriately tailor treatment decisions, further understanding of the pathophysiology behind aneurysm growth and rupture is needed. In recent years, a growing body of literature has identified inflammation as a key player in the pathogenesis of intracranial aneurysms (IAs), from aneurysmogenesis and vascular remodeling to aneurysm destabilization and rupture. Here, we will review the pathology of IAs along with the literature supporting a role for inflammation in this pathology; we will also examine potential inflammatory targets for noninvasive treatment of IAs.

STRUCTURAL CHARACTERISTICS OF INTRACRANIAL ANEURYSMS

Intracranial aneurysms are believed to be acquired vascular lesions; they are exceedingly rare in children and their incidence increases with age.^[4,5] As IAs are preferentially located at bifurcations and sharp curves, hemodynamics (e.g. various shear stressors) are believed to trigger aneurysmogenesis. From a structural perspective, compared to extracranial vessels, intracranial vessels have less elastic fiber in the tunica media and adventitia, less smooth muscle in the media, and a thinner adventitia.^[6] At vessel bifurcations, the apical portion of the intracranial vessel lacks smooth muscle cells (SMCs), a gap referred to as the “medial raphe”.^[7] During the initiation of aneurysms, the luminal surface of the vessel becomes

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10.4103/2347-8659.153982

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irregular and often damaged, even denuded, a probable consequence of disturbed hemodynamic stress.^[8]

Although shear stressors likely trigger the initial injury, further degradation and disorganization of the vascular wall leading to the aneurysmal growth is likely the result of an inflammatory cascade.^[9-11] In general, the vessel wall is transformed into a disorganized array, with fragmentation/loss of the internal elastic lamina, myointimal hyperplasia, and disorganization of muscle fiber structure.^[12-14] SMCs transition from a contractile phenotype to a pro-remodeling, pro-inflammatory synthetic phenotype, and finally to a dedifferentiated phenotype prior to aneurysm rupture.^[15] Though the initial vascular injury was from high shear stress, the cavity of the aneurysm is subjected to low, atheroprone-like shear stress, the type conducive to inflammatory cell adhesion and infiltration.^[16] In large aneurysms (e.g. those prone to rupture), there are often advanced atherosclerotic changes, phenotypically modified SMCs, lipid-laden macrophages, and lymphocytes.^[17]

INFLAMMATORY MEDIATORS OF ANEURYSM WALL REMODELING

The histological findings in the walls of IAs, those of degeneration and pathologic vascular remodeling, are similar to the findings evident in inflammatory atherosclerotic lesions. Summarized here and depicted in Figure 1 are the mediators of inflammation likely to play a role in IA pathogenesis.

Endothelial dysfunction

Flow-mediated endothelial dysfunction is likely pivotal in aneurysm formation.^[18] Several mechanosensors, such as ion channels, integrins, cell adhesion molecules, G-protein-coupled receptors, have been identified at the apical and basal surfaces of the endothelium;^[14] these sensors can identify variations in wall shear stress and adapt lumen diameter accordingly. High

shear stress can result in activation of inflammatory mediators, such as the master regulator of inflammation, nuclear factor-kappaB (NF- κ B).^[19,20] Mechanical stressors can denude the endothelium, triggering the expression of chemoattractants, pro-inflammatory cytokines, and cell adhesion molecules at the surface of endothelial cells.^[21] Absent from normal control arteries, monocyte chemoattractant protein-1 (MCP-1) and interleukin-8 (IL-8) are expressed in human and experimental IAs^[22] and vascular cell adhesion molecule-1 (VCAM-1) is expressed in the walls of human and rat model IAs.^[23]

Macrophages and other inflammatory infiltrates

Numerous studies have demonstrated the presence of inflammatory cell infiltrates, particularly macrophages, in IAs.^[24] In one study, inflammatory infiltrates were present in half of all unruptured aneurysms (10/20) versus 100% of all ruptured aneurysms (40/40).^[25] And in a study by Frösen *et al.*^[26] whereby 42 ruptured IAs were histologically compared with 24 unruptured IAs, infiltration of the aneurysm wall by macrophages correlated strongly with aneurysm rupture. Macrophages are thought to be a key mediator of IA vascular remodeling as they release matrix metalloproteinases (MMP) such as MMP-9 and MMP-2.^[27,28] In one study by Kanematsu *et al.*,^[29] macrophage-depleted mice had a substantially lower risk of IA development compared with control mice (10% vs. 60%).

Extracellular matrix remodeling

An essential feature of IAs is fragmentation of the internal elastic lamina (IEL) and thinning of the arterial media. These changes alter the mechanical properties of the aneurysm wall; in response to further shear stress, the destabilized arterial wall may progressively balloon. MMPs are proteolytic enzymes secreted by activated macrophages and by phenotypically modified SMCs. MMPs are capable of degrading the principal structural components in the artery wall, collagen, and elastin, and are, therefore, likely responsible for the structural

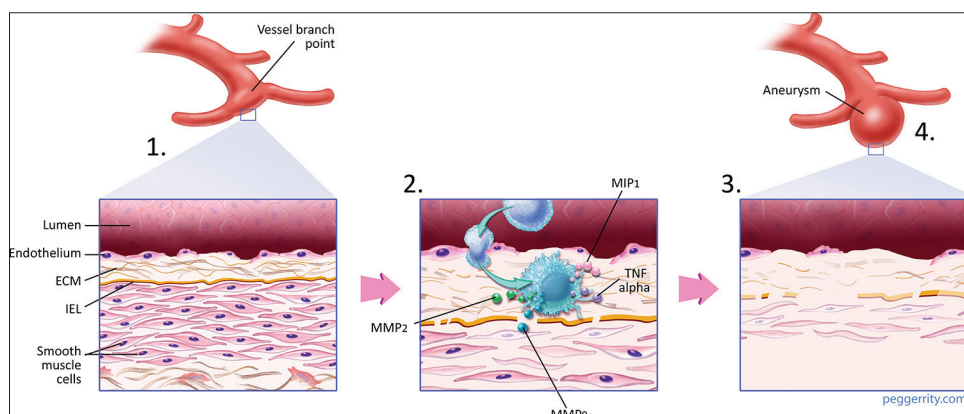


Figure 1: (1) Flow-related endothelial injury; (2) triggers an inflammatory response whereby cells (macrophages) infiltrate the arterial wall and secrete pro-inflammatory cytokines and metalloproteinases; (3) the mounting inflammatory response results in proteolytic destruction of the extracellular matrix and smooth muscle cell phenotypic modulation; (4) macroscopically, the arterial wall is remodeled into an aneurysm wall with progressive aneurysmal ballooning

changes in the internal elastic lamina and media in IAs.^[30,31]

Thrombus formation

Normally, an intact endothelial wall protects the luminal surface from thrombosis and platelet aggregation, in part via the expression of CD39, nitric oxide, and prostacyclin.^[32] In contrast, damaged or denuded endothelial cells may instead activate thrombosis and platelet aggregation pathways. Altered hemodynamic flow within the IA cavity may also promote thrombus formation.^[33] Neutrophils and macrophages are recruited to the site of endothelial injury and thrombosis. These cells often release proteolytic enzymes, MMPs, cathepsin G, and elastase, to try and promote fibrinolysis and thrombus degradation. Instead, these proteolytic enzymes may further degrade the IA wall.^[34] SMCs and myofibroblasts may invade the thrombus, incorporating the thrombus into the IA wall itself.^[35]

Complement cascade

The complement cascade has also been studied as a contributor to the pathogenesis of IAs. Activation of complement leads to robust and efficient proteolytic cascades, typically terminating in opsonization and lysis of pathogens as well as in the generation of the classic inflammatory response through the production of potent pro-inflammatory molecules. Immunostaining of IA walls both in humans and animal models have identified complement components, particularly C3 and C9.^[36] Two studies using microarray analysis have demonstrated variable expression of complement-related genes in IAs as compared with control arterial tissue.^[37,38] And in another study comparing ruptured with unruptured aneurysms, the expression of the complement cascade end product (the membrane attack complex) was greater in ruptured samples and correlated significantly with aneurysm wall degeneration and inflammatory cell infiltration.^[39] It continues to be unclear, though, how complement activation results in IA rupture, further studies are needed to define the exact pathways linking the two.

IMAGING OF ARTERIAL WALL INFLAMMATION

Noninvasive imaging of vascular inflammation within the aneurysm wall may in the future help differentiate stable IAs from destabilized IAs at greater risk for rupture. For instance, protocols have been developed to visualize arterial wall inflammation in patients with intracranial atherosclerosis. Preliminary studies of atherosclerotic plaques suggest vulnerable plaques prone to rupture have arterial wall imaging profiles separate from stable, asymptomatic plaques.^[40-42] Regarding IAs, DeLeo *et al.*^[43] published a pilot study

whereby active inflammation was imaged *in vivo* in a rabbit model of common carotid artery aneurysms. This group utilized a myeloperoxidase-specific paramagnetic contrast agent in conjunction with magnetic resonance imaging (MRI). Several years ago, Hasan *et al.*^[44] reported on the use of ferumoxytol-enhanced MRI to image macrophages within aneurysm walls in 11 patients with unruptured IAs. Ferumoxytol is an iron oxide nanoparticle theoretically macrophage-selective as it is cleared by reticuloendothelial system macrophages. Interestingly, early ferumoxytol-associated imaging changes (24 h postinfusion) were identified in five patients, and several of these patients had “symptomatic” IAs (progressive headache; rapid aneurysmal enlargement; aneurysm rupture). Further studies with larger sample sizes are needed to confirm whether ferumoxytol-associated imaging changes correlate with greater IA rupture risk.

POTENTIAL ANTI-INFLAMMATORY PHARMACOLOGIC TARGETS

Advances in our understanding of the inflammatory cascade leading to aneurysm destabilization and rupture may result in the designing of novel therapies individualized to specific patients. Preliminary data in animal models of IA suggest therapies targeting the inflammatory response may have efficacy in the future treatment of IA. For instance, in their rat model of IA, Aoki *et al.*^[45,46] demonstrated reduction of IA wall inflammation and cessation of aneurysm progression via various statin agents. The expression of MCP-1, VCAM-1, IL-1 β , inducible nitric oxide synthase, and MMP-9 were all reduced in statin-treated rats, likely via inhibition of NF- κ B. However, other studies have demonstrated dose-dependent effects of statins on IAs, including aneurysm growth and/or rupture with high doses of statins.^[47] A case-control study by Marbacher *et al.*^[48] did not find a reduction in the incidence of IAs in patients with a history of statin use. Additional prospective studies are needed to clarify the role statins may play in patients with IAs. Other promising therapeutics include edavarone, a synthetic free radical scavenger, and nifedipine, a calcium channel antagonist.^[45,49] In an experimental model of IA, nifedipine inhibited DNA binding of NF- κ B, preventing progression of IA wall degeneration and limiting IA size.

Recently, aspirin has emerged as a candidate for noninvasive pharmacotherapy in patients with unruptured IAs. Depending on the dose, aspirin can inhibit several inflammatory mediators via its irreversible inhibition of cyclooxygenase-2. Among patients enrolled in ISUIA, those with a history of aspirin use 3 times weekly, or greater had a lower risk of cerebral

aneurysm rupture and subarachnoid hemorrhage compared with those who never used aspirin.^[50] These findings were reproduced in a retrospective study of 747 patients with IAs by Gross *et al.*^[51] subarachnoid hemorrhage occurred in 28% of patients with a history of aspirin use versus 40% of patients without a history of aspirin use. In the previous section, we described an imaging protocol (ferumoxytol-enhanced MRI) whereby macrophages in IA walls can be visualized. In one ferumoxytol-enhanced MRI study, patients were imaged pre- and post-daily aspirin therapy (for several months). Ferumoxytol signal within the IA walls was decreased in these patients postaspirin therapy, suggesting IA inflammation may have decreased.^[52]

CONCLUSION

Inflammation has emerged as a probable key mediator of both aneurysmogenesis and aneurysmal destabilization and rupture. The inflammatory cascade is likely interrelated with mechanical flow-induced vascular dysfunction. Further studies will, hopefully, further define these pathways, aid in our prediction of the natural history of an IA in a patient-specific manner, and identify novel pharmacologic targets to prevent aneurysm growth and rupture.

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Cite this article as: Turkmani AH, Edwards NJ, Chen PR. The role of inflammation in cerebral aneurysms. *Neuroimmunol Neuroinflammation* 2015;2(2):102-6.

Source of Support: Nil. **Conflict of Interest:** No.

Received: 23-10-2014; **Accepted:** 12-12-2014

The role of leukocytes in the formation and rupture of intracranial aneurysms

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ABSTRACT

Ruptured intracranial aneurysms (IAs) affect a small proportion of the population; however, the morbidity and mortality is disproportionately high. Although little is known about IA formation, progression, and rupture, mounting evidence suggests that inflammation may play an important role in IA pathogenesis. There is emerging evidence to suggest that leukocytes play a key role in generating and maintaining a pathologic inflammatory response that leads to aneurysm formation and rupture. We present the current literature pertaining to the role of leukocytes in aneurysm formation, progression, and rupture. The contributions of individual cell types are detailed, with special attention paid to the cytokine and molecular profiles. The role of magnetic resonance imaging as a means by which to evaluate aneurysm-associated inflammation is reviewed. Finally, we discuss leukocytes as potential targets of pharmacologic intervention.

Key words: Aneurysm, inflammation, inflammatory cells, leukocytes, lymphocytes, macrophages, mast cells, neutrophils

INTRODUCTION

Stroke is the fourth leading cause of death in the United States and is a prominent cause of long-term disability.^[1] The prevalence of stroke among adults age 20 or older is estimated at 6.8 million, with 795,000 individuals experiencing a new or recurrent stroke annually.^[1] Subarachnoid hemorrhage (SAH), secondary to ruptured intracranial aneurysms (IAs) comprises 1-7% of all strokes.^[2] On an average 3.6-6% of the adult population harbor IAs; however, the rate of rupture is estimated to be between 0.05% and 0.5%.^[3] The small number of IAs that do rupture have a poor prognosis with a mortality rate of roughly 50%.^[3] Of those that survive the initial hemorrhage, approximately 30% remain severely disabled, resulting in a poor quality of life.^[4]

The mechanisms of aneurysm genesis, maturation, and eventual rupture remain incompletely defined, yet new studies highlight multiple genetic and environmental factors that may contribute to the pathogenesis.

Chronic hypertension, binge drinking, and cigarette smoking have all been linked to aneurysm development and rupture.^[5-7] Inflammation represents a potential common endpoint through which these diverse environmental stimuli enact pathologic changes in the intracranial vasculature, thus leading to aneurysm formation.

Animal aneurysm models, as well as analysis of human aneurysms, suggest that inflammation is a key mediator in the formation, progression, and rupture.^[5,8-19] Multiple studies have demonstrated the inflammatory response to be associated with persistent pathologic vascular remodeling in response to an insult to the vessel wall. Abnormal blood flow, chronically elevated blood pressure, and shear stress have all been linked to the induction of the inflammatory response as well as IA pathogenesis.^[6,12,20-29] Central to the process of inflammation-driven vascular remodeling is endothelial and vascular smooth muscle cell (VSMC) dysfunction resulting in vessel weakening.^[30] The inflammatory response associated with vascular remodeling is composed of multiple complex cellular and biochemical processes. VSMCs, endothelial cells, and inflammatory cells participate in intercellular signaling, resulting in the recruitment of immune cells, such as leukocytes, to the vessel walls.

We review the current literature pertaining to the role of leukocytes in aneurysm formation, progression,

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10.4103/2347-8659.153972

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and rupture. The contributions of individual cell types are detailed, with special attention paid to the cytokine and molecular profiles. The role of magnetic resonance imaging (MRI) as a means by which to evaluate aneurysm-associated inflammation is reviewed. Finally, we discuss leukocytes as potential targets of pharmacologic intervention.

DETECTION AND INVESTIGATION OF LEUKOCYTES IN HUMAN INTRACRANIAL ANEURYSM PATHOGENESIS

Currently, the literature suggests that leukocyte infiltration of the intracranial vasculature may play various roles in the prolonged formation and acute rupture of IAs. Frösen *et al.*^[12] reported that IA walls obtained less than 12 h after rupture demonstrated T-cell and macrophage infiltration, as well as VSMC proliferation, indicating a chronic process that preceded rupture. In addition, the observation of leukocytes spread throughout aneurysm walls supports their role in the global deterioration of the vessel and suggests that their prominence in ruptured aneurysms is not an acute response to the sudden event.^[31]

Transcriptome analysis of control vessels and IAs demonstrates upregulation of gene expression of the pro-inflammatory cytokines associated with leukocyte infiltration within aneurysm walls.^[15,32-36] Weinsheimer *et al.*^[35] in an analysis of IAs obtained from autopsies within 24 h of death, showed upregulation of several pro-inflammatory genes, including, adherens junction, the mitogen-activated protein kinase pathway, and Notch signaling. Krischek *et al.*^[36] investigated gene expression on 10 IAs (6 ruptured and 4 unruptured) and determined that the most significantly upregulated pathway was antigen processing. Shi *et al.*^[15] interrogated 6 IAs using the illumina microarray platform and determined focal adhesion, extracellular matrix receptor interaction, cell communication, inflammatory response, and apoptosis to be the most significant functional pathways involved in IA pathogenesis. These findings indirectly implicate leukocyte infiltration as a major contributor to aneurysm genesis and progression.

Finally, immunohistochemical analysis of the animal model and human aneurysms has repeatedly demonstrated leukocytes within the aneurysmal walls. Chyatte *et al.*^[20] reported the presence of macrophages and T-lymphocytes within the walls of unruptured aneurysms. Ruptured aneurysms have also been found to harbor T-lymphocytes and macrophages within their walls.^[12,31] In a study of ruptured and unruptured IAs, Kataoka *et al.*^[31] observed leukocyte infiltration, particularly macrophages, in 50% of the unruptured

and in all of the ruptured IAs. Using electron microscopy, the authors were able to demonstrate an association between advanced deterioration in the wall of ruptured aneurysms and the infiltration of leukocytes and macrophages.^[31] Frösen *et al.*^[12] also observed more prominent leukocyte infiltration in ruptured IAs when compared to unruptured IAs. These findings suggest that the structural architecture of ruptured aneurysms differs from that of unruptured aneurysms. Furthermore, leukocyte invasion appears to be a mediator of this change and a potential driving impetus behind the progression to aneurysm rupture.

ROLE OF MACROPHAGES

Animal and clinical studies have identified macrophages as important contributors to the formation and rupture of aneurysms. These cells participate in the synthesis and secretion of matrix metalloproteinases (MMPs) and elastases, which play significant roles in the degradation of the extracellular matrix and internal elastic lamina. Histopathological analysis of both unruptured and ruptured aneurysms has repeatedly identified macrophage infiltration within the aneurysm walls.^[12,20,37] In addition, Ruzevick *et al.*^[38] observed the pro-inflammatory haptoglobin 2-2 genotype to be associated with larger aneurysms and increased macrophage infiltration within the aneurysm walls.

Macrophage-depleted mice have been shown to have a moderate protective advantage from aneurysm formation and rupture, suggesting macrophages play a critical role in the aneurysm pathogenesis.^[13,39] Corroborating this hypothesis are two animal studies investigating the role of monocyte chemoattractant protein 1 (MCP-1), an important macrophage chemoattractant that has been studied in atherosclerosis and abdominal aortic aneurysms (AAA).^[40,41] By using MCP-1 knockout (KO) mice, both Aoki *et al.*^[9] and Kanematsu *et al.*^[13] were able to demonstrate a decrease in aneurysm formation and macrophage accumulation. Aoki *et al.*^[9] also reported that inhibiting MCP-1 activity using a dominant negative mutant of MCP-1 resulted in the inhibition of aneurysm progression in rats. MCP-1 deficient mice also demonstrated decreased macrophage accumulation and expression of MMP-2 and MMP-9.^[9] A recent study conducted by Chalouhi *et al.*^[42] surveyed the cytokines and chemokines in aneurysm lumen blood and found an increase in chemoattractant cytokines interleukin-7 (IL-7), IL-8, and MCP-1, suggesting active recruitment of inflammatory cells into the aneurysm.

Nuclear factor- κ B (NF- κ B) is a family of transcriptional factors involved in regulating the expression of a variety of inflammatory factors including MCP-1. Aoki *et al.*^[43] investigated the role of NF- κ B in the

initiation and progression of IAs using animal models. The authors were able to demonstrate that NF- κ B participates in the initiation of IA formation through transactivation of many downstream genes related to macrophage recruitment and vascular inflammation, such as MCP-1, vascular cell adhesion molecule 1 (VCAM-1), MMP-2, MMP-9, IL-1 β , and inducible nitric oxide synthase (iNOS).^[43] In addition, NF- κ B decoy oligodeoxynucleotides (ODNs), which inhibit NF- κ B, abrogated the upregulation of inflammatory factors, including MCP-1. In an additional study conducted by the same group, Aoki *et al.*^[44] linked MCP-1 expression in VSMCs with Ets-1, a transcription factor implicated in many vascular inflammatory diseases. Ets-1 binds to the promoter region of MCP-1 resulting in increased Ets-1 expression. Utilizing the knowledge obtained from previous studies on the role of NF- κ B and Ets-1, Aoki *et al.*^[45] showed that treating rats with chimeric decoy ODNs, designed to simultaneously inhibit NF- κ B and Ets-1, reduced aneurysm size while thickening aneurysm walls of preexisting aneurysms. Furthermore, decreased expression of MCP-1 and reduced macrophage infiltration was observed in rats treated with the decoy ODNs.

Additional molecular signaling molecules associated with macrophage-induced aneurysm formation include tumor necrosis factor alpha (TNF- α) and stromal cell-derived factor-1 (SDF-1). Several studies have suggested that TNF- α is a key mediator in aneurysm development through the activation of several cytokines and MMPs.^[17-19,46] TNF- α has been shown to upregulate MCP-1, which in return attracts macrophages, thereby leading to additional TNF- α expression in a positive feedback loop.^[18] Using TNF- α KO mice, Starke *et al.*^[18] demonstrated a reduction in IA formation and rupture. Additional studies using a synthesized TNF- α inhibitor 3, 6'dithiothalidomide (DTH) substantiated the results from the KO experiments. Starke *et al.*^[18] also showed DTH to inhibit IA progression with fewer ruptured IA in the treatment group compared to the control group. Furthermore, using tumor necrosis factor receptor superfamily member 1a (TNFR1) deficient mice, Aoki *et al.*^[46] demonstrated suppressed IA formation with decreased NF- κ B activation, reduced MCP-1 and cyclooxygenase 2 (COX-2) expression, and fewer infiltrating macrophages. These results suggest that TNF- α /TNFR1 signaling is critical in IA pathogenesis.

Stromal cell-derived factor-1 is an important chemokine that promotes inflammation directly as well as through angiogenesis.^[47] Macrophage recruitment and retention around new blood vessels has been shown to be mediated by SDF-1.^[48] Expression of SDF-1 in IAs was recently evaluated in a study conducted by Hoh *et al.*,^[47] wherein

SDF-1 was present in the walls of both human and mouse aneurysms. Hoh *et al.*^[47] also found SDF-1 promotes aneurysm wall angiogenesis through endothelial cell tube formation and macrophage infiltration. Inhibiting SDF-1, using anti-SDF-1 blocking antibodies, suppressed murine aneurysm wall angiogenesis and resulted in the development of significantly fewer IAs compared to control mice.

Macrophages mediate flow-induced vascular remodeling, in part, through the release of MMPs, a process that under physiologic conditions, preserves vascular integrity and health.^[39,49] However, increased levels of MMP expression, particularly MMP-2 and MMP-9, have been reported in IAs.^[10,11,50,51] Studies using broad-based MMP inhibitors, such as doxycycline, have shown significant reductions in the incidence of IAs in animal models.^[49,52] Tolylsam, a selective inhibitor for MMP-2, -9, and -12 also abolished the progression of IA, although it did not reduce the incidence of total aneurysmal changes.^[10] Using more refined inhibition techniques, a greater understanding for the role of MMPs has materialized. Nuki *et al.*^[52] showed that MMP-9 KO mice, but not MMP-2 KO mice, diminished the incidence of IAs. A separate study by Ota *et al.*^[49] also demonstrates a reduced incidence of IAs in MMP-9 KO animals but not in MMP-12 KO animals.

Whereas MMP-9 is the main gelatinase, MMP-12 is the main elastase secreted from macrophages. Since MMP-12 appears to have no effect on aneurysm formation and rupture, other sources of elastases are likely. Neutrophil elastase is involved in atherosclerotic plaques and AAA and is produced by not only neutrophils but also macrophages and vascular endothelial cells.^[53,54] Furthermore, neutrophil depletion studies inhibited AAA development through a non-MMP-2 and non-MMP-9-mediated mechanism, implying other mediators must exist, including the possibility of neutrophil elastase.^[55]

Another protease that is of interest is the cathepsin family (B, D, K, and S), which have been shown to be expressed in IAs and promote their progression.^[56,57] Specifically, histological analysis of ruptured aneurysms exhibited a cluster of macrophages expressing cathepsin D within the aneurysm wall where there was evidence of collagen erosion.^[31] Multiple studies suggest that a polarized macrophage population is associated with a variety of diseases including atherosclerosis, inflammatory lung disease, and inflammatory diseases of the nervous system.^[58-62] Two populations of macrophages, the M1 (pro-inflammatory) and M2 (anti-inflammatory) subtypes, have been identified. Predominance of the M1 subtype has been implicated in aneurysm progression and rupture.

The M1 population is pro-inflammatory and secretes high levels of IL-2, IL-23, IL-6, IL-1, and TNF- α .^[63] The M2 population is anti-inflammatory and secretes high levels of IL-10. Hasan *et al.*^[63] examined 10 patients with IAs (5 unruptured and 5 ruptured) for the presence of M1 and M2 macrophage populations. The authors demonstrated a predominance of M1 over M2 macrophages within the walls of ruptured aneurysms and observed an increase in mast cells in ruptured aneurysms compared to unruptured aneurysms. The authors hypothesized that the imbalance between M1 and M2 may be in part due to the effects of mast cells. Given these results, the interplay between M1 and M2 phenotypes appears to be important in the aneurysm pathogenesis and warrants further investigation.

ROLE OF MAST CELLS

Mast cells are resident leukocytes that contain cytoplasmic granules rich in histamine and heparin, as well as, the pro-inflammatory cytokines, TNF- α , IL-1, IL-3, IL-4, IL-5, IL-8 and IL-13, and transforming growth factor-beta.^[64,65] Mast cell degranulation and release of cytokines has been linked with vascular inflammatory processes, such as, atherosclerosis and AAAs.^[66,67] Recent investigations have targeted mast cells as contributors to IA genesis and progression. In a study conducted by Ishibashi *et al.*,^[65] an increase in the total number of mast cells during IA formation was observed using a rat model. Mast cell degranulation inhibitors suppressed IA progression through attenuation of the local chronic inflammatory response, as was evident from decreased NF- κ B activation, macrophage infiltration, and expression of MCP-1, MMPs, and IL-1 β .^[65] In addition, Ollikainen *et al.*^[68] demonstrated that mast cells in the wall of IAs were associated with histopathological changes consistent with wall remodeling, lipid accumulation, and inflammatory cell infiltration. Finally, Hasan *et al.*^[63] observed increased mast cells in ruptured IAs relative to unruptured IAs. Taken together, these studies indicate that mast cell degranulation play a critical role in aneurysm formation and may contribute to IA rupture.

ROLE OF NEUTROPHILS

Neutrophils are recruited to sites of injury and are a hallmark of acute inflammation. Although the contribution of neutrophils in IA formation is largely undefined, evidence from investigations into AAA pathogenesis offers insight into their role. Animal models have demonstrated progressively increasing neutrophil infiltration into the walls of AAAs over the course of aneurysm development.^[55,69]

Neutrophil recruitment to the vascular wall may be associated with macrophage infiltration. Mice

treated with an antineutrophil-antibody showed a decreased number of macrophages compared to wild-type (WT) mice.^[55] Furthermore, depletion of neutrophils attenuated the size and incidence of AAA. Diminished macrophage infiltration in aneurysms of neutrophil-depleted mice is not associated with a decrease in chemoattractants such as MCP-1 and MIP-1 α .^[55] This suggests that additional mediators are contributing to this complex interaction. Importantly, there was no difference in expression of MMP-2 and MMP-9, despite a decrease in macrophage infiltration.

The presence of neutrophils was recently reported by Marbacher *et al.*^[70] using a decellularized rat aneurysm model. This rat model simulated the loss of mural cells (endothelial and VSMCs), a hallmark of ruptured cerebral aneurysms.^[12,31] The ruptured aneurysms displayed marked adventitial fibrosis and inflammation, complete wall disruption, and increased neutrophil accumulation in unorganized intraluminal thrombus.^[70] Neutrophils trapped in unorganized thrombus are a major source of matrix-degrading proteases. Intraluminal thrombus is a site of protease and cytotoxic compound release leading to wall inflammation and subsequent matrix degradation.^[55,71]

Myeloperoxidase (MPO) is a peroxidase enzyme that catalyzes the formation of a number of reactive oxidant species and is primarily produced by neutrophils.^[72] Along with a well-known role in host mechanisms against pathogens, MPO has recently been implicated in the initiation and destabilization of atherosclerotic plaques.^[73] In a study conducted by Gounis *et al.*,^[74] MPO was detected in all three ruptured IAs and 10 out of 20 unruptured IAs.^[74] Additionally, Gounis *et al.*^[74] demonstrated that MPO positivity was a significant predictor of 5-year aneurysm rupture rate. An emerging picture suggests a key factor in aneurysm formation and rupture is the ongoing inflammatory process mediated by infiltration of leukocytes. This suggests that MPO may play an important role in the aneurysm pathogenesis. Therefore, MPO may also serve as a potential biomarker.

Neutrophils represent a potential therapeutic target for pharmacologic interventions designed at preventing aneurysm progression and rupture. Hannawa *et al.*^[69] demonstrated suppressed AAA formation in L-selectin KO mice. L-selectin, an adhesion molecule expressed on the surface of most leukocytes, is responsible for the recruitment of immune cells.^[75-77] Hannawa *et al.*^[69] postulated that the diminished AAA formation seen in WT mice compared with the L-selectin KO mice is most likely due to the impaired recruitment of neutrophils

and macrophages. Specifically, since neutrophils are present in the aortic wall before macrophages in WT mice, a decrease in neutrophils in the L-selectin KO mice is most likely due to the lack of L-selectin.^[69]

ROLE OF LYMPHOCYTES

The contribution of T and B lymphocytes to IA formation is an additional avenue of exploration. B lymphocytes are rarely detected, and their role in IA pathogenesis is unclear.^[20] However, T-lymphocytes have been documented within aneurysm walls^[12,20] and CD8+ T-cells have been linked with AAA development.^[78] T-lymphocytes have been shown to secrete pro-inflammatory cytokines including TNF- α , IFN- γ , and IL-6.^[79] T-lymphocytes were detected within the walls of ruptured aneurysms and were associated with increased infiltration in samples taken < 12 h from rupture. These results indicate that this observation was not reactive.^[12] Based on these observations, T-lymphocytes may play an important role in not only aneurysm formation, but also rupture. Additional studies focused on the role of lymphocytes in IA formation and rupture are necessary to further our understanding of the aneurysm pathogenesis.

DETERMINING INFLAMMATORY STATUS USING IMAGING

The apparent relationship between inflammation and aneurysm rupture is of clinical significance and may provide an avenue through which more accurate predictions of aneurysm rupture can be made. MRI is currently being explored as a noninvasive modality with the potential to evaluate the inflammatory state of aneurysms.

Hasan *et al.*^[80] have reported on ferumoxytol-enhanced MRI images to evaluate aneurysm walls for macrophage infiltration. Ferumoxytol, which is used to treat iron deficiency anemia in patients with chronic renal failure, is a Food and Drug Administration approved drug consisting of an iron oxide nanoparticle.^[81,82] The investigators imaged 19 unruptured aneurysms in 11 patients and determined that images acquired 72 h postinfusion of ferumoxytol were optimal for detecting macrophages within the aneurysm wall.

In a follow-up study, Hasan *et al.*^[83] found that early uptake (within 24 h of infusion) of ferumoxytol in unruptured aneurysm walls suggested an active inflammatory process leading to aneurysm instability, ultimately resulting in rupture within 6 months. This hypothesis was validated with increased expression of COX-2 and mPGES-1 and an increased number of

macrophages in aneurysms with early MRI signal changes. These results showed similar expression patterns to ruptured aneurysms. Unruptured aneurysms with late uptake (72 h postinfusion), did not rupture or increase in size after 6 months of follow-up. As a result, these studies show that ferumoxytol signal changes may indicate a greater risk of aneurysm rupture and suggest macrophage infiltration as a potential marker of aneurysms more likely to rupture.

Myeloperoxidase-specific paramagnetic magnetic resonance (MR) contrast agents, which are specific for MPO activity, have been evaluated in animal and tissue culture studies to examine their utility for imaging active inflammation.^[74,84,85] Rabbit studies have shown promise for the use of an MPO-specific paramagnetic MR contrast agent, di-5-hydroxytryptamide of gadopentetate dimeglumine, in detecting local inflammation.^[86] Since MPO has been detected in IAs, specially ruptured IAs, using MPO-specific contrast agents to monitor MPO within IAs will predict active inflammation and may aid in the management of unruptured aneurysms.

FUTURE DIRECTION AND THERAPEUTIC APPROACHES

Despite advances in microsurgical and endovascular therapy, outcomes following IA rupture remain poor. Thus, the identification of indicators of pending rupture and the development of pharmacologic interventions designed at limiting aneurysm progression and rupture are of great clinical interest. A better understanding of the relationship between inflammation and IA pathogenesis is a promising avenue of exploration, as there are multiple cellular and molecular targets for potential exploitation. Pharmacologic interventions targeting inflammation-driven IA formation and progression have shown promise in animal and human studies.^[5] These drugs target inflammatory molecules such as TNF- α (DTH),^[18] NF- κ B (decoy ODN),^[43] Ets-1 (decoy ODN),^[45] SDF-1 (blocking anti-SDF-1 antibodies),^[47] MMPs (tolylsam and doxycycline),^[10,49,52] MCP1 (7ND),^[9] and cathepsins (NC-2300)^[56] [Table 1]. In addition, mast cell degranulation inhibitors (tranilast and emedastine difumarate)^[65] have also been tested. All these therapeutic agents have shown to decrease aneurysm size in experimental animal models. Ferumoxytol-enhanced and MPO-specific paramagnetic MRI appear to offer a possible means by which to evaluate the inflammatory profile of individual aneurysms. Additional investigations into the role of inflammation and IA formation, progression, and rupture are required to better elucidate potential clinically relevant pathways for intervention.

Table 1: Major leukocyte inflammatory mediators and targeted therapies for intracranial aneurysms

Major molecules	Targeted therapies for intracranial aneurysms
Common inflammatory mediators	
TNF- α	DTH ^[18]
NF- κ B	Decoy ODN ^[45]
Ets-1	Decoy ODN ^[45]
VCAM-1	
iNOS	
L-selectin	
MCP-1	7ND ^[9]
SDF-1	Blocking anti-SDF-1 antibody ^[47]
Macrophages	
IL-1 β , IL-2, IL-6, IL-23	
MIP-1 α	
Cathepsins	NC-23000 ^[56]
MMP-2, MMP-9	Tolylsam (selective) and doxycycline (broad) ^[10,49,52]
Neutrophils	
IL-1 β	
Neutrophil elastase	
Lymphocytes	
IFN- γ	
IL-6	
Mast cells	
IL-1, IL-3, IL-4, IL-5, IL-8, IL-13	Degranulation inhibitors (tranilast and emedastine difumarate) ^[65]
TGF- β	

TNF- α : tumor necrosis factor- α ; DTH: 3,6'dithiothalidomide; NF- κ B: nuclear factor- κ B; ODN: oligodeoxynucleotide; VCAM-1: vascular cell adhesion molecule; iNOS: inducible nitric oxide synthase; MCP-1: monocyte chemoattractant protein-1; 7ND: N-terminal deletion variant of MCP-1; SDF-1: stromal cell-derived factor 1; MMP: matrix metalloproteinase; MIP-1 α : macrophage inflammatory proteins-1- α ; IFN- γ : interferon gamma; TGF- β : transforming growth factor beta; IL: interleukin

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Cite this article as: Strong MJ, Amenta PS, Dumont AS, Medel R. The role of leukocytes in the formation and rupture of intracranial aneurysms. *Neuroimmunol Neuroinflammation* 2015;2(2):107-14.

Source of Support: Nil. **Conflict of Interest:** No.

Received: 29-08-2014; **Accepted:** 06-09-2014

Astrocytes: everything but the glue

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The current knowledge in neuroscience indicates that the neural tissue has two major cell populations: neurons and glia (term derived from the Greek word for glue). Neuronal population is characterized by the capacity to produce action potentials, whereas glial cells are typically identified as the subordinate cell population of neurons. Till date, this point of view has changed dramatically, and growing evidence indicates that glial cells play a crucial role in normal mental functions and the pathogenesis of neurological diseases. Classically, glial cells include four major populations clearly discernible in the adult's brain: astrocytes, oligodendrocytes, microglia cells and NG2 glia. Astrocytes, also referred as to astroglia, are by far the most abundant cell lineage in the adult's brain and display a very diverse morphology, which varies during neural development and adulthood [Figure 1]. These cells are in close contact with several tissue components of the brain parenchyma including neurons, vasculature, extracellular matrix and other glial populations. Hence, the number and strategic position of astrocytes provide them with exceptional capacity for modulating multiple functions in the neural tissue.

During neural development, the origin of astrocyte varies according to the anatomical region, but the most accepted viewpoint suggests that astrocytic cells originate from radial glial cells (in the ventricular zone) and some progenitor cells located in the subventricular zone (SVZ). Therein, astrocyte lineage is directed by the expression of notch receptors and their ligands (delta and serrate-jagged) that efficiently repress neuronal fate and promotes gliogenesis.^[1] At this developmental stage, astrocytes are crucial for the synapse formation by improving the neuron's ability to receive synaptic contacts, which consequently initiate neuronal

communication.^[2] Postnatal synapse refinement is also associated with astrocyte maturation suggesting that the astroglia is an important modulator of neuronal plasticity.^[3]

Astrocytes are ubiquitous brain cells that outnumber neuronal population in the proportion of 10:1 and comprise approximately 50% of the neural tissue. Before 1980's, the role of astrocytes was basically associated with supporting and nourishing neurons (primarily considered as a "real" functional cells). However, current evidence indicates that this perspective is quite limited and incorrect. Today, it is well accepted that astrocytes have a more major role in neurological functioning, neural homeostasis and pathogenesis than previously thought.^[4]

Astroglia is the main source of glycogen that delivers energy to the brain interstitial tissue and nourishes neurons by providing lactate.^[5] Other astrocytic functions include: the uptake or release of neurotransmitters,^[6] modulation of synaptic plasticity and neuronal transmission,^[3] regulation of interstitial ions,^[7] cerebral regulation of blood flow and blood-brain barrier,^[8] myelin regulation,^[9] tissue repair,^[10] neuroprotection^[11] and drug metabolism.^[12]

In addition, astrocytes have astonishing enzymatic machinery that includes cyclooxygenase, lipoxygenase and cytochrome P450 epoxygenase pathways.^[12] Remarkably, these enzymes allow astrocytes to deplete several drugs and alcohol, a property that was previously thought to be exclusive of hepatocytes (liver cells). Astroglia can also produce metabolic intermediates of polyunsaturated fatty acid arachidonic acid and epoxide metabolites that may help protect against stroke. Nevertheless, some gene mutations on astrocytes produce defective proteins that upon accumulation may lead to neurodegenerative diseases. Besides, the phospholipase-C (PLC) enzymatic family and the phosphoinositide signaling system are essential for molecule transduction from the cell membrane to the nucleus.^[12] This system appears to be involved

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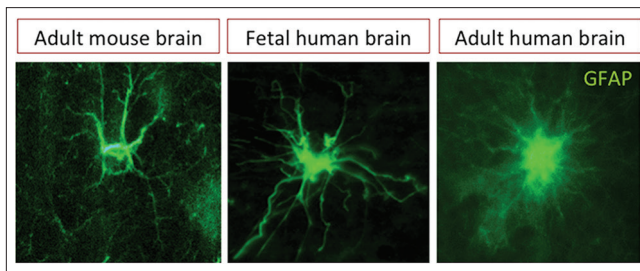


Figure 1: Immunofluorescence images of parenchymal astrocytes. Astrocytic processes in the adult human brain are more profuse and thinner than in the rodent or fetal brain, glial fibrillary acidic protein

in a number of neuronal and glial functions. Up- or down-regulation of different phosphoinositide-specific PLC isoforms (PI) can drive astrocyte reactivity associated with brain inflammation and tumor progression.^[13] PI-PLC β 1, PI-PLC β 4 and PI-PLC 1 have been found in reactive astrocytes, whereas PI-PLC β 3, PI-PLC 2, PI-PLC 2, PI-PLC PI-PLC have been identified in brain tumor cells (astrocytoma). However, the clinical significance of these findings remains to be clarified.^[14] In this regard, the expression of nicotinamide adenine dinucleotide phosphate⁺-dependent isocitrate dehydrogenase-1 gene and epidermal growth factor (EGF) appear to distinguish reactive astrocytes from glioma, but further validation is required to confirm these findings.^[15]

Neurotropic viruses are infectious particles that can preferentially infect neural cells by evading the brain immune response and infecting astroglia. Such viral infection in astrocytes alters their functions and lead to serious neural damages with severe neurological complications. In response to the viral infection, current evidence indicates that activated astrocytes promote innate immune response through the expression of toll-like receptors and secretion of cytokines, that is, astrocytes appear to direct immune response and neuroinflammation.^[13]

Astrocytes contain inward-rectifier K⁺ (Kir) channels that efficiently maintain potassium (K⁺) homeostasis during neuronal activities.^[16] Changes in the expression and functioning of astrocytic Kir channels modify K⁺ spatial buffering. The reduced Kir4.1 channel expression or Kir4.1 channel misallocations promote astrocyte depolarization, which decreases their ability to clear extracellular glutamate. This mechanism is responsible for the neural damage in epilepsy and ischemia. Hence, novel therapeutic approaches have been suggested to involve the regulation of K⁺ spatial buffering by astrocytes.

Since astrocytes are responsible for the optimal ionic homeostasis and neurotransmission balance, astrocytic atrophy has been associated with early alterations of the neuronal transmission and synaptic networks observed

in several neurodegenerative diseases including Alzheimer's disease (AD). In later alterations of AD, senile plaques trigger glial reactivity that, in turn, advances neuronal death and cognitive deficit. Recently, it has been demonstrated that reactive astrocytes produce the inhibitory gliotransmitter GABA, which in consequence reduces the synaptic plasticity and the performance of learning and memory in a mouse model of AD.^[17] Nevertheless, the role of astrocytes in the pathogenesis or progression of neurodegenerative diseases is not completely understood.

A subpopulation of astrocytes can also function as neural stem cells in the adult's brain. Neural stem cells reside in the ventricular-SVZ (V-SVZ), a cell niche lining the lateral wall of the lateral ventricles.^[18] Therein a subpopulation of astrocytes functions as bona fide neural stem cells that generate a number of neuroblasts that rostrally migrate and differentiate into interneurons at the olfactory bulb.^[18] V-SVZ astrocyte progenitors can also promote the genesis of oligodendrocytes that populate white matter tracts of corpus callosum and striatum.^[19] Interestingly, the intraventricular administration of EGF increases dramatically the production of V-SVZ oligodendrocytes and NG2-platelet-derived growth factor receptor- α expressing cells, which temporarily disrupt the neighboring white matter.^[19] Oligodendrocyte dysfunction has been found across most psychiatric conditions, including mood disorders.^[20] However, the role of V-SVZ oligodendrogenesis in the pathophysiology of neuropsychiatric disorders remains to be elucidated.

In the adult hippocampus, another subpopulation of astrocytes gives rise to new neurons that populate the granular layer of the dentate gyrus.^[21] These multipotent astrocytes help control several behavioral tasks, such as episodic-like and spatial memory, object recognition tasks and cognitive performance.^[22] Interestingly, morphological changes and local distribution of astrocytes are dependent on aging and enriched environments. Therefore, astrocytic neural stem cells in the V-SVZ and dentate gyrus help preserve cellular homeostasis and exert modulatory roles on neural circuitry in the adult's brain.

Astrocytes not only preserve the oligodendrocyte population,^[23] but also modulate the myelination process of oligodendrocytes.^[24] Some mutations in astrocytes are associated with leukodystrophies, a group of neurological disorders characterized by imperfect myelin ensheathing. Therefore, although the origin of megalencephalic leukoencephalopathy was initially related to the malfunction of oligodendrocyte, emerging evidence indicates that the pathophysiology is associated with astrocytic dysfunction.

In summary, astrocytes are vital cell mediators for the cerebral homeostasis and play an important role in the pathophysiology and progression of a number of brain disorders. Every day, new evidence emerges to reveal that the glial cells are much more than supporting and nourishing cells in the brain. Thus, astrocytes are everything plus the glue.

ACKNOWLEDGMENTS

The National Institute of Health and the National Institute of Neurological Disorders and Stroke (NIH/NINDS; R01-NS070024), Consejo Nacional de Ciencia y Tecnologia (CONACyT; INFR2014-224359), Programa de Desarrollo del Personal Docente (PRODEP-Redes 2014).

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Cite this article as: Gonzalez-Perez O, Lopez-Virgen V, Quiñones-Hinojosa A. Astrocytes: everything but the glue. *Neuroimmunol Neuroinflammation* 2015;2(2):115-7.

Source of Support: Nil. **Conflict of Interest:** No.

Received: 15-09-2014; **Accepted:** 10-11-2014

Concurrent occurrence of both intracranial and intramedullary tuberculomas

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ABSTRACT

Tuberculosis involving spinal cord in the form of intramedullary tuberculoma is uncommon, and the concurrent occurrence of cranial and intramedullary tuberculomas is extremely rare. We report a case of disseminated tuberculoma involving brain and spinal cord with miliary tuberculosis in a 32-year-old male presenting with fever, cerebellar signs and motor weakness of both upper and lower extremities. Based on magnetic resonance imaging and polymerase chain reaction, we diagnosed as tuberculoma. He completely recovered with conventional antituberculous treatment and steroids. The follow-up of the patient showed disappearance of signs and symptoms.

Key words: Intracranial tuberculoma, intramedullary tuberculoma, magnetic resonance imaging, polymerase chain reaction

INTRODUCTION

Mycobacterium tuberculosis is a serious pathogen worldwide. Central nervous system (CNS) tuberculoma is a rare form of extrapulmonary tuberculosis and is often the result of hematogenous spread from a primary focus, mostly the lung.^[1] Most common manifestations of tuberculosis in the CNS are tuberculous meningitis and intracranial tuberculoma. Brain is far more commonly affected than the spinal cord.^[2] Infratentorial tuberculomas are more frequent in children, whereas lesions are mostly supratentorial in adults.^[3] Intramedullary tuberculoma of the spine is a rare manifestation of disseminated tuberculosis, usually in young people and most commonly involve the thoracic spinal cord.^[4] Its incidence is only 2 out of 100,000 cases of all tuberculosis.^[5] We report a case of combination of intramedullary and intracranial tuberculoma which is extremely rare, so far, only five cases have been reported in the literature.

CASE REPORT

A 32-year-old male immunocompetent patient came with severe signs of unsteadiness of gait, swaying, slurred speech and heaviness in both upper and lower limbs

for 10 days. There was also the history of the evening rise of temperature, loss of weight and appetite. On examination, he had quadriparesis with a power 4/5 in all four limbs, tremors, dysarthria, dysmetria, dyssynergia, dysdiadochokinesia, hypotonia in all limbs, plantar response was equivocal without sensory involvement.

On the evaluation, erythrocyte sedimentation rate (ESR) was 40 mm/1st h, chest X-ray showed military mottling [Figure 1]. Sputum examination for acid fast-bacilli (AFB) could not be done as a patient had no cough. Mantoux test was positive and cerebrospinal fluid (CSF) was clear, with 4 white blood cell/dL (100% lymphocytes), sugar 52 mg/dL, proteins 37 mg/dL, chloride 89 mmol/L. AFB staining was negative in CSF with adenosine deaminase activity levels of 7 IU/L. Magnetic resonance imaging (MRI) brain showed multiple well-defined ring-enhancing lesions with perilesional edema in the bilateral cerebral and cerebellar hemispheres, midbrain, pons, medulla suggestive of tuberculomas [Figures 2 and 3]. Spine MRI showed intramedullary ring enhancing lesions affecting the cervical and dorsal regions and edema also noted [Figures 4-6]. Polymerase chain reaction (PCR) test was positive for Mycobacterium tuberculosis.

Based on chest X-ray, MRI and PCR, the diagnosis of multiple tuberculomas was made.

DISCUSSION

Central nervous system tuberculosis is rare, affecting 0.5-2% of patients with systemic tuberculosis.

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10.4103/2347-8659.153980

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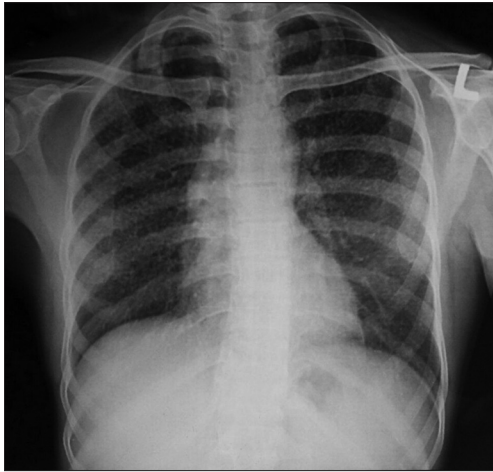


Figure 1: Chest X-ray showed miliary mottling suggestive of miliary tuberculosis

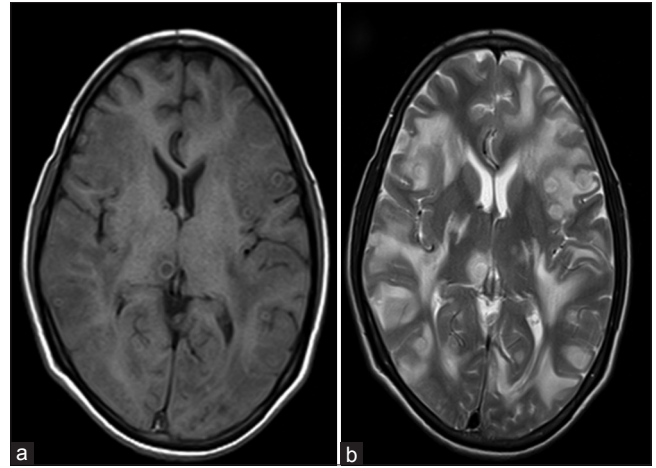


Figure 2: (a) T1W axial image shows multiple ill-defined ring shaped lesions in right thalamus, left parietal, and bilateral temporal lobes; (b) T2W axial images show multiple ill-defined lesions with disproportionate edema

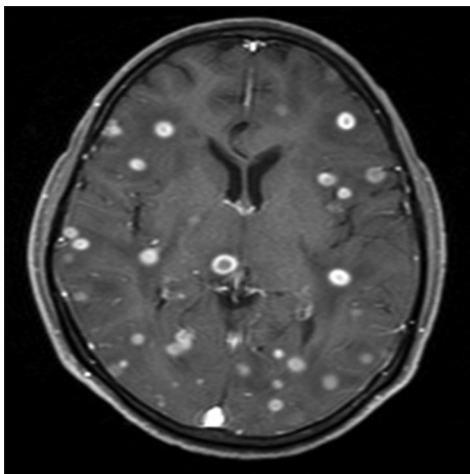


Figure 3: T1 axial postgadolinium shows multiple ring enhancing lesions



Figure 4: T1 postgadolinium sagittal images show multiple ring enhancing lesions in pons, cerebellum, medulla and intramedullary cervical region



Figure 5: D spine T1 postgadolinium sagittal image show intramedullary ring enhancing lesion at D10, and D12, L1 edema



Figure 6: T1 postgadolinium sagittal images of entire spine show multiple ring enhancing lesion at cervical and dorsal with vertebral edema (D1, D2, D4, D12)

Tuberculoma is a peculiar manifestation of tuberculosis that might occur in any solid organ of the body. It is, usually, formed by conglomeration of several miliary tubercles, with the centre of the conglomeration becoming caseous. Caseous material gets inspissated and sometimes liquefied. A thick capsule may form around these lesions.^[6] Concurrent occurrence of

intracranial tuberculomas along with intramedullary spinal tuberculomas is extremely rare.^[7,8]

The case presented here was diagnosed in the background of miliary tuberculosis. The chest X-ray



Figure 7: Magnetic resonance imaging of brain and spine reveal absence of the lesion and normal function

of this patient showed miliary mottling. The patient had elevated ESR and was symptomatic in the form of loss of weight and appetite with occasional evening rise of temperature.

Magnetic resonance imaging is the optimal measure because it shows location, size, and number of lesions and the presence of degeneration and necrosis.^[9] The MRI brain showed multiple ring-enhancing lesions with moderate perilesional edema in the bilateral cerebral hemispheres, bilateral cerebellar hemispheres, midbrain, pons and medulla suggestive of tuberculomas. Spine MRI showed ring enhancing lesions at C2-C3, C6, C7 levels and elongated lesion at D10 level indicating tuberculomas.

The differential diagnosis of tuberculomas includes granulomas such as cysticercal granulomas and neoplastic lesions such as astrocytoma, metastasis or lymphoma. In this case, the clinical presentation and size of the lesion combined with the classical ring enhancement and surrounding edema was thought to be typical of a tuberculous granuloma. Clinical

improvement and resolution of the lesion in the brain as well as spinal cord as seen on the MRI [Figure 7] after the institution of 12 months (2 months intensive phase + 10 months of continuation phase) using anti-tuberculous treatment along with dexamethasone in intensive phase confirmed our diagnosis.

In conclusion, we report a case of concurrent occurrence of intramedullary and intracranial tuberculomas in a patient of military tuberculosis. This case is being presented because of extreme rarity. Medical therapy is generally advocated as the initial treatment.

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Cite this article as: Diguvinti S, Damam S, Ubara KK, Dara C. Concurrent occurrence of both intracranial and intramedullary tuberculomas. *Neuroimmunol Neuroinflammation* 2015;2(2):118-20.

Source of Support: Nil. **Conflict of Interest:** No.

Received: 17-09-2014; **Accepted:** 14-10-2014

Primary supratentorial intracerebral malignant paraganglioma

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ABSTRACT

Paragangliomas are extra-adrenal neuroendocrine tumors that derive from neural crest. In general, they are benign tumors but few cases had shown a tendency to metastasize. Malignant forms have been reported previously with intracranial metastasis from duodenal origin, but primary intracranial origin represents a rare and unusual location for such tumors. Here, we report a rare case of a 48-year-old lady who presented with symptomatic right-sided insular mass with negative metastatic work up. A complete surgical resection had been done with an unexpected diagnosis of primary gangliocytic paraganglioma with malignant features.

Key words: Brain, chemodectoma, gangliocytic paraganglioma, intracerebral, intracranial

INTRODUCTION

Paragangliomas are rare neuroendocrine tumors. They arise from the paraganglia distributed along the paravertebral sympathetic chains and related ganglia, as well as from the parasympathetic paraganglia such as aortic body, carotid body, and vagal nerve. As a result of their neuroectodermal origin, few paragangliomas can be functional. Hence, autonomic dysfunctional symptoms may occur such as excessive sweating, hypertension, and tachycardia secondary to vasoactive substances release. They are known to be benign tumors with WHO Grade I. However, around 5-10% of them may transform malignant along the course of the disease.^[1]

Paraganglioma may also arise from intracranial or intra-spinal origin. Several explanations were proposed for their existence which include possible growth from ganglionic cells, growth from paraganglia associated with blood vessels or growth from embryonic neuroepithelial rest.^[2] They have a tendency to grow in the sellar/parasellar region or cauda equina region. However, the intraparenchymal growth of paraganglioma is uncommon with seldom reported cases in the literature. An unusual case of primary

intraparenchymal paraganglioma with malignant features was encountered lately at our center. The case is considered to be the first of its kind, and it does reflect the potential risk for malignant primary growth of paraganglioma within the brain parenchyma. A review of the literature for intracranial supratentorial paragangliomas along with the predisposing genetic mutation will be discussed.

CASE REPORT

A 48-year-old healthy female presented to the emergency department with 1-week history of headache and slurred speech. She noticed that her balance had got worse 3 days prior to presentation. On examination, she was noticed to be ataxic with left sided pronator drift and left lower facial asymmetry. The initial computed tomography (CT) scan of her brain showed an iso-dense mass in the right sub-insular area measuring 3.6 cm × 4.1 cm × 3.7 cm, along with perilesional vasogenic edema and mass effect over adjacent structures [Figure 1]. There was no evidence of calcification or hemorrhagic foci within the lesion. It carried slightly low intensity signal in T1-weighted image with heterogenous signal intensity in T2-weighted image. The lesion was well-demarcated and had homogenous gadolinium uptake with restricted diffusion in diffusion-weighted image/apparent diffusion coefficient map [Figure 2].

The patient was admitted to the hospital for a right-sided fronto-parieto-temporal craniotomy for tumor resection guided by neuronavigation. Intraoperatively, the tumor

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10.4103/2347-8659.154431

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was firm in consistency and very vascular in nature but eventually we were able to achieve gross total resection. The early postoperative course was unremarkable for any neurological deficits until patient deteriorated few hours thereafter and had asymmetric blown pupils. Owing to the urgency of the case, the patient was taken for surgery with a provisional diagnosis of postoperative bleeding. Surgical re-exploration was done, and large intracerebral hematoma within the tumor bed was evacuated. The patient, unfortunately, woke up postoperatively with dense left sided hemiplegia [Figure 3].

Microscopic examination of the tumor revealed nests of highly pleomorphic, spindle, epithelioid,

and large atypical cells with prominent nuclei and nucleoli [Figure 4a]. Abnormal mitotic figures and apoptotic nuclei were common. The tight collections of neoplastic cells were surrounded by rich fibrovascular stroma forming the architectural patterns of “Zellballen”. The majority of the neoplastic cells were strongly positive for chromogranin, synaptophysin (SYN), and neuron-specific enolase, and focally for tyrosine hydroxylase [Figure 4b]. Ganglionic cells displayed strong cytoplasmic reaction for SYN and less often tubulin [Figure 4c]. Glial fibrillary acidic protein showed very strong reaction in the cells outlining the edges of the neoplastic congregates, in a pattern seen in sustentacular cells of extracranial paragangliomas [Figure 4d]. The rest of the immunohistochemistry is outlined in Table 1.



Figure 1: Preoperative plain computed tomography head shows a right-sided isodense lesion within the insular region

At this point, an extensive metastatic work up had been done which included CT of her chest, abdomen, and pelvis, as well as mammogram. Due to the fact that this tumor has been frequently originating from the duodenum, a duodenal scope was also performed. The analysis resulted negative for any primary lesions. As some paragangliomas can be functionally active, serum and urine metanephrines, and catecholamines were investigated and resulted also negative. Given these results and the pathological features of the resected tumor, the assumption was made being a primary malignant gangliocytic paraganglioma. The patient was approached in a multidisciplinary team including the radiation oncology and offered local tumor bed radiation. Due to low karnofsky performance

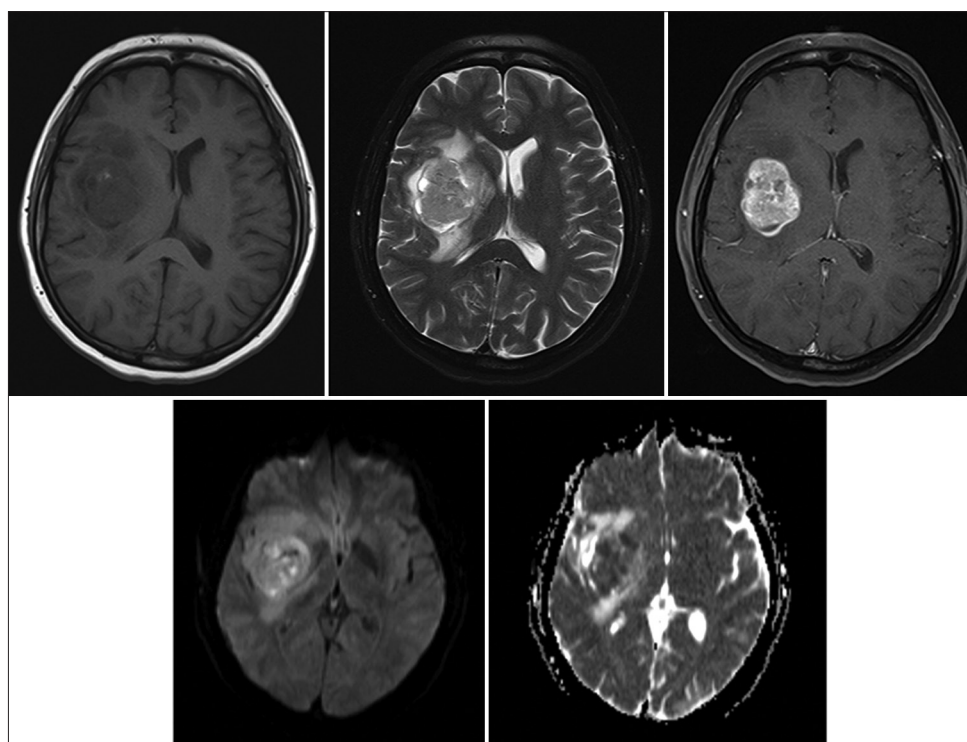


Figure 2: A well-circumscribed tumor that is hypointense in T1-weighted image with heterogeneous texture in T2-weighted image. The lesion was homogeneously enhancing post-gadolinium with restricted diffusion

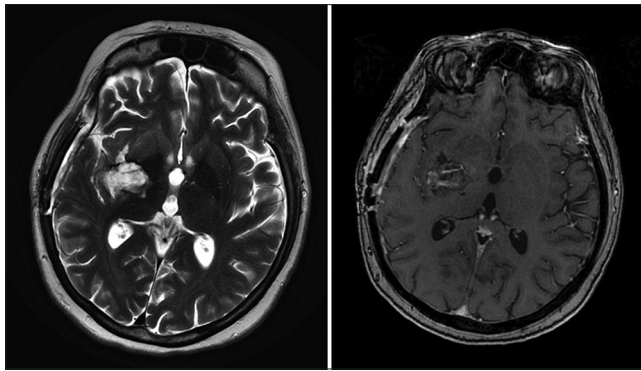


Figure 3: Brain magnetic resonance imaging after 6 weeks from surgery depicts a tumor bed cavity with complete resection. A slight gadolinium uptake was seen within the tumor cavity which represents remnant of fibrillary surgi-cells packing (ethicon) from the second surgery

scale (KPS) of 30, the radiation treatment was deferred until the patient gains more functional strength. She remained stable along with no tumor recurrence on serial follow-up magnetic resonance imaging.

Unfortunately, 12 months postoperatively she presented with a 5 cm × 5 cm ipsilateral enhancing lesion associated with mass effect. Although she was asymptomatic, we opted for surgical resection given the size of the mass that would interfere with radiation therapy. As expected, the pathological work up revealed similar findings to previously resected tumor. Although her surgical resection went uneventful, she continued to decline her functions with lower KPS, which affected the delivery of radiation treatment. She was referred eventually into palliative care unit and died after 2 months from her second surgery.

DISCUSSION

Paragangliomas are neural crest-derived tumors that originate in the paraganglia of the autonomic nervous system. Although they are usually considered benign growths, but occasionally malignant forms may occur. They can be functional lesions with active release of catecholamines upon manipulation to result in hemodynamic alterations. The incidence of paraganglioma is frequently reported in combination with pheochromocytoma owing to its rareness along with analogous histological features. The combined annual incidence in United States, for instance, is 500-1600 cases per year with 50% of them present with hypertension.^[3] Paragangliomas are usually sporadic but genetic and syndromic associations have been described. In about 27-32% cases of paraganglioma, genetic mutations have been discovered among which the succinate dehydrogenase complex mutation is considered one of the most commonly affected genes.^[4,5] In addition, several recent works have also correlated the SDHB gene mutation and the malignant behavior of paragangliomas.^[6-9]

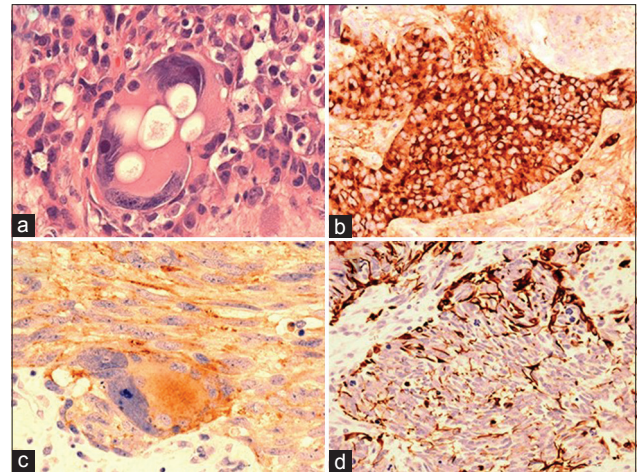


Figure 4: (a) Large neoplastic ganglionic cell with prominent nucleoli and cytoplasmic vacuolation HE; (b) strong chromogranin immunoreactivity in epithelioid tumor cells; (c) cytoplasmic synaptophysin immunoreactivity in a large ganglionic cell and the processes of the adjacent tumor cells; (d) glial fibrillary acidic protein immunoreaction accentuated at the periphery of the tumor nest characteristically seen in sustentacular paraganglioma cells

There have been only 37 reported cases of primary intracranial supratentorial paragangliomas since 1960, and they all harbor benign growth features [Table 2].

In this manuscript, we have excluded from our review all intracranial metastatic paragangliomas and lesions originated in the infratentorial compartment. We found that the mean age of presentation is 47 with 1:1.3 female:male ratio. In around three-quarter of these cases, the sellar/parasellar region was the most common location with symptoms of headache, ophthalmoplegia, and endocrinopathy.^[43] Again, the benign behavior was a character of all of them with few cases required radiation therapy for incomplete resection.

In term of intraparenchymal origin, only three cases had been reported and were harboring benign pattern. Their location within the brain represents a rare condition. Reithmeier *et al.*^[23] had reported the first case of primary paraganglioma in the insular region. This patient was successfully treated with a complete gross total resection, but dense left sided hemiplegia had occurred postoperatively secondary to cerebral vasospasm. In another case, a left temporal melanotic paraganglioma had developed in a patient who was treated previously with chemotherapy and radiotherapy for langerhans cell histiocytosis.^[35] The lesion was partially resected followed by postoperative radiation treatment. The third case had occurred in premotor region, and it was resected completely with the patient remaining stable at short-term follow-up.^[38]

In this report, we presented a unique case of paraganglioma in which primary intraparenchymal growth with malignant features and ganglionic

Table 1: Immunohistochemical evaluation result for primary malignant ganglionic paraganglioma

Antibody	Distributor	Abbreviation (dilution)	Immunoreactivity
Neurofilament MAb	DAKO	NF (1:400)	-
Neural CAM	DAKO	CD56 (undiluted)	+++
β-tubulin	SIGMA	βTUB (1:5000)	F++
Synaptophysin MAb	DAKO	SYN (1:25)	++
Chromogranin A MAb	DAKO	CHRG (1:100)	++
Calretinin Poly Ab	Invitrogen	CAL (1:200)	F+
Neuron-specific enolase	DAKO	NSE (undiluted)	+, ++
S100 protein	DACO	S100 (1:4000)	-
Epithelial membrane antigen MAb	DACO	EMA (1:100)	-
Keratins	DACO	CAM5.2, AE1/AE3 (undiluted)	-
GFAP Poly Ab	DAKO	GFAP (1:3000)	++ sustentacular c.
Anti-human melanosome	DAKO	HMB 45 (undiluted)	+ sustentacular c.
Desmin	DACO	DSM (undiluted)	-
p53	DAKO	p53 (1:200)	+
Ki-67 MAb	DAKO	Ki-67 (1:1000)	+++

GFAP: glial fibrillary acidic protein; CAM: cell adhesion molecule; MAb: monoclonal antibody

Table 2: Literature review for intracranial supratentorial primary paraganglioma

Author	Age (gender)	Presentation	Location	Treatment	Outcome
Kruse, 1960 ^[10]	68, male	Behavioral changes	Middle fossa	Resection	Improved
Smith <i>et al.</i> , 1966 ^{*[11]}	17, male	Headache	Pineal region	Resection	Moderate disability
Chytil, 1967 ^[12]	46, male	Visual loss, hypopituitarism	Sellar/suprasellar	Resection + RT	No progression
Bilbao <i>et al.</i> , 1978 ^[13]	37, male	Delayed growth	Sellar	Resection	-
Ho <i>et al.</i> , 1982 ^[14]	65, male	Diplopia	Cavernous sinus	Resection	Moderate disability
Prabhakar <i>et al.</i> , 1984 ^[15]	7, female	Ophthalmoplegia	Parasellar	Resection + RT	-
Steel <i>et al.</i> , 1993 ^[16]	44, female	Headache	Sellar	Resection + RT	No progression
	41, female	Headache, ptosis	Sellar	Resection + RT	No progression
Flint <i>et al.</i> , 1993 ^[17]	17, female	Visual defect	Sellar	Resection	-
Scheithauer <i>et al.</i> , 1996 ^[18]	14, male	Visual defect	Sellar/parasellar	Resection + RT	Left hemiparesis
Nishitani <i>et al.</i> , 1996 ^{*[19]}	41, female	Amenorrhea	Parasellar	Resection	Good recovery
Noble <i>et al.</i> , 1997 ^[8]	71, male	Visual defect	Sellar	Resection	-
Mokry <i>et al.</i> , 1998 ^[9]	76, female	Visual defect	Sellar	Resection	Unchanged
Caro <i>et al.</i> , 1998 ^[20]	84, male	Memory loss	Sellar/suprasellar	Resection	-
Sambaziotis <i>et al.</i> , 1999 ^[21]	54, male	Visual defect	Sellar	Resection	No progression
Yamauchi <i>et al.</i> , 1999 ^[22]	56, female	Headache	Frontal fossa	Resection	No progression
Reithmeier <i>et al.</i> , 2000 ^[23]	42, male	Seizure	Insula	Resection	Hemiparesis
Laquis <i>et al.</i> , 2001 ^[24]	15, female	Oculomotor palsy	Middle fossa	Resection + RT	Improved
Salame <i>et al.</i> , 2001 ^[25]	48, female	Oligomenorrhea	Sellar/parasellar	Resection	No progression
Hertel <i>et al.</i> , 2003 ^[26]	51, female	Facial paresis	Middle fossa	Resection + RT	Oculomotor palsy
Yokoo <i>et al.</i> , 2003 ^[27]	52, female	Behavioral changes	Suprasellar	Resection	-
Arkha <i>et al.</i> , 2003 ^[28]	58, female	Endocrine dysfunction	Sellar/parasellar	Resection	-
Riopel <i>et al.</i> , 2004 ^[29]	66, male	Diplopia	Parasellar	Biopsy	-
Naggara <i>et al.</i> , 2005 ^[30]	47, male	Visual defect	Suprasellar	Resection	-
Zorlu <i>et al.</i> , 2005 ^[31]	37, male	Visual defect	Sellar/suprasellar	Resection + RT	-
Boari <i>et al.</i> , 2006 ^[32]	52, male	Brain ischemia	Sellar	Resection	Pituitary dysfunction
Peltier <i>et al.</i> , 2007 ^[33]	51, female	Oculomotor palsy	Parasellar	Resection	-
Sinha <i>et al.</i> , 2008 ^[34]	18, male	Visual defect	Sellar	Resection + RT	Skull, scalp and femur metastasis
Yoo <i>et al.</i> , 2008 ^[35]	21, female	Headache	Temporal lobe	Resection + RT	-
Ozüm <i>et al.</i> , 2008 ^[36]	70, male	Headache	Sellar/parasellar	Resection + RT	-
Lu <i>et al.</i> , 2009 ^[5]	81, male	Visual change	Sellar/suprasellar	Resection	Died 4 months after (esophageal cancer)
Haresh <i>et al.</i> , 2009 ^[37]	17, male	Visual change	Sellar/suprasellar	Resection + RT	Skull and femur metastasis
Thakar <i>et al.</i> , 2011 ^[38]	40, male	Visual defect	Frontal lobe	Resection	Recurrence (6 months)
Prajsnar <i>et al.</i> , 2011 ^[39]	53, female	Trigeminal neuralgia	Meckel's cave	Resection	Recurrence (2 years)
Albert <i>et al.</i> , 2011 ^[40]	63, male	Proptosis	Sellar/parasellar	Resection + RT	Improved
Nascimento <i>et al.</i> , 2012 ^[41]	33, female	Endocrine dysfunction	Sellar	Resection	Diabetes insipidus
Chaudhry <i>et al.</i> , 2013 ^[42]	44, male	Visual defect	Sellar/suprasellar	Resection	No recurrence

*Found in Yamauchi *et al.* review of literature, 1999. RT: radiation therapy

component makes it unique and the first of this type. The radiological features were not conclusive for primary malignant paraganglioma as only extracranial

primary malignant forms are known. In addition, the tumor was not functional, and it did not associate with any hemodynamic changes intra-operatively to alert for

possible active paraganglioma. Although the resection was complete, but the functional deconditioning that can be attributed to postoperative bleeding impeded early adjuvant therapy.

Radiation therapy for local control has been proposed for incomplete resection of benign paraganglioma. There seems to be a better response to radiosurgery than external beam radiation therapy for such lesions. The treatment is being delivered at a range of 12-15 Gy to the tumor margin.^[44] We would have planned similar treatment for our patient if she was in a better functional state. Chemotherapy appears to play a role in terms of stabilizing the disease and in some it may lead to reduction in tumor size. This could not be applied to our case given the low KPS and stable tumor bed on serial imaging follow-up.

Even with treatment, the prognosis of malignant paraganglioma remains dismal with 50% reported mortality in 5 years.^[45] However, such data were reported for extracranial paraganglioma/pheochromocytoma and we still do not know if this can be applied for intracerebral primary lesions, which are similar to our case. We suggest that such tumors should be treated with upfront postoperative radiation therapy, preferably radiosurgery. Similarly, it is worth considering delivering chemotherapy based on postoperative resection and risk for future recurrence.

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Cite this article as: Al Jishi AA, Lach B, Elgheriani A, Kachur E, Cenic A. Primary supratentorial intracerebral malignant paraganglioma. *Neuroimmunol Neuroinflammation* 2015;2(2):121-6.

Source of Support: Nil. **Conflict of Interest:** No.

Received: 13-09-2014; **Accepted:** 23-12-2014

The role of glutamate excitotoxicity and neuroinflammation in depression and suicidal behavior: focus on microglia cells

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Major depressive disorder (MDD) is associated with a significant disability worldwide, relevant psychosocial impairment, and increased risk of suicidal behavior.^[1] Although multiple psychoactive compounds are now available,^[2] more than 20% of MDD patients treated with traditional antidepressant drugs do not benefit from complete recovery and are affected by treatment-resistance.^[3] Traditional antidepressant medications may be ineffective and sometimes worsen depressive symptoms in a vulnerable subpopulation of patients with subthreshold hypomanic symptoms that may be better included in the bipolar spectrum rather than MDD.^[4]

The most recent years have been characterized by the paradigm shift from the monoamine conceptualization of depression to neuroplasticity hypothesis mainly focused on glutamatergic dysfunctions.^[5] There are consistent evidence reporting that abnormalities of glutamatergic neurotransmission are common in depressed individuals.^[6] Specifically, N-methyl-D-aspartate receptors (NMDAR) overactivation seems to play a critical role in the pathogenesis of MDD as reducing their functioning may be associated with mood recovery. For instance, ketamine has been recently investigated for its potential antidepressant effects^[7] and improvement of suicidal ideation^[8] beyond the monoamine hypothesis.

In addition, the existence of abnormalities in inflammatory processes in depressed patients suggests

the immunological origin of major depression.^[9] Inflammatory mediators and oxidative stress may lead to glutamate excitotoxicity playing a significant role in the pathogenesis of MDD.^[10] Notably, immunological differences have been frequently observed in patients with MDD and suicidal behavior.

Glial cells have been proposed as potential candidate targets for both glutamatergic and inflammatory-mediated alterations underlying MDD and suicidal behavior.^[11,12] Historically, glial cells may be grouped in astrocytes, oligodendrocytes, and microglia.

Microglial cells derive from the immune system and may be considered the immunologic sentinel cells of the brain. As the activation of microglial cells was associated with the abnormal production of inflammatory mediators,^[13,14] these cells have been proposed as possible effectors of the abnormal immune response in MDD. They provide immunomodulatory functions,^[15] and functionally support neural plasticity-processes.^[9]

Importantly, the abnormal activation of microglial cells reflected long-lasting depression- and anxiety-like behavioral effects.^[11] This has been also confirmed by the fact that minocycline with anti-inflammatory properties and microglia inactivation is not only able to reverse microglial alterations,^[16] but is also associated with antidepressant-like activity in rats exposed to learned helplessness.^[17]

Unfortunately, some of the existing studies supporting the link between abnormal glial activation and MDD/suicidal behavior are limited by small sample size. As an example, Bayer *et al.*^[18] in a postmortem study found elevated microglial cells in both frontal cortex and hippocampus of 6 depressed and 14 psychotic

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10.4103/2347-8659.157955

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individuals compared to 13 healthy controls. Steiner *et al.*^[19] observed increased microglial cell numbers in the anterior cingulate cortex and mediodorsal thalamus of two individuals who committed suicide during acute psychosis. However, no effect of diagnosis on the microglial density but only significant microgliosis was reported in dorsolateral prefrontal cortex, anterior cingulate cortex, mediodorsal thalamus, and hippocampus of 16 schizophrenic and 14 depressed subjects died by suicide.^[20] The authors hypothesized that the link between microglial activation and suicidal behavior may be mediated by neuroendocrine factors such as inflammatory cytokines and oxidative stress. Finally, Dean *et al.*^[21] showed that CD11b (a potential microglia/macrophages marker) was not increased in the cortex of ten subjects with MDD, and ten with bipolar disorder.

To the best of our knowledge, there are no reports in the current literature concerning the association between microglial glutamatergic abnormalities and MDD/suicidal behavior with the exception of the review of Niciu *et al.*^[22] suggesting that glial-mediated glutamatergic dysfunction is a common neuropathological pathway in patients with substance use disorders and MDD.

It is currently unclear whether microglial abnormal activation may directly induce psychopathological conditions, should be considered an epiphenomenon of other related processes associated, in turn, with psychopathological conditions, or alternatively a nonspecific tissue reaction independent of psychopathology. Another controversial issue concerns the exact relationship between inflammatory stressful stimuli, autoimmunity, and abnormalities in glutamatergic activity.

The sequence of molecular events underlying MDD and suicidal behavior is still poorly understood. Microglia and monocytes are usually involved in the integration of sensory information within the peripheral sensory nerves and endocrine system.^[23] Stress and other signaling molecules (e.g. cytokines, oxidative free radicals) may activate the oxidation sensitive transcription factor nuclear factor- κ B (NF- κ B), which is highly expressed in microglia with the final result of increased NF- κ B-DNA binding and transcription of genes encoding for chemokines, cytokines, and oxidases/proteases. As suggested by the same authors,^[24] microglia reported morphological changes in response to exposure to both environmental and internal stimuli.

Furthermore, antibodies against serotonin have been commonly found in more than 50% of depressed patients and importantly, in all those conditions

in which increased inflammatory cytokines were observed.^[24] They cannot affect brain functions until inflammatory mediators do alter the integrity of blood-brain barrier. However, when a blood-brain barrier alteration occurs, antibodies may presumably cross-react with the subunits of NMDAR on glial cells inducing the abnormal release of glutamate. This enhanced activation of glial cells is associated with glutamatergic excitotoxicity, apoptosis, and clinically significant behavioral changes.^[25,26] Also, as suggested by Santello *et al.*,^[27] tumor necrosis factor alpha (TNF- α) controls the neuromodulatory action of dentate granule cell synapses in astrocytes, through Ca²⁺-dependent glutamate release and pre-NMDAR activation. Therefore, gliotransmission together with its synaptic effects seem to be controlled not only by astrocyte Ca²⁺ elevations but also by homeostatic factors such as TNF- α . Previous studies have shown that both the excitatory neurotransmitter glutamate and the proinflammatory cytokine TNF- α may be considered as effectors of microglial-stimulated death.^[28]

Recently, Schnieder *et al.*^[29] also found a 18% greater density of perivascular cells in dorsal white matter prefrontal cortex of 11 subjects died by suicide suggesting the induction of important alterations in the characteristics of blood-brain barrier in microglia cells of these individuals. Autoimmune activity directed against serotonin may directly compromise serotonergic axons and their functioning with the final result of relevant deficits in serotonergic neurotransmission. Serotonin may be disrupted by abnormal (e.g. hyperactivated) pathways such as that of kynurenine inside microglial cells due to the enhanced metabolism of tryptophan to quinolinic acid.^[30,31]

Elevated levels of kynurenic acid, an astrocyte-derived metabolite of the kynurenine pathway has been reported to significantly reduce glutamate release in some brain regions such as the hippocampus.^[32,33] Also, increased micromolar levels of kynurenic acid have been suggested to inhibit α -amino-3-hydroxy-5-methyl-4-isoxazole propionic acid and kainate receptors.^[34,35]

Inflammatory cytokines may further induce the abnormal release of quinolinic acid in microglia^[36] related to aberrant stimulation of neurons in the ventral prefrontal cortex and altered connectivity between cortical structures and limbic system.

The critical role of quinolinic acid in microglia-abnormal activation has been also demonstrated by the elevated levels of indoleamine 2,3-dioxygenase and kynurenine monooxygenase in the quinolinic acid biosynthesis pathway in CX3CR1 knockout mice.^[37] Notably, recent compounds with antidepressant properties

such as ketamine^[38] as well as selective serotonin reuptake inhibitors^[39] and tricyclic^[40,41] may reverse the neurodegenerative activation of microglia induced by pathologically increased inflammatory cytokines.

Furthermore, increased glucocorticoids levels may affect the integrity of microglial cells in the early phases of depression.^[42] Abnormal extracellular glutamate concentrations may spill into microglial cells exerting neurotoxic effects on γ -aminobutyric acid neurons.^[43] The uptake of glutamate is progressively decreased, and the density of glutamatergic pyramidal neurons is reduced in depressed individuals^[44] with the final result of reduced cortical levels of glutamate in the later phases of depression.

Overall, microglial cells are able to exert significant immunomodulatory functions in the central nervous system. Structural changes induced by chronic stress and MDD on glial cells may contribute to the pathophysiology of these conditions, but they may be significantly reversed using modern antidepressant medications. Aberrant levels of quinolinic acid produced by abnormal pathways inside microglia cells represent a valid intracellular mediator of pathologically inflammatory- and glutamatergic-related changes. A complex interaction between dysfunctional inflammatory pathways, increased oxidative stress, altered neuroplasticity in glial cells and neuronal abnormalities are involved in both MDD and suicidal behavior.

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Cite this article as: Serafini G, Rihmer Z, Amore M. The role of glutamate excitotoxicity and neuroinflammation in depression and suicidal behavior: focus on microglia cells. *Neuroimmunol Neuroinflammation* 2015;2(3):127-30.

Source of Support: Nil. **Conflict of Interest:** No.

Received: 25-09-2014; **Accepted:** 18-01-2015

Microglia and astroglia: the role of neuroinflammation in lead toxicity and neuronal injury in the brain

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ABSTRACT

Lead (Pb²⁺), a ubiquitous environmental toxicant, may widely affect the function of many organs or systems of human beings, especially the brain. Although lead is believed to transport into the brain through the blood-brain barrier (BBB) and cause direct neuronal injury, growing data have shown that lead exposure could induce brain dysfunction by triggering microglial and astroglial activation, pro-inflammatory cytokine production and inflammatory response, generation of reactive oxygen species and oxidative stress, and finally result in BBB dysfunction and neuronal damage. This review summarizes recent studies regarding microglial and astroglial reaction, neuroinflammation, and neuronal death in the brain following lead insult, suggesting that reactive glial cells may represent a potential target for manipulation of lead-induced neuroinflammatory injury of the brain.

Key words: Astroglia, brain, lead toxicity, microglia, neuroinflammation

INTRODUCTION

Lead (Pb²⁺) is a widely distributed heavy metal and environmental toxicant, and overexposure to lead due to pollution or accident can impair the function of the nervous system, especially learning and memory abilities of developing brains during childhood.^[1-4] Epidemiologic data indicate that learning impairment may be caused by moderate lead exposure in young individuals.^[2,3] This impairment is largely related to neuronal injury caused by lead toxicity, but the detailed mechanism by which lead exposure induces neuronal injury, neuronal death, and brain dysfunction still remains elusive.^[4,5] Several studies have indicated that lead exposure may interfere with calcium signaling, suppress neurogenesis and neuronal differentiation, inhibit formation of long-term potentiation (LTP), influence secretion of neurotransmitters, and even

enhance production of amyloid protein.^[6-13] Lead can also bind to key metabolic enzymes such as pyruvate kinase, induce reactive oxygen species (ROS), impede the supply of energy to neurons, and cause neuronal apoptosis.^[14-17] Moreover, recent studies have shown a crucial involvement of microglial and astroglial cells in neuroinflammatory injury induced by lead exposure.^[10,11] Microglia and astrocytes are two major types of glial cells involved in the regulation of the immune response to pathological processes in the brain.^[18] Functional activation of microglia and astrocytes and the resulting neuroinflammation are associated with infection, autoimmunity, and pathogenesis of neurodegenerative diseases. In response to lead exposure, microglia and astrocytes can increase the production and release of inflammatory cytokines, enhance ROS generation, impede antioxidant activity, and result in neuronal injury or neuronal loss in the brain or other parts of the central nervous system (CNS).^[10,11,19-22]

MICROGLIAL ACTIVATION, PRO-INFLAMMATORY CYTOKINES, AND NEUROINFLAMMATION

It is generally regarded that microglial cells are derived from blood monocytes that reset in the CNS during embryonic development and are functionally

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10.4103/2347-8659.156980

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involved in neuronal maintenance, injury, and repair in a manner similar to peripheral macrophages.^[23] Microglial cells are a predominant source of various inflammatory cytokines, that is, interleukin-1 beta (IL-1 β), tumor necrosis factor-alpha (TNF- α), and interferon-gamma (IFN- γ), which can then induce a broad spectrum of inflammatory reactions. The activation of microglia and astrocytes in response to internal and external stimuli or insults might further increase the release of cytotoxic substances, pro-inflammatory cytokines, ROS, and excitatory amino acids, thus causing further neuronal injury in the brain.^[22]

Lead-induced inflammatory cytokines in microglial cells

Obvious morphological change and higher synthesis of cytokines have been observed in activated microglial cells after lead exposure.^[10,11,24] For instance, elevated expression of IL-1 β and TNF- α is found in the cerebral cortex after lead exposure, as well as increased expression of IL-1 β and IL-6 in the hippocampus.^[25,26] *In vitro* experiments have also confirmed the elevation of TNF- α expression after lead exposure.^[27] Gene expression analysis has shown that levels of the pro-inflammatory factors IL-6 and TNF- α are significantly perturbed by the lead insult in multiple brain regions.^[19,20] These cytokines are co-expressed in glial cells in response to lead crossing the blood-brain barrier (BBB) and might also represent a mechanism for lead toxicity to the immature brain. Conversely, anti-inflammatory factors such as IL-10 and transforming growth factor beta (TGF- β) are decreased in the cortex in response to lead, as detected by real time-polymerase chain reaction.

Lead-induced reactive oxygen species generation in microglial cells

Lead exposure might destroy the glial support of neuronal cells by increasing ROS and other toxins in microglial cells.^[28] The microglial inflammatory response is also associated with the production of ROS and nitric oxide (NO)-dependent reactive nitrogen species (RNS).^[19] Nicotinamide adenine dinucleotide phosphate (NADPH) oxidase (NOX), which is ubiquitously expressed in microglia, contributes much to the production of superoxide and the induction of ROS.^[29] Furthermore, NOX could be activated in monocytes and microglial cells by IL-1 β , TNF- α , IFN- γ , and other pro-inflammatory cytokines.^[19] The inducible NO synthase (iNOS) is also prevalent in microglia, and microglial NO generation regulates vascular relaxation and initiates rapidly induced, transiently regulated signaling events.^[30] On the other hand, lead also increases NOX, which causes superoxide production and inhibits antioxidant production, and increases the accumulation of ROS in the brain.^[22] It is well-known

that neurons in the brain are vulnerable to excess ROS and RNS. Oxidative stress could result in the death of newly-born neurons by disrupting signaling processes, dysfunction of ion homeostasis, and protein misfolding.^[29]

The signaling pathways involved in lead-induced microglial activation, however, need more investigation. In response to various environmental toxins including lead, microglia could enter the activated state and release ROS.^[31] Pattern recognition receptors expressed on microglia might be one common signaling pathway. For example, toll-like receptors act as initiators of the nuclear factor kappa B (NF- κ B) pathway when exposed to several toxins, such as lipopolysaccharide (LPS), resulting in the release of pro-inflammatory cytokines.^[32] However, it is still not clear how lead could induce microglial activation and trigger inflammatory cytokine production, which remains a critical question to be answered.

ASTROCYTIC ACTIVATION AND THE NEUROINFLAMMATORY RESPONSE

The neurovascular unit in the brain comprises of neurons, blood vessels and their adjacent astrocytes.^[33,34] The concept of a functional unit is a new one, and emphasizes the interaction between neurons and astrocytes under both normal and pathological physiological conditions. Astrocytes play a critical role in neuron function, including energy support, metabolism, and synapse formation.^[35,36] Astrocytes maintain the trans-endothelial electric resistance (TEER) of the BBB.^[37] Under pathological conditions, astrocytes might remove toxic substances and balance electrolyte and water levels.^[33] It has been found that lead interferes with astrocyte functions such as energy metabolism, immune response, and ROS removal. Furthermore, astrocytes could collaborate with microglia to switch on neuroinflammatory reactions in the brain, and each of these effects can result in BBB dysfunction and injury to neurons.

Lead exposure leads to the insufficient supply of energy from astrocytes to neurons. Astrocytes contain a large number of mitochondria for energy and glutamate metabolism. Neurons in the brain show a preference for lactose and glutamine provided by astrocytes via shuttle routes.^[38] Glycogen is exclusively localized in astrocytes in the adult brain^[39] and can be metabolized to pyruvate, which is converted to lactate by lactate dehydrogenase mainly in astrocytes and then transported to neurons. When energy is insufficient, astrocytes can also use glycolysis from stored glycogen for the use of neurons.^[40,41] Glycogen metabolism in astrocytes is also required for long-term

memory formation in the brain.^[42] Lead exposure causes morphological and functional changes in astrocytic mitochondria.^[9] Creatine kinase and pyruvate kinase are two key enzymes in astrocytes that are involved in the production of pyruvate and lactate, and lead can bind to their sulfhydryl groups and decrease activity, resulting in an insufficient supply of pyruvate and lactate from astrocytes to neurons.^[16] Lead can also act on cytochrome C and adenosine triphosphate synthase to cause dysfunction of the electron transport chain in mitochondria and generation of free radicals.^[14,43] Mitochondrial dysfunction in astrocytes affects the survival of motor neurons.^[44] Accumulation of free radicals and ROS might enhance the lack of energy and glutamine, eventually causing neuronal apoptosis.^[15]

Lead triggers inflammation through a collaboration of astrocytes with microglia. The functional collaboration between astrocytes and microglia might play an important role in neuroinflammation and BBB dysfunction in the brain.^[45] Overexpression of inflammatory stimuli in the neurovascular unit may start a response to clear antigenic material, leading to destruction of the BBB as well as neuronal damage. Following lead exposure, astrocytes secrete a number of inflammatory cytokines such as TNF- α , IL-6, and IL-10 into surrounding tissues.^[45] These cytokines further mediate the immune response, including activation of microglia and macrophages, and induce other adverse reactions, which might eventually result in the destruction of BBB tight junctions. Matrix metalloproteinases (MMPs) are an important family of proteins composed of a variety of zinc-dependent enzymes that are capable of degrading extracellular matrix proteins such as collagen, gelatin, viscous protein, fibronectin, and proteoglycans.^[46] It has been hypothesized that inflammatory cytokines induce production of MMP-2 and MMP-9, two proteinases that degrade the extracellular matrix and basement membrane, in astrocytes, resulting in increased permeability of the BBB.^[47] Other studies have shown that low concentrations of pro-inflammatory cytokines (such as TNF- α or IL-1 β) or lead did not influence MMP-9 expression when administered separately, but combined administration of lead and cytokines could induce a marked synergistic elevation in MMP-9 expression.^[48]

FUNCTIONAL CROSSTALK BETWEEN MICROGLIA AND ASTROCYTES IN NEUROINFLAMMATION

The start of an inflammatory reaction to lead exposure depends on the interaction between the inflammatory responses of astrocytes and microglial cells. Following lead exposure, activation of astrocytes surrounding blood vessels is indicated by increased expression of

glial fibrillary acidic protein (GFAP).^[49,50] Therefore, the response to lead in astrocytes may affect the BBB. It has been shown that lead in the brain accumulates predominantly in astrocytes, as opposed to neurons.^[51,52] Another culture experiment has shown that younger astrocytes accumulate and retain more lead than older astrocytes.^[53] To protect neurons against lead, astrocytes serve as a lead pool in the process of neurogenesis. However, because astrocytes are not able to remove lead from their own cytoplasm effectively, the accumulated lead will finally cause progressive damage of astrocytes, the BBB, and nearby neurons.

The response of microglia and astrocytes to neuroinflammation

Liu *et al.*^[18] has proposed that activation of microglia in response to pathological conditions such as trauma, stroke, or neurodegenerative disorders occurs before activation of astrocytes. For instance, the activation of astrocytes occurs subsequently to microglial activation in respect to the cytokine expression sequence in Alzheimer's disease.^[54,55] A study with trimethyltin (TMT) treated rats, a model of neurodegenerative disease, revealed that GFAP significantly increases following microglial activation and that microglial activation requires lower concentrations of TMT than activation of astrocytes.^[56] Considering that astrocytes are closer to the peripheral environment anatomically and more easily store toxic substances like lead, it may also be an imperceptible inflammatory signal released from astrocytes such as low amounts of TNF- α , free radicals, or ROS/NO that further initiates activation of microglial cells, leading to an inflammatory response.

The role of inflammatory cytokines and receptors in microglial-astrocytic interactions

Reciprocal activation of microglia and astrocytes mainly depends on inflammatory cytokines or their receptors.^[57] Previous studies have shown that cytokines secreted from activated microglia also promote activation of astrocytes. Among those cytokines, IL-1 is a key mediator. IL-1 β , mainly from microglia, can be rapidly expressed and may work to increase the secretion of other cytokines such as IL-6, mainly from astrocytes, in order to promote inflammation.^[35] Moreover, IL-1 might decrease the ability of astrocytes to reabsorb glutamic acid and promote the release of free radicals.^[58,59] Experiments have shown that IL-1 receptor antagonists prevent pathological damage to astrocytes,^[60] indicating that microglia might indirectly affect the function of astrocytes. In addition, microglial activation also promotes astrocytes to secrete TGF- β 1 and IL-10.^[61] When the severity of the immune response reaches a certain extent, however, TGF- β initiates a feedback loop to reduce the level of IL-1, inhibiting microglial activation and resulting in suppression of inflammation in the CNS.^[62]

BBB DYSFUNCTION RESULTING FROM LEAD INSULT AND NEUROINFLAMMATION

Inflammatory cytokines and the inflammatory response are critical in the neurovascular unit and may result in alteration of BBB function. Brain microvascular endothelial cells (BMECs) are considered to be the anatomical and functional basis of the BBB.^[63] As they are in direct contact with the circulating blood, BMECs are highly vulnerable to the impact of the blood environment. Studies have revealed that lead toxicity in the BBB or BMECs might influence tight junction proteins.^[64] Tight junctions are key functional structures that bond BMECs together. Adhesion proteins are a component of tight junctions, and the zonula occludens (ZO) family plays a key role in connecting transmembrane proteins with actins inside the ECs to complete the structure of tight junctions.^[65] In the cultured brain microvessel endothelial cell line RBE4, lead reduces the expression of tight junction proteins and lowers TEER, causing changes in ion permeability at the BBB and brain interstitial fluid ion regulation. As ZO-1 and ZO-2 are intracellular proteins, this suggests that cytoplasmic mechanisms may be associated with this process. Increased permeability of endothelial cells along with a decrease in occludin proteins has been detected following lead exposure.^[65] The ZO family also seems to be susceptible to oxidative stress, and tight junctions are destroyed by lead-induced inflammation and ROS, leading to long-term BBB damage. However, other *in vitro* and *in vivo* experiments have revealed that claudin-1 mRNA and protein levels are downregulated without significant changes to ZO-1 and atresia proteins.^[65,66]

The divalent iron ion channel [divalent metal transporter (DMT)] is a key element for the transport of iron across the BBB.^[67] Many experiments have indicated that lead could also pass through DMT in a competitive way and may occupy this transporter when iron is deficient. Lead affects the offset of iron-regulated proteins, which allows it to more easily access endothelial cells.^[68,69] When the concentration of iron is elevated, the transport of lead is effectively inhibited.^[70] Interestingly, expression of fractalkine (CX3CL1), a mediator of neuron-glia signaling, is also enhanced after exposure to lead, especially in the hippocampus and forebrain.^[10] In addition, lead also passes through and interferes with calcium channels, suggesting that lead might be able to cross the BBB in multiple or unknown other ways.

In one model involving exposure to lead, increased β -amyloid (A β) levels were found in the choroid plexus.^[2] On the choroid epithelial cell surface, a critical transporter known as lipoprotein receptor-related protein-1 (LRP-1) is responsible for transporting A β out

of the brain. LRP-1 knockout mice show higher levels of amyloid protein following lead exposure.^[71] Lead could induce a significant reduction in LRP-1 expression by interfering with the LRP-1 gene promoter. These studies, therefore, suggest that lead neurotoxicity might also be related to memory deficits in the pathogenesis of Alzheimer's disease.^[72]

NEURONAL DAMAGE INDUCED BY LEAD EXPOSURE AND NEUROINFLAMMATION

Lead-induced inflammatory reaction cascades within the neurovascular unit may cause neuronal damage.^[21] It has been hypothesized that TNF- α , IL-1 β , and IL-6 could cause neuronal apoptosis through glial activation.^[73] Possible mechanisms of injury might be ROS production due to the pro-inflammatory cytokine IL-1 β or increased glycogen consumption in astrocytes due to TNF- α and IL-1, thereby causing increased levels of toxic substances and affecting the metabolism of the cells.^[74] TNF might also be involved in the expression of NO, suggesting another way by which could inactivate LTP. Furthermore, IL-1 β acts on endothelial cell tight junction proteins, reducing the amount and location of occludin and increasing the permeability of the BBB.^[75] Inflammatory reactions could also change the transport of multiple substances by affecting the role of glutamate receptors.^[76] Lead-induced chemokines, mainly secreted from neurons, have been shown to act on microglial receptors and participate in the interactions between neurons and glial cells, resulting in changes in microglial and astrocyte morphology.^[77]

Oxidative damage is fatal to brain neurons. In pathological conditions such as hypoxia, traumatic injury, and lead insult, these toxic free radicals might be over-generated and cause secondary injuries to neurons. Compared with neurons, astrocytes have higher levels of antioxidants such as glutathione (GSH), heme-oxygenase 1 and GSH S-transferase.^[78] Neurons may maintain their antioxidant capacity by transporting and utilizing these substances, among which the GSH shuttle pathway is likely to be paramount.^[79,80] GSH, the most abundant antioxidant in the brain, is mainly generated in astrocytes. Astrocytes store a much higher content of GSH-related enzymes in order to guarantee a supply to neurons.^[78] GSH-depleted astrocytes display a reduced ability to protect neurons against oxidative injury.^[78]

When lead enters astrocytes, it could directly deplete NADPH. More importantly, it affects glucose 6 phosphate dehydrogenase, a key enzyme of the pentose phosphate pathway, reducing the production of NADPH.^[15] Both effects might result in a lack of GSH support from astrocytes to neurons. Lead is able to bind to GSH sulfhydryl groups and disable its

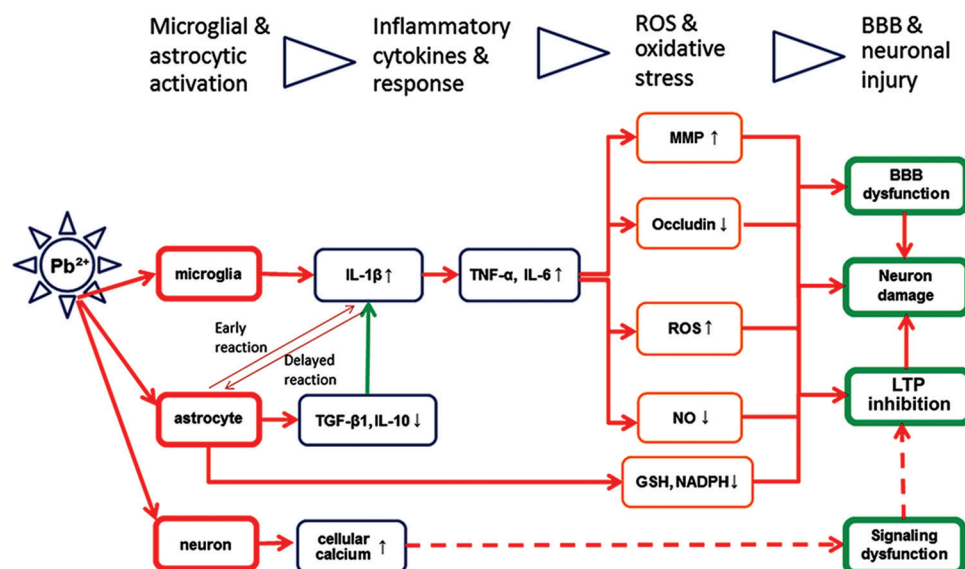


Figure 1: Correlative function of microglial and astroglial cells in the generation of inflammatory cytokines and neuronal injury. The lead (Pb^{2+}) may contact and trigger microglial and astrocytic activation, enhance inflammatory cytokines and response, increase reactive oxygen species and oxidative stress, and finally result in blood-brain barrier dysfunction, long-term potentiation inhibition and inflammatory injury of various neurons in the brains

ability as a ROS scavenger. Lead exposure results in an accumulation of ROS and a decrease in antioxidants. Increased levels of ROS contribute to higher BBB permeability, inducing oxidative damage to cellular molecules, activation of inflammatory mediators, and the destruction of tight junctions.^[81] ROS also inhibit glutamate transporters and cause a secondary glutamate metabolism exception,^[82] increasing the role of lead in the destruction of neurons. In addition, studies have shown that lead reduces many antioxidant molecules such as superoxide dismutase and catalase in adult mouse and rat brain.^[68,83]

Finally, the phosphorylated cyclic-AMP response element binding (pCREB) is an important transcription factor for long-term memory, and lead could block the cAMP-CREB pathway by reducing pCREB, resulting in a decline in long-term memory.^[6,7,13] The effect of lead exposure on (CREB) protein expression and phosphorylation in the cerebral cortex and hippocampus during postnatal development has been studied. Lead exposure did not affect total CREB levels, but decreased pCREB levels by about 30-38% in both cortex and hippocampus.^[13] Disruptions in pCREB expression levels and the binding activity of CREB proteins may decipher intracellular mechanisms of lead neurotoxicity in developing brains.^[12,13] In addition, the protein kinase C (PKC)/NF- κ B pathway might be involved in lead-induced neuroinflammatory injury to brain neurons, as it represents a key stress response signal to inflammation.^[84] The PKC-NF- κ B pathway might also play a critical role in cell defense reactions and cell apoptosis. The PKC-NF- κ B pathway has been shown to be involved in the regulation of NO and pro-inflammatory cytokine production in the LPS model

of inflammation.^[85] PKC-NF- κ B pathway downstream products such as tumor necrosis factor-related apoptosis inducing ligand, caspase-1, and NOS2 are enhanced in animal models after lead exposure.^[86]

CONCLUSION

In summary, microglial and astroglial responses might be critically involved in neuroinflammation and lead neurotoxicity in the brain. Microglia and astrocytes may have crosstalk or mutual activation by inflammatory cytokines and receptors. Lead (Pb^{2+}) has been shown to contact and interfere with microglia and astrocytes, which may trigger microglial and astrocytic activation, enhance inflammatory cytokine generation and release, increase ROS and oxidative stress, and finally result in BBB dysfunction and neuronal injury [Figure 1]. Further extensive studies are still needed, however, to elucidate the specific signaling pathways for microglia and astrocytes partaking in neuroinflammation in the brain and to find new targets of manipulation for the prevention and treatment of lead neurotoxicity in human beings.

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Cite this article as: Liu JT, Dong MH, Zhang JQ, Bai Y, Kuang F, Chen LW. Microglia and astroglia: the role of neuroinflammation in lead toxicity and neuronal injury in the brain. *Neuroimmunol Neuroinflammation* 2015;2(3):131-7.

Source of Support: The National Basic Research Program (2012CB525002, 2011CB504103) and National Natural Science Foundation of China (81272346, 31371374). **Conflict of Interest:** No.

Received: 31-08-2014; **Accepted:** 20-02-2015

Neuroinflammation and neurological alterations in chronic liver diseases

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ABSTRACT

Several million people with chronic liver diseases (cirrhosis, hepatitis) show neurological alterations, named hepatic encephalopathy (HE) with cognitive and motor alterations that impair quality of life and reduces life span. Inflammation acts synergistically with hyperammonemia to induce cognitive and motor alterations in patients with chronic liver disease and minimal hepatic encephalopathy (MHE). Previous studies in animal models have suggested that neuroinflammation is a major player in HE. This would also be the case in patients with liver cirrhosis or hepatitis C with HE. Rats with MHE show microglial activation and neuroinflammation that is associated with cognitive impairment and hypokinesia. The anti-inflammatory drug ibuprofen reduces microglial activation and neuroinflammation and restores cognitive and motor functions in rats with MHE. Chronic hyperammonemia *per se* induces neuroinflammation. Both peripheral inflammation and hyperammonemia would contribute to neuroinflammation in chronic liver failure. Therefore, neuroinflammation may be a key therapeutic target to improve the cognitive and motor alterations in MHE and overt HE. Identifying new targets to reduce neuroinflammation in MHE without inducing secondary effects would serve to develop new therapeutic tools to reverse the cognitive and motor alterations in patients with HE associated with chronic liver diseases.

Key words: Cognitive impairment, hepatic encephalopathy, hyperammonemia, inflammation, motor function, neuroinflammation

INTRODUCTION

Current evidence suggests that chronic inflammatory diseases lead to neuroinflammation. Increased neuroinflammation can result in neurological impairment with deficits in cognition and motor function. For example, patients with diabetes, rheumatoid arthritis, obesity or chronic kidney disease can develop neurological deficits.^[1-9] Inflammation and neuroinflammation are major contributing factors to cognitive and motor deficits in situations such as postoperative cognitive dysfunction, ageing and in some mental (e.g. schizophrenia) and neurodegenerative (e.g. Alzheimer's disease) diseases.^[10-16]

Patients suffering from chronic liver diseases (mainly cirrhosis and/or hepatitis) also show chronic inflammation which can lead to hepatic encephalopathy (HE): any alteration in cerebral function which is a consequence

of previous liver failure.^[17] There are two main types of liver diseases which induce HE: acute liver failure and chronic liver diseases. The effects and mechanisms underlying the cerebral alterations in acute and chronic liver failure are completely different.^[17] This review will focus only on the mechanisms involved in HE in chronic liver diseases, mainly in liver cirrhosis.

Chronic liver diseases affect more than 5 million people in USA and a similar number in the European Union.^[18] Patients with the chronic liver disease do not show neurological alterations at the beginning of the disease. However, with the progression of liver failure, most of these patients will suffer from some grade of HE. There are two main forms of HE in chronic liver disease: (1) minimal hepatic encephalopathy (MHE), in which the symptoms are not evident but can be unveiled using psychometric tests and (2) clinical or overt HE, in which the symptoms are evident. Once the symptoms are evident, clinical HE is graded in four stages according to the West Haven criteria.^[19]

Approximately, 40-50% of cirrhotic patients present with MHE with mild cognitive impairment, attention deficits, psychomotor slowing, reduced mental processing speed and bimanual and visuomotor incoordination.^[20-22] This incidence means that more

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10.4103/2347-8659.160845

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than two million people suffer from MHE in USA, in the European Union and even more in the rest of the world.

Minimal hepatic encephalopathy has important consequences on daily life of patients with chronic liver diseases. Attention, mental processing speed, visuomotor and bimanual coordination are necessary for many daily tasks such as driving cars or dressing. Patients with MHE have impaired driving ability, which is associated with peripheral inflammation, with increased levels of interleukin-6 (IL-6) and IL-18.^[23-25] They also have increased risk of traffic, home and work accidents and more falls, fractures, and hospitalizations, which pose a high economic burden to health systems. In addition, MHE predisposes patients to clinical HE with more serious neurological impairment that can lead to coma and death and reduces life span. Early diagnosis and treatment of MHE would improve the quality of life and survival of patients and economic burden.^[26-29]

INFLAMMATION AND NEUROINFLAMMATION IN THE NEUROLOGICAL ALTERATIONS IN PATIENTS WITH CHRONIC LIVER DISEASE AND MHE

Inflammation is a main contributor to the changes in cognitive and motor functions found in MHE patients suffering from chronic liver disease. For example, the serum levels of the pro-inflammatory cytokines IL-6 and IL-18 are increased in cirrhotic patients with MHE compared with cirrhotics without MHE. Moreover, the levels of IL-6 and IL-18 correlate with the grade of MHE evaluated using psychometric tests.^[30]

Hyperammonemia plays a synergistic role with inflammation in the induction of the neurologic impairment [Figure 1]. Hyperammonemia developed

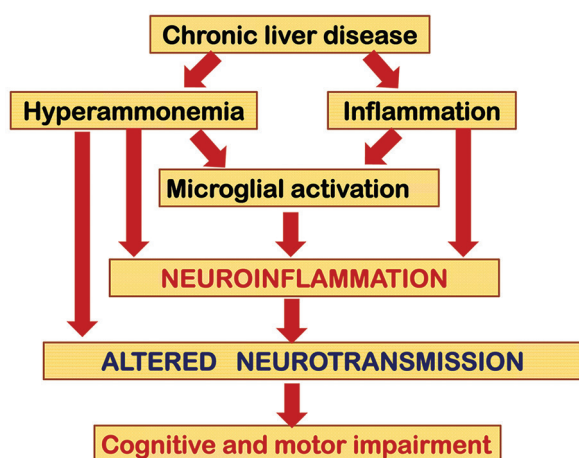


Figure 1: Hyperammonemia and inflammation act synergistically to induce neurological alterations in chronic liver disease. Chronic liver failure induces both hyperammonemia and peripheral inflammation, leading to microglial activation and neuroinflammation, which alters neurotransmission, leading to cognitive and motor impairment

in cirrhotic patients by ingestion of an amino acid mixture induces neuropsychological impairment during inflammation, but not after its resolution.^[31] The contribution of inflammation and hyperammonemia to the neurological impairment in different pathological situations with different grades of inflammation and hyperammonemia was assessed by Felipo *et al.*,^[32] who showed that the joint presence of inflammation and hyperammonemia, is enough to induce mild cognitive impairment, even in the absence of liver failure. The studies by Shawcross *et al.*^[31] and Felipo *et al.*^[32] support the idea that, as occurs in other chronic diseases, in chronic liver diseases, peripheral inflammation is involved in the induction of the cognitive and motor alterations associated to MHE and to overt HE.

Peripheral inflammation may lead to neuroinflammation by different mechanisms. The presence of neuroinflammation in patients with chronic liver diseases has not been thoroughly studied. One of the main methods to assess neuroinflammation is to assess by positron emission tomography (PET); the binding of ligands to the translocator protein (18 kDa) (TSPO), previously known as peripheral type benzodiazepine receptor.^[33] Cagnin *et al.*^[34] showed using PET techniques that cirrhotic patients with HE show increased binding of [¹¹C](R)-PK11195 to TSPO in brain.^[34] Moreover, the patient with the most severe cognitive impairment had the highest increases in regional [¹¹C](R)-PK11195 binding. The authors suggested that an altered glial cell state might be causally related to impaired brain functioning in HE. These data suggest the presence of neuroinflammation in patients with HE, correlating with the grade of cognitive impairment.^[34]

In support for the role of microglia, Dennis *et al.*^[35] performed immunohistochemistry studies and cytokine assays in fixed postmortem brain tissue from chronic alcoholics with cirrhosis and HE, alcoholics without HE and controls. About half of the patients with HE showed what they called “proliferative HE (pHE)” and the other half did not.^[35] The microglia in pHEs displayed an activated morphology with hypertrophied cell bodies and short, thickened processes. These patients also showed increased levels of IL-6. In contrast, the microglia in white matter regions of the non-proliferative HE cases were less activated and appeared dystrophic. The authors suggest that microglial proliferation may form part of an early neuroprotective response in HE that ultimately fails to halt the course of the disease because underlying etiological factors such as high cerebral ammonia and systemic inflammation remain.^[35] In support, Zemtsova *et al.*^[36] also analyzed markers of neuroinflammation in postmortem brain tissue from patients with cirrhosis that presented with and without HE and noncirrhotic

controls. They found an up-regulation of the microglial activation marker ionized calcium-binding adaptor molecule-1 (Iba-1) in the cerebral cortex from patients with cirrhosis who have HE but not from patients with cirrhosis who do not have HE. In this study, mRNA and protein expression of iNOS and cyclooxygenase (COX-2) or mRNA expression of pro-inflammatory cytokines and chemokine monocyte chemoattractive protein-1 was analyzed in cerebral cortex. They were not different in patients with liver cirrhosis and HE than in patients with cirrhosis who did not have HE or control patients without cirrhosis. This report shows increased microglial activation in patients with HE but did not find increased levels of pro-inflammatory markers.

Neuroinflammation has been also reported in patients with chronic hepatitis C (HC). However, in these patients a main contributor to neuroinflammation would be the presence of the virus into the brain. Postmortem studies of HC virus indicate that the viral replication might occur in the brain and microglia may be the locus for infection and subsequent neuroinflammatory activity.^[37,38] Bokemeyer *et al.*^[39] assessed the possible contribution of neuroinflammation to cognitive dysfunction in patients with HC who have mild liver disease. They performed an extensive neuropsychological examination and a magnetic resonance spectroscopy study in a group of patients with HC showing only mild liver disease but differing degrees of neuropsychiatric alterations. Choline, creatine and N-acetyl-aspartate and N-acetyl-aspartyl-glutamate concentrations in the basal ganglia were increased in the patients compared to controls. Fatigue correlated negatively with the metabolic changes.^[39] As the increase of choline, creatine and myo-inositol are usually interpreted to indicate glial activation and macrophage infiltration in chronic inflammation they conclude that HC virus infection may induce neuroinflammation and brain dysfunction. The negative correlation with fatigue suggests a cerebral compensatory process after infection. Grover *et al.*^[38] used two independent *in vivo* imaging techniques to assess the presence of neuroinflammation in patients with mild chronic HC and control subjects. Using magnetic resonance spectroscopy, they found that basal ganglia myo-inositol/creatine and choline/creatine ratios were significantly elevated in patients with chronic HC compared to controls. Using PET with a ligand for microglial/brain macrophage activation, ¹¹C-(R)-PK11195, Grover *et al.*^[38] found evidence of microglial activation, which positively correlated with HC viremia and altered cerebral metabolism. This suggests that the HC virus induces neuroinflammation, which would contribute to the cognitive alterations in patients with HC.

Although in patients with HC neuroinflammation seems to be caused mainly by the presence of the virus into the brain,^[37,38] the studies from Cagnin *et al.*,^[34] Dennis *et al.*^[35] and Zemtsova *et al.*^[36] suggest that neuroinflammation could also be present in patients with liver cirrhosis in the absence of virus infection.

ROLE OF NEUROINFLAMMATION IN THE COGNITIVE AND MOTOR ALTERATIONS IN ANIMAL MODELS OF MHE

Neuroinflammation would be a main contributor to the cognitive and motor alterations in minimal and clinical HE associated to chronic liver disease. This possibility is clearly supported by studies in animal models. Cauli *et al.*^[40] showed that rats with portacaval shunts (PCS), a main model of MHE,^[41] show neuroinflammation. Following PCS, rats showed increased levels of inflammatory markers such as IL-6 levels or COX activity in the brain. PCS rats show impaired ability to learn a Y maze task due to reduced function of the glutamate-nitric oxide (NO)-cGMP pathway in cerebellum.^[42] Cauli *et al.*^[40] showed that chronic treatment with the anti-inflammatory ibuprofen reduced neuroinflammation and restored the ability to learn the Y maze task because it restores the function of the glutamate-NO-cGMP pathway in cerebellum *in vivo*. This report showed for the first time that neuroinflammation is a main contributor to the cognitive impairment in MHE [Figure 1].^[40]

Neuroinflammation also contributes to other types of cognitive and motor alterations by altering other specific mechanisms modulating them. For example, neuroinflammation is a main contributor to hypokinesia in rats with MHE and chronic treatment with ibuprofen restored motor function.^[43] The mechanisms and brain areas involved in neuroinflammation-induced hypokinesia are different from those involved in impairment of learning in the Y maze. Hypokinesia in PCS rats is due to the increased level of extracellular glutamate in substantia nigra pars reticulata (SNr). Glutamate activates metabotropic glutamate receptor 1 (mGluR1) and induces an increase extracellular GABA in ventromedial thalamus (VMT). This, in turn, reduces extracellular glutamate in motor cortex, which reduces motor activity. Blocking mGluR1 in SNr with an antagonist normalizes GABA in VMT, glutamate in motor cortex and motor activity.^[44] A down-regulation of glutamate transporters contributes to the increased levels of extracellular glutamate in SNr.^[44] Chronic treatment of rats with MHE with ibuprofen normalizes the amount of glutamate transporters, reduces extracellular glutamate in SNr and normalizes motor

activity.^[43] The presence of neuroinflammation and its contribution to cognitive and motor alterations have also been demonstrated in the other main animal model of MHE: rats with bile duct ligation (BDL). Rodrigo *et al.*^[45] showed that BDL rats have microglial activation, mainly in cerebellum, as demonstrated by immunohistochemistry. This is associated with increased levels of inflammatory markers [inducible nitric oxide synthase (iNOS), IL-1 β , prostaglandin E2] and with cognitive impairment and hypokinesia. Similar to the previous studies, chronic treatment with ibuprofen reduced microglial activation and inflammatory markers and restored cognitive and motor functions in the BDL rats.^[45] The above reports clearly support the idea that MHE in chronic liver failure is associated with neuroinflammation that affects different cerebral processes resulting in different types of cognitive and motor alterations [Figure 1].

CHRONIC HYPERAMMONEMIA INDUCES ACTIVATION OF MICROGLIA AND NEUROINFLAMMATION

Concerning the mechanisms by which chronic liver failure induces neuroinflammation, it seems that two main contributors would be involved: chronic hyperammonemia and peripheral inflammation.

In addition to PCS and BDL rats, a well-recognized rodent model of MHE are rats with “pure” hyperammonemia without liver failure induced by feeding an ammonium-containing diet.^[41] As described above for rat model of MHE induced by PCS, rats with chronic hyperammonemia also show reduced function of the glutamate-NO-cGMP pathway in cerebellum^[46] and reduced ability to learn the Y maze task,^[47] which is restored when the function of the pathway is restored, for example, by treatment with sildenafil.^[42] This suggests that hyperammonemia and neuroinflammation impair learning the ability by the same mechanism. This idea led Rodrigo *et al.*^[45] to hypothesize that hyperammonemia would induce neuroinflammation in the brain, which would be responsible for impairment of the glutamate-NO-cGMP pathway and of cognitive function. To assess whether chronic hyperammonemia *per se* induces neuroinflammation, Rodrigo *et al.*^[45] assessed in the brains of hyperammonemic rats without liver failure activation of microglia by immunohistochemistry and the levels of inflammatory markers [iNOS, IL-1 β , and prostaglandin E 2 (PGE2)]. Hyperammonemic rats show microglial activation [Figure 1], mainly in cerebellum, and increased levels of inducible iNOS, IL-1 β , and PGE2 which are associated with impaired function of the glutamate-NO-cGMP pathway and with cognitive impairment and hypokinesia.

Chronic treatment with ibuprofen reduced microglial activation and inflammatory markers and restored the function of the glutamate-NO-cGMP pathway in cerebellum and cognitive and motor functions in hyperammonemic rats.^[44] In summary, this indicates that: (1) chronic hyperammonemia *per se* induces neuroinflammation; (2) neuroinflammation mediates the effects of chronic hyperammonemia on the function of the glutamate-NO-cGMP pathway in cerebellum and on cognitive and motor functions in hyperammonemic rats. As neurological alterations are mainly due to altered glutamatergic and GABAergic neurotransmission,^[48,49] this suggests that neuroinflammation-mediated alterations in neurotransmission are mainly responsible for changes in cognitive and motor function in MHE and clinical HE [Figure 1].

Hyperammonemia may also alter neurotransmission by other mechanisms. For example, Thrane *et al.*^[50] have shown that ammonia, at high concentrations, alters astrocyte potassium buffering, increasing extracellular potassium concentration and over-activating the Na⁺-K⁺-2Cl⁻ co-transporter isoform 1 (NKCC1) in neurons. The consequent depolarization of the neuronal GABA reversal potential selectively impairs cortical inhibitory networks. This altered GABAergic neurotransmission may contribute to the neurological alterations in hyperammonemia. The alterations in glutamatergic and GABAergic neurotransmission in hyperammonemia and HE have been recently reviewed by Cauli *et al.*^[48] In addition to the contribution of hyperammonemia, peripheral inflammation would also contribute to induction of neuroinflammation in chronic liver disease [Figure 1].

The mechanisms by which hyperammonemia induces microglial activation and neuroinflammation have been poorly studied for the moment. Zemtsova *et al.*^[36] studied the effects of large ammonia concentrations on rat microglia *in vitro* and *in vivo*. In cultured rat microglia, ammonia-stimulated cell migration and induced oxidative stress and an up-regulation of the microglial activation marker ionized calcium-binding adaptor molecule-1 (Iba-1). Up-regulation of Iba-1 was also found in the cerebral cortex from acutely ammonia-intoxicated rats. However, ammonia had no effect on microglial glutamate release, prostaglandin synthesis, and messenger RNA (mRNA) levels of iNOS, COX-2, and IL-1 α/β , tumor necrosis factor α (TNF α), or IL-6, whereas in cultured astrocytes ammonia induced the release of glutamate, prostaglandins, and increased levels of IL-1 β mRNA. Although this study was performed using ammonia levels much higher than those that can be found in chronic liver disease *in vivo*, these data suggest that ammonia *per se*

may activate microglia. Additional factors would act synergistically with hyperammonemia to induce pro-inflammatory factors and neuroinflammation in chronic liver failure.

THERAPEUTIC IMPLICATIONS

The *in vivo* studies reported above in animal models show that neuroinflammation would be a key therapeutic target to improve the cognitive and motor alterations in MHE and overt HE. However, different approaches can be used to reduce neuroinflammation in chronic liver disease, but it is important to take into account the possible secondary effects of each treatment, which may depend on the type and grade of liver disease in each patient. For example, several studies show that ibuprofen ameliorates cognitive and motor function in rats with MHE.^[40,43,45] However, ibuprofen is a non-steroidal anti-inflammatory drug (NSAID), and this type of anti-inflammatory is not recommended in cirrhotic patients because they may induce kidney damage and, in patients prone to portal hypertension (a common liver disease complication), the risk of gastrointestinal bleeding is intensified with NSAIDs.^[51,52] Therefore, alternative therapeutic approaches are needed to reduce neuroinflammation that would present no secondary side-effects. As activation of microglia is a main contributor to neuroinflammation in animal models of HE, Agusti *et al.*^[53] hypothesized that reducing directly microglial activation would be useful to improve cognitive and motor function in HE without inducing secondary effects in the kidney. Inhibiting the p38 MAP kinase antagonizes microglial activation^[54] and inhibitors of p38 are being tested as therapeutic tools in different inflammatory and autoimmune diseases.^[55] Taking this into account, Agusti *et al.*^[53] assessed whether inhibiting p38 with SB239063 reduces neuroinflammation and improves cognitive and motor function in rats with MHE due to PCS without affecting the kidney. This group showed that treatment with SB239063 reduced microglial activation and inflammatory markers (PGE₂, COX activity, iNOS, IL-1 β , TNF α) in brain and completely restored learning ability, motor activity and coordination in PCS rats without inducing kidney damage. This suggests that inhibitors of p38 would improve the neurological status in HE without inducing secondary effects in the kidney. This type of inhibitors is being developed by different pharmaceutical companies to treat different chronic inflammatory diseases. It is likely that they can be developed further and following extensive pre-clinical and clinical evaluation become available to patients suffering from MHE.

Additional studies to identify and test new targets to reduce neuroinflammation in MHE without inducing secondary effects would be very useful to develop new therapeutic tools to reverse the cognitive and motor alterations in patients with MHE and clinical HE associated with chronic liver diseases.

ACKNOWLEDGMENTS

Supported by grants from Ministerio de Ciencia e Innovación (SAF2011-23051, CSD2008-00005), Conselleria Educació Generalitat Valenciana (PROMETEO-2009-027, PROMETEOII/2014/033, ACOMP/2012/066, and ACOMP/2013/101).

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Cite this article as: Montoliu C, Llansola M, Felipo V. Neuroinflammation and neurological alterations in chronic liver diseases. *Neuroimmunol Neuroinflammation* 2015;2(3):138-44.

Source of Support: Supported by grants from Ministerio de Ciencia e Innovación (SAF2011-23051, CSD2008-00005), Conselleria Educación Generalitat Valenciana (PROMETEO-2009-027, PROMETEOII/2014/033, ACOMP/2012/066, and ACOMP/2013/101). **Conflict of Interest:** No.

Received: 02-01-2015; **Accepted:** 19-05-2015

Transcranial magnetic stimulation research on reading and dyslexia: a new clinical intervention technique for treating dyslexia?

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ABSTRACT

Nowadays, several noninvasive neuroimaging techniques, including transcranial magnetic stimulation (TMS), exist. The working mechanism behind TMS is a rapidly changing magnetic field that generates an electric current via electromagnetic induction. When the coil is placed on the scalp, the magnetic field generates a physiological reaction in the underlying neural tissue. The TMS-induced change in the participant's behavior is used by researchers to investigate the causal relations between specific brain areas and cognitive functions such as language. A variant of TMS has been developed, which is called rapid-rate TMS (rTMS). In this review, three databases (Medline, Educational Resources Information Center, and Scopus) were searched for rTMS studies on normal reading and dyslexia with a cut-off date of October 31, 2014. rTMS was found to be a valuable tool for investigating questions related to reading research, both on the word and the sentence level. Moreover, it can be successfully used in research on dyslexia. Recently, (high-frequency) rTMS has been used as a "clinical" intervention technique for treating dyslexia and for improving reading performance by exciting underactive reading pathways in the brain. Finally, we end the paper with a discussion of future directions in the field of rTMS research and dyslexia, for instance, the promising prospect of combining TMS with simultaneous electroencephalographic imaging.

Key words: Clinical intervention, dyslexia, sentence reading, transcranial magnetic stimulation, word reading

INTRODUCTION

Transcranial magnetic stimulation

Nowadays, (cognitive) neuroscientists can choose from several noninvasive neuroimaging techniques, and one of them is transcranial magnetic stimulation (TMS). The working mechanism behind TMS is a rapidly changing magnetic field that generates an electric current via electromagnetic induction.^[1] When the coil is placed on the scalp, the magnetic field generates a physiological reaction in the underlying neural tissue,^[2] which can be a spiking and/or a depolarizing reaction,^[3,4] and specific or general areas of the brain can be affected. Transient noise is introduced into the

neural computation being performed, often leading to longer reaction times or higher error rates.^[2]

As early as 1985, the first successful TMS study on human participants was conducted by Barker *et al.*^[5] The authors described the use of a pulsed magnetic field focused over specific regions of the cerebral cortex to induce muscle action potentials (see also the publication by Barker *et al.*^[6] later that year). In fact, their pioneering study turned out to be the beginning of a whole new research field. This TMS-induced change in the participant's behavior is an interesting opportunity for researchers to study the causal relations between specific brain regions and cognitive functions.^[7]

Rapid-rate transcranial magnetic stimulation

Later, a variant of TMS was developed, which is called rapid-rate TMS (rTMS).^[8,9] Further improvements of the stimulators, which are now able to provide discharges at frequencies of up to 60 Hz, have greatly increased the value of TMS as a tool in cognitive neuroscience

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10.4103/2347-8659.157967

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research.^[10] By varying the intensity, stimulation frequency, and duration of rTMS, the researcher can now transiently inhibit or block the function of a specific cortical structure or enhance the excitability of particular cortical areas under the coil.^[10-12] Moreover, in addition to the useful applications of rTMS in basic cognitive neuroscience research, clinicians have recently started to use it as a therapeutic technique.^[13] Later, in this paper, we will look into the possibility of using rTMS as a therapeutic intervention for treating dyslexia.

First transcranial magnetic stimulation studies on language

The first TMS study on language was conducted by Pascual-Leone *et al.*,^[14] who induced speech arrest in presurgical patients with epilepsy. They were particularly interested in whether TMS could be used as an alternative to intracarotid amobarbital testing, also known as the WADA-test,^[15] which is a test that is clinically used to determine language representation in presurgical patients, but can lead to several substantial negative side effects (like seizures, encephalopathy, strokes, *etc.*).^[16]

In the last two decades, TMS studies on language have further focused on language representation issues;^[17] moreover, the identification of language areas and an understanding of their underlying functions have become key research topics.^[18] TMS is used to either inhibit or facilitate language processes and may operate directly on a specific language-related cortex area or indirectly via the intra-cortical networks. With TMS, reversible temporary lesions have been made in order to investigate the cerebral cortical areas that are thought to be responsible for language function. Interestingly, the TMS results differ from those predicted by classical models of language organization: speech production in the left inferior frontal region^[19] and reception of language in the superior temporal gyrus.^[20] RTMS over the left inferior frontal region was found to block speech output while speech arrest was obtained most easily over the facial motor-cortex, a structure located anterior to the central sulcus and superior to the perisylvian fissure. Surprisingly, in general, the rTMS results show limited proof for aphasia as a result of impairment of the classical Broca's left inferior frontal region^[19] and of Wernicke's superior temporal gyrus,^[20] whereas right-hemisphere or bilateral lateralization was often found.^[18]

Aim of the study and methodology used

The aim of the present paper was to review the use of TMS in the study of, as well as its role in developing a better understanding of, one specific language area, namely, reading. What are the contributions of TMS to the understanding of the different reading

modalities? After discussing the main findings of TMS research on normal readers, we will look deeper into the field of TMS and dyslexia. We will finish our paper by answering the question whether rTMS can be used as a future therapeutic modality for treating dyslexia.

The following methodology was used in the present review. Three databases (Medline,^[21] Educational Resources Information Center,^[22] and Scopus^[23]) were searched with a cut-off date of October 31, 2014. In addition, the reference lists of all studies that were found in the databases were further checked in order to find additional suitable studies (also known as the snowball method).^[24] Two authors (van den Noort and Struys) independently performed the literature search; moreover, the study selection and data extraction were also independently conducted by two authors (van den Noort and Struys). The extracted data included the authors, the title, the journal in which the study had been published, the publication year, the number of participants involved in the study, the exact methodology used, the effects of TMS on normal reading and in treating dyslexia that were found, and the conclusions that were drawn. In addition, Bosch was contacted in case of disagreement regarding the study selection and/or data extraction, and in all cases, a consensus was reached.

TMS RESEARCH ON NORMAL READING

First, we will discuss several influential TMS studies involving normal readers and focusing on the processing of words and Chinese characters. Then, we will move on to the sentence-level. Note that the processing of words/characters is a simplification of reading in daily life, where complete sentences and texts are processed. However, for reading research, these studies are relevant because they give important insights into how normal reading works, and as we will see later in this paper, can explain what has gone wrong in individuals with dyslexia.

Word-level

Visual word recognition

Before the advent of TMS, behavioral studies of visual word recognition had already suggested that the left cerebral hemisphere was more critically involved in visual word recognition than the right cerebral hemisphere. Participants showed slower reading times for longer words than for shorter words (a phenomenon known as the "word-length reading effect") when the words were presented in the left-hemi-field, as a result, were processed in the right hemisphere (note that the human visual system is contra-lateral in nature^[25]), but this was not the case when the words

were presented in the right-hemi-field, and as a result, were processed in the left-hemisphere (a phenomenon known as “length-independent reading”),^[26] proving the hypothesis that the left cerebral hemisphere is more critically involved in visual word recognition.

Skarratt and Lavidor^[27] were the first to test these behavioral findings^[26] with rTMS. They were particularly interested in how expert readers were able to identify arrays of several letters quickly and in parallel. They found that left occipital cortex rTMS disrupted processing in the right visual hemi-field of experts, resulting in the previously-discussed word-length reading effect. RTMS of the right occipital cortex, however, did not disrupt the processing of right visual hemi-field words in experts nor did it affect the word-length reading effect that was already visible in the left visual hemi-field. To conclude, Skarratt and Lavidor’s study^[27] were the first to demonstrate that TMS-induced impairment in the left-hemisphere led to a word-length reading effect, providing neuroscientific evidence for the hypothesis that the left-hemisphere is more specialized in word recognition [Table 1].

In addition, several studies have tested these visual hemi-field word processing findings^[26,27] by using foveally-presented lexical stimuli^[28,29] and have revealed that the different right and left-hemispheric processing styles have contra-lateral influences on the responses driven by the right and the left halves of the lexical stimuli (also known as the split fovea theory).^[30] In sum, research has shown the importance of human foveal splitting for the visual recognition of words,^[31] but the question of what would happen in

Chinese character recognition remained? Therefore, an intriguing study on human foveal splitting with rTMS was conducted by Hsiao *et al.*,^[32] in which not words, but Chinese characters were used as stimuli. As in Skarratt and Lavidor’s rTMS study,^[27] Hsiao *et al.*^[32] conducted rTMS on the right and the left occipital cortexes. Hsiao *et al.*^[32] found neuroscientific proof for the split fovea theory.^[30] Moreover, with respect to visual word recognition, they showed that fovea splitting was not a unique characteristic of European languages, but could also be found in Chinese, which belongs to a completely different language family and uses characters instead of Arabic letters.^[32]

In their TMS study, Stoeckel *et al.*^[33] were particularly interested in the supramarginal gyrus. Previous neuroimaging research had shown that the supramarginal gyrus played a role in visual word recognition.^[34] Stoeckel *et al.*^[33] were the first to use the TMS technique to investigate the role of the supramarginal gyrus in word recognition. They used three different tasks: a phonological, a semantic, and a visual control task. Their results showed that the supramarginal gyrus contributed to reading, regardless of the specific task requirements. The supramarginal gyrus automatically seemed to compute the sound of the word, even when it was not needed for the task.^[33] Thus, the visual perception of words automatically seemed to activate the auditory representation of their spoken forms.^[33]

Nakamura *et al.*^[35] further investigated the above issue by conducting TMS on both the left superior temporal

Study	Participants	Brain area	Main finding
Skarratt and Lavidor ^[27]	Twelve right-handed, healthy, volunteers	Occipital cortex	A word-length effect was found after rTMS had been conducted on the left occipital cortex. Evidence was found for the hypothesis that the left-hemisphere was more specialized in word recognition
Hsiao <i>et al.</i> ^[32]	Eight right-handed, healthy, volunteers, who were all native speakers of Chinese	Occipital cortex	The findings of the Chinese character study confirmed the split fovea hypothesis. Moreover, it showed that fovea splitting was not solely found for reading in European languages, but seemed to be a universal processing constraint
Stoeckel <i>et al.</i> ^[33]	Twenty-two right-handed, healthy volunteers, who were all native English speakers	Supramarginal gyrus	The authors found that the supramarginal gyrus clearly contributed to reading; moreover, a conclusion was that the supramarginal gyrus automatically computed the sound of a word and that this occurred even when it was not really required to perform the task
Nakamura <i>et al.</i> ^[35]	In total, 30 healthy, native Japanese speakers	Superior temporal gyrus and inferior parietal lobe	A clear double dissociation was discovered; the repetition priming during the pronunciation task was eliminated when TMS was conducted on the left inferior parietal lobe, but not when it was conducted on the left superior temporal gyrus, whereas the priming during the lexical decision task was eliminated when the left superior temporal gyrus, but not the left inferior parietal lobe, was stimulated
Tomasino <i>et al.</i> ^[36]	Twenty right-handed, healthy men, who were all native speakers of German	Primary motor-cortex	The authors showed that the primary motor-cortex was critically involved in processing action verbs, but that this was only the case when participants were simulating the corresponding movement
Hoffman <i>et al.</i> ^[37]	Thirteen right-handed, healthy, native speakers of English	Ventrolateral prefrontal cortex	The results suggested that the ventrolateral prefrontal cortex worked as a kind of executive regulator in the processing of abstract words. However, this was less the case when abstract words were presented in a specific context and when concrete words were processed

rTMS: rapid-rate transcranial magnetic stimulation; TMS: transcranial magnetic stimulation

gyrus and the inferior parietal lobe. In their study, they used auditory and visual targets and a pronunciation and lexical decision task. Nakamura *et al.*^[35] discovered a clear double dissociation. On the one hand, the repetition priming during the pronunciation task was eliminated when TMS was conducted on the left inferior parietal lobe, but not when it was conducted on the left superior temporal gyrus. On the other hand, the priming during the lexical decision task was eliminated when TMS was conducted on the left superior temporal gyrus, but not when it was conducted on the left inferior parietal lobe [Table 1].^[35]

Reading action verbs

So far, we have discussed TMS studies on general visual word recognition; however, from a neurolinguistic point of view, the study of what happens in the brain when a specific type of verb is read, namely, an “action verb”, is also interesting. These verbs all express some kind of action. Tomasino *et al.*^[36] applied TMS to the hand area of the left primary motor cortex during experimental trials of three different tasks (silent reading of action verbs, motor imagery of the action, and frequency judgment) and to the vertex during the control trials of these tasks. The authors found neuroscientific evidence for the hypothesis that the primary motor cortex was critically involved in processing action verbs but that this was only the case when the participants were simulating the corresponding movement.^[36]

Abstract versus concrete words

Another important neurolinguistic word class distinction, besides the previously-discussed “action verbs”, can be made between the so-called “concrete” words (an example is the word tree) and “abstract” words (an example is the word love). In a rTMS study, Hoffman *et al.*^[37] investigated the idea that abstract words depended on the ventrolateral prefrontal cortex for understanding, as was previously suggested based on neuroimaging findings.^[38] The authors hypothesized that an increase in the executive regulation would be

needed as a result of the various meanings abstract words could have, depending on the context. Their results, indeed, suggested that the ventrolateral prefrontal cortex worked as a kind of executive regulator in abstract word processing. However, this was less the case when the abstract words were processed within a particular context because then the system was already guided in the direction of a specific meaning or interpretation. In contrast, regulation played a smaller role in the processing of concrete words because in the processing of concrete words, the number of possible meanings are already decreased as a result of their physical referents; moreover, their meanings did not differ in various contexts.^[37]

Sentence-level

Having discussed the main results that were found on the word-level in normal readers using TMS, we will now discuss the main findings that were found on the sentence-level. Note that these studies are closer to real language situations, for instance, situations in which people are reading books or newspapers.

Sentence comprehension

Manenti *et al.*^[39] conducted an rTMS study on sentence reading [Table 2]. They were particularly interested in if and how a specific area in the brain, the dorsolateral prefrontal cortex, was involved in the understanding of sentences. It had previously been suggested that dorsolateral prefrontal cortex engagement might reflect the working memory load in sentence processing.^[40,41] Manenti *et al.*^[39] found that when rTMS was conducted on the left dorsolateral prefrontal cortex, the participants needed more time to complete a semantic task (i.e. was the meaning of the sentence correct or not), but not to complete a syntactic task (i.e. was the grammar of the sentence correct or not). Furthermore, when rTMS was conducted on the right dorsolateral prefrontal cortex, the opposite pattern was visible, and the participants needed more time to finish the syntactic task, but did not need more time

Table 2: TMS findings on sentence-reading in normal readers

Study	Participants	Brain area	Main finding
Manenti <i>et al.</i> ^[39]	Twelve right-handed, native Italian speakers	Dorsolateral prefrontal cortex	A double dissociation between the type of task (semantic vs. syntactic) and the rTMS effects was found, supporting the idea that the underlying working memory resources in sentence comprehension were processed differently by the two hemispheres. The activity of the motor areas was affected by the motor component of the verb. This phenomenon was visible when fictive and metaphorical motion sentences were processed. Early activation of the hand-related motor system was found after reading phrases with concrete verbs whereas a delay in the same region was visible after reading phrases with abstract verbs. Their results supported the idea that the middle temporal gyrus was involved in the retrieval of lexical-syntactic information whereas the inferior frontal gyrus played a key role in the unification processes required in order to understand sentences.
Cacciari <i>et al.</i> ^[42]	Nine healthy participants	Motor area	
Scorolli <i>et al.</i> ^[43]	Sixteen healthy, right-handed, native Italian speakers	Primary motor-cortex	
Acheson and Hagoort ^[44]	Twenty participants in the TMS group and 20 in the control group	Inferior frontal gyrus and middle temporal gyrus	

rTMS: rapid-rate transcranial magnetic stimulation; TMS: transcranial magnetic stimulation

on the semantic task. In sum, a double dissociation between the type of task (semantic versus syntactic) that was performed and the rTMS effects was found in this study and provided neuroscientific proof for the hypothesis that the underlying working memory resources in sentence comprehension were processed differently by the two hemispheres.^[39]

Processing of specific sentence types

So far, we have discussed general sentence comprehension in normal readers. In contrast to Manenti *et al.*,^[39] Cacciari *et al.*^[42] were interested in how readers process specific kinds of sentences. They investigated three neurolinguistic classes of sentences, “literal”, “nonliteral” (i.e. metaphorical, idiomatic), and “fictive” motion sentences, and wondered how these different types of sentences affected the excitabilities of the motor areas in the brain. Larger motor-evoked potentials were found when individuals read literal, fictive, and metaphorical motion sentences than when they read idiomatic motion or mental sentences. Cacciari *et al.*^[42] found neuroscientific evidence for the hypothesis that the activity of the motor areas was affected by the motor component of the verb when reading fictive and metaphorical motion sentences.

In line with the study by Cacciari *et al.*,^[42] Scorolli *et al.*^[43] were also interested in the motor-cortex involvement underlying sentence comprehension; more precisely, the focus of their study was on the specific role of abstract versus concrete verbs in this process. As Scorolli *et al.*^[43] had hypothesized, early activation of the hand-related motor system was found after reading phrases with concrete verbs, whereas a delay in the same region was visible after reading phrases with abstract verbs.^[43]

Finally, in their TMS study on normal readers, Acheson and Hagoort^[44] were interested in the processing of different kinds of sentences, namely, the so-called: “ambiguous” and “unambiguous” sentences. More precisely, they tested the hypothesis that the middle temporal gyrus played a significant role in the selection and the integration of lexical-syntactic information whereas the inferior frontal gyrus was involved in the unification processes needed for the successful understanding of sentences. Their results,^[44] indeed, supported the idea that the middle temporal gyrus was involved in the retrieval of lexical-syntactic information and that the inferior frontal gyrus was involved in the unification processes underlying the successful understanding of sentences.

So far, we have seen that rTMS has become a valuable neuroscientific tool for answering questions related to reading research, both on the word and

the sentence-level. However, all the studies and the findings that we have discussed so far were rTMS studies conducted on normal readers who were not experiencing any reading problems. In the second part of our paper, we will address whether rTMS can also be successfully applied to research on individuals that are known to have reading problems? In order to do so, we will discuss TMS studies on dyslexia. In addition, we will go one step further and address whether rTMS can be used as a clinical intervention technique to overcome reading problems?

TMS AND DYSLEXIA

Dyslexia

Dyslexia (also referred to as specific reading disability)^[45,46] occurs when a child or adult has significant difficulty with the speed and the accuracy of word decoding, which may lead to decreased text comprehension.^[47] In addition, spelling difficulties are common in dyslexia [Figure 1].^[48,49] Previous research showed that dyslexia was stable, meaning that children who were identified as dyslexic were likely to continue suffering from reading difficulties throughout their lives.^[50,51] The exact prevalence of dyslexia worldwide is unknown; however, in most studies, the prevalence of dyslexia is estimated to be somewhere between 5% and 10% of the population.^[52] There is no cure for dyslexia, but phonics-based treatments seem to be most successful.^[53] Furthermore, in recent years, several treatment variants using temporal-auditory, articulatory, or multisensory exercising programs have been developed in order to help individuals with dyslexia.^[54]

TMS research on dyslexia

One of the first TMS studies on dyslexia was conducted by Coslett and Monsul^[55] who investigated the hypothesis that the right-hemisphere mediated the reading of patients with acquired dyslexia. A 57-year-old man with (partially recovered) pure alexia participated in the study. The participant’s task was to read aloud words that were presented briefly, while receiving TMS on either the right or the left hemisphere. The results of the study supported the right-hemisphere reading hypothesis because stimulation of the right, but not the left, hemisphere affected oral reading. Moreover, this study showed that TMS could be used successfully to answer experimental research questions on dyslexia.

	Word-level	Sentence-level
Normal reader	reading	It is easy to read this sentence
Reader with dyslexia	reabing	If is easy to reab fhis senfence

Figure 1: Examples of how a normal reader and a reader with dyslexia would read the same words and sentences

However, long time would elapse before researchers started to use TMS not only for basic research on dyslexia but also for clinical applications.

TMS as clinical intervention technique

Within TMS protocols, Frye *et al.*^[56] were the first to hypothesize that high-frequency repetitive TMS could improve reading performance in people suffering from dyslexia by exciting underactive reading pathways in the brain. Previous neuroscientific research had shown that an improvement in reading in dyslexics was mediated by an increase in the activations of typically hypoactive left-hemisphere areas (also referred to as “normalization”) and by additional activation in the right hemisphere regions (also referred to as “compensation”).^[57]

Costanzo *et al.*^[58] conducted an intriguing study with high-frequency rTMS on 10 dyslexic adults,

who were native speakers of Italian, to test the hypothesis of exciting underactive reading pathways in dyslexics.^[56] They conducted 5-Hz TMS over both the left and the right inferior parietal lobules and the superior temporal gyrus (note that these areas had previously been found to improve reading in a TMS study on nondyslexics^[59]) in advance of reading words, nonwords, and text aloud. The results of the study showed that on the one hand, high-frequency rTMS stimulation over the left inferior parietal lobule led to a better performance in nonword reading; that is, the individuals with dyslexia made fewer errors. On the other hand, high-frequency rTMS stimulation over the left superior temporal gyrus resulted in faster word reading and better text reading. Interestingly, after the right inferior parietal lobule had been stimulated, the performances for nonword reading also increased. This intriguing study led

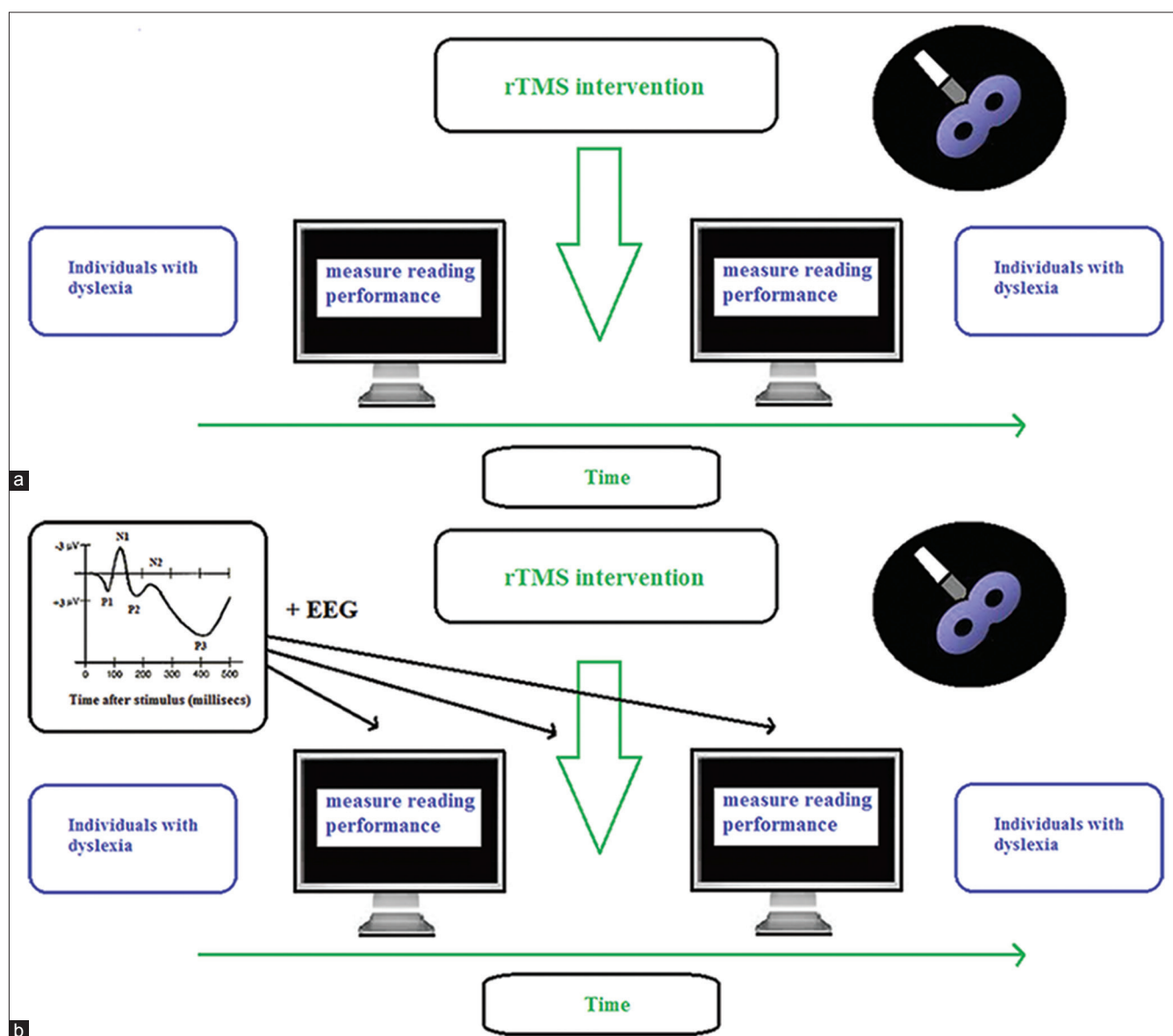


Figure 2: (a) One direction for future research aims to investigate the clinical usefulness of the rapid-rate transcranial magnetic stimulation intervention technique for treating individuals with dyslexia; (b) The second direction for future research aims to investigate the underlying neural working mechanisms (by using simultaneous electroencephalographic and transcranial magnetic stimulation) behind the rapid-rate transcranial magnetic stimulation intervention technique for treating individuals with dyslexia

to several important insights. First, these findings indicated that in individuals with dyslexia, the left superior temporal gyrus, and the left inferior parietal lobule did not have the same role when words, nonwords, and texts were read. Second, an important finding is that not only were left-lateralized improvements found in individuals with dyslexia, as one would expect, but also right inferior parietal lobule involvement, suggesting that additional compensatory recruitment^[57] exists in this area, were found in those individuals. For the first time, these results showed that distinctive facilitation of specific neural pathways (that were previously found to be less active in individuals with dyslexia)^[57] transitorily improves the reading of words and texts, which is a fascinating finding, and could have far-reaching implications, for instance, the development of new treatments for dyslexia.^[58]

CONCLUSION

The primary aim of this study was to determine the contributions that TMS has made to different reading modalities. The second goal was to investigate whether TMS might be used as a future intervention technique to overcome reading problems associated with dyslexia. We have seen that rTMS turned out to be a valuable tool for investigating questions related to reading research, both on the word and the sentence-level. Moreover, it can be applied successfully in research on dyslexia. Recently, (high-frequency) rTMS has been used as a “clinical” intervention technique for treating dyslexia by improving the reading performance by exciting underactive reading pathways in the brain. This seems to be a very promising direction for developing new and better treatments for dyslexia [Figure 2a], as long as the safety of the individuals with dyslexia can be guaranteed and strict guidelines on brain stimulation are followed.^[60,61]

Moreover, a new development, the combination of brain stimulation by TMS with simultaneous electroencephalographic (EEG) imaging,^[62,63] offers new prospects for research on reading and dyslexia. The integration of TMS with EEG is able to give information on the causal link between brain activity and its underlying function and cortical reactivity and its connection with other areas in the brain. More importantly, it also gives a better time window on when particular neural actions occur in the brain.^[63] Therefore, this integration of TMS with EEG will give important additional neural information on reading abnormalities in individuals with dyslexia, as well as on the efficiencies and the underlying working mechanisms of future TMS dyslexia treatments [Figure 2b].

ACKNOWLEDGMENTS

We thank Prof. Jenny Thomson, Harvard Graduate School of Education, Cambridge, MA, USA, for vivid discussions on dyslexia that eventually resulted in the writing of the present review paper. Moreover, we thank Dr. Heike Staudte from the LVR-Klinik Bedburg-Hau, Kleve, Germany, for her insights regarding the possible clinical applications of high-frequency rTMS in individuals with dyslexia.

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Cite this article as: Noort Mv, Struys E, Bosch P. Transcranial magnetic stimulation research on reading and dyslexia: a new clinical intervention technique for treating dyslexia?. *Neuroimmunol Neuroinflammation* 2015;2(3):145-52.

Source of Support: Nil. **Conflict of Interest:** No.

Received: 05-11-2014; **Accepted:** 23-12-2014

Brain abscess: surgical experiences of 162 cases

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ABSTRACT

Aim: Brain abscess still poses a public health challenge in spite of the advent of modern neurosurgical techniques and antibiotics. Here, we present our surgical experiences and ultimate outcome in the management of brain abscess. **Methods:** Totally, 162 patients with proved brain abscess who underwent surgical treatment were included in this study. The prospectively recorded data of surgical management of brain abscess and the ultimate outcome (by Glasgow outcome scale) were studied retrospectively. **Results:** Total number of cases was 162, of which 113 were acute pyogenic abscess while 49 were chronic abscess. Among the chronic abscess, 29 were chronic pyogenic abscess, 14 were tubercular, 3 aspergillus, and 3 abscesses were in malignant brain metastases. In acute cases, common clinical features were headache, fever, vomiting, focal deficit and seizure. In chronic abscesses, common clinical features were mild to moderate headache and progressive focal deficit. Seventy-three (45.06%) patients had adjacent localized sinus, middle ear or cranial infection. The common predisposing factors included postneurosurgery, postpenetrating injury to brain, chronic suppurative otitis media, and congenital heart disease, infective endocarditis, sinusitis and sub optimum immuno-status. Frontal lobe involved in 30.2% cases, temporal lobe is next to involved. Single time burr hole aspiration in 111 (68.5%) cases, two or more times burr hole aspiration were done in 34 (21%) cases. Pus culture was negative in 129 (79.62%) cases. Total number of death was 22 (13.58%) cases. Complete resolution of abscess with complete recovery of preoperative neuro-deficit was seen in 80.86% cases and recovery with major neuro-deficit was observed in 5.55% cases. There is a significant association between Glasgow coma scale (GCS) on admission and mortality in brain abscess. **Conclusion:** In most of the cases, pus culture did not yield growth of any causative organism. Mortality was not directly related to surgical intervention, but GCS on admission has a significant association with mortality. Early diagnosis, optimum follow-up and timely surgical interventions are the keys in the proper management of brain abscess.

Key words: Acute and chronic brain abscess, brain abscess, outcome, surgical management

INTRODUCTION

Brain abscesses often occur in the developed world, and they are even more common in developing countries.^[1] In spite of the advent of modern neurosurgical techniques, including stereotactic brain biopsy and aspiration, better culturing techniques to identify the infectious agent, new antibiotics, and modern noninvasive neuroimaging procedures, brain abscess still poses a public health challenge, especially in developing countries.^[2,3] There are enormous diagnostic and therapeutic challenges and controversies in the management of brain abscess.

Here, we report our experiences including preoperative clinical features, radio-imaging findings, surgical interventions, postoperative course, complications, risk factors and causes, infectious agent and ultimate outcome in the management of brain abscess.

METHODS

Totally, 162 patients with proved (peroperative and postoperative) brain abscess who underwent surgical treatment in the Department of Neurosurgery, Mitford Hospital, Dhaka Medical College Hospital, and some private hospitals (Ibn Sina specialized hospital, popular specialized hospital, Islami Bank Central Hospital and Pan Pacific Hospital) in Dhaka, Bangladesh, from July 1999 to June 2013, were included in this study. The local review board in Dhaka approved this study. The prospectively recorded data of clinical presentation, neurological status at admission, radiological imaging, predisposing factors, anatomical location, number of lesions, surgical techniques, complications, cultured organisms, and the neurological outcome were

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10.4103/2347-8659.160851

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studied by Glasgow outcome scale (GOS). Patients with evidence of neurological symptoms unrelated to brain abscess were excluded from the study as, there was evidence showing the patient had not undergone a drainage procedure or intraoperative pus sampling and the patient was lost to follow-up within the first year after operation.

Patients with features of suspected brain abscess were undergone preoperative computed tomography (CT) and/or magnetic resonance imaging (MRI) scans with contrast enhancement. The normal CT scan of brain finding was hypodense lesion with thick contrast enhancing capsule with surrounding edema. By conventional MRI, pyogenic brain abscesses were identified by hypointense signal in T1-weighted and hyperintense signal in T2-weighted, with ring-shaped enhancement and extensive surrounding edema. Conventional MRI with diffusion-weighted imaging, and magnetic resonance spectroscopy (MRS) were performed when it was difficult to discriminate brain abscesses from cystic or necrotic tumors in our later cases of the series. MRS spectra in patients with abscess showed lactate, amino acids (including valine, alanine, and leucine), and acetate peaks while spectra for patients with cystic or necrotic tumors showed only lactate peaks. Hyperintensity was detected in all the pyogenic abscess cavities, and hypointensity was observed in all the cystic and necrotic tumors on diffusion-weighted images. A predisposing factor was considered as any conditions or events which were directly related to the onset of a brain abscess. The neurological status at admission was evaluated using the Glasgow coma scale (GCS) and the outcome of the patients was assessed using the GOS on discharge and 12 months after the operation. Chi-square test was done to see the association between GCS on admission and mortality in brain abscess. Standard laboratory tests including a complete blood count, erythrocyte sedimentation rate (ESR), C-reactive protein, blood cultures, and serum chemistry were conducted in all cases. Case findings were based on the review of microbiology laboratory data for all intracranial samples. All collected intracranial pus with or without abscess wall samples were transported promptly to laboratory microscopy, aerobic, anaerobic and fungal culture and sensitivity and histopathological study. Initial empirical antimicrobial therapies were selected in accordance with the portal of entry and the anatomical location of the abscess. Initial empirical antimicrobial therapy included a combination of high dose of ceftriaxone/cefuroxime/meropenam, flucloxacillin/vancomycin and metronidazole. Between 4 and 6 days later, treatment either remained the same or was changed based on the results of antimicrobial sensitivity. Antibiotic therapy lasted for 4-8 weeks

in accordance with the therapeutic response and neuroimaging findings.

Low-dose corticosteroid was used to manage perilesional edema in first 5-7 days. Seizure prophylaxis or antiepileptic medication was applied in all cases and continued for at least 2 years.

Surgical intervention

Burr hole aspiration was performed under local or general anesthesia for abscesses larger than 2.5 cm, signs of brain herniation secondary to space-occupying lesions (SOL) or ventricular proximity, abscess growth during medical therapy or SOL of uncertain etiology associated with neurological deterioration. If the size of the abscess on CT or MRI obtained after the first aspiration increased or was not reduced despite antibiotic therapy, aspiration was repeated. During surgical procedure, the abscess was drained completely and rinsed with saline containing gentamycin until the effluent was clear. Patients with poor response to repeated aspirations (with three aspirations) and medical treatment underwent complete excision of abscesses through open craniotomy excision. Postoperative abscesses where burr hole aspiration would hinder the fusion of the bone flap also underwent complete abscess excision through open craniotomy excision. Patients with otomastoiditis and brain abscess underwent radical mastoidectomy in a same time or the second session.

RESULTS

Of 221 cases of clinico-radiologically diagnosed brain abscess, 162 cases were surgically managed. Types of abscess [Table 1], predisposing factors [Table 2], site of abscess [Table 3] and types of operations, residual neuro-deficit and outcome [Table 4] are shown.

One hundred and thirteen cases were acute pyogenic abscess [Figures 1-4] and 49 were chronic abscess. Among the chronic abscess, 29 were chronic pyogenic abscess, 14 were tubercular [Figures 5 and 6], 3 aspergillus [Figure 7] and 3 abscesses were in malignant brain metastases.

Age range was 3-72 (average 42.5) years. The male-to-female ratio in our study was 3.37:1. Gender

Table 1: Types of abscess

Types of chronic abscess	Number of different type of chronic abscess	Chronic brain abscess (%)	Acute pyogenic abscess (%)	Total
Chronic pyogenic	29	49 (30.24)	113 (69.76)	162
Tubercular	14			
Aspergillus	3			
Abscess in metastases	3			

Table 2: Predisposing factors of brain abscess				
Types of predisposing factors	Number of predisposing factors	Total predisposing factors (%)	Without predisposing factors (%)	Total
Postsurgery	8	73 (45.06)	89 (54.94)	162
Penetrating injury	11			
CSOM/mastoiditis	22			
Congenital heart disease	10			
Infective endocarditis	3			
Frontal sinusitis	12			
Ethmoidal sinusitis	4			
Immunocompromised	3			
CSOM: chronic suppurative otitis media				

Table 3: Site distribution						
Types of abscess	Frontal	Temporal	Parietal	Occipital	Cerebellar	Ganglio-thalamic zone
Acute pyogenic (113)	34	25	16	18	13	7
Chronic pyogenic (29)	10	9	4	3	3	0
Tubercular (14)	3	3	2	1	4	1
Aspergillus (3)	2	-	-	1	-	-
Abscess in metastasis (3)	-	-	-	1	1	1
Total (%)	49 (30.2)	37 (22.8)	22 (13.6)	24 (14.8)	21 (12.96)	9 (5.5)

Table 4: Types of operations, residual neuro-deficit and outcome				
Operations	Number	Mortality	Residual major neuro-deficit	Complete recovery
Single burr hole aspiration	111 (68.5%)	11	4 (hemiparesis, motor dysphasia, hand weakness, footdrop)	96
Multiple aspiration	34 (21%)	9	3 (monoparesis, sensory dysphasia, visual field defect)	22
Third ventriculoscopic (endoscopic) drainage and ETV [Figure 6]	1 (0.62%)	0	0	1
Excision of abscess by craniotomy	16 (9.87%)	2	2 (nominal dysphasia, monoparesis)	12
Total	162	22 (13.58%)	9 (5.55%)	131 (80.86%)

ETV: endoscopic third ventriculostomy

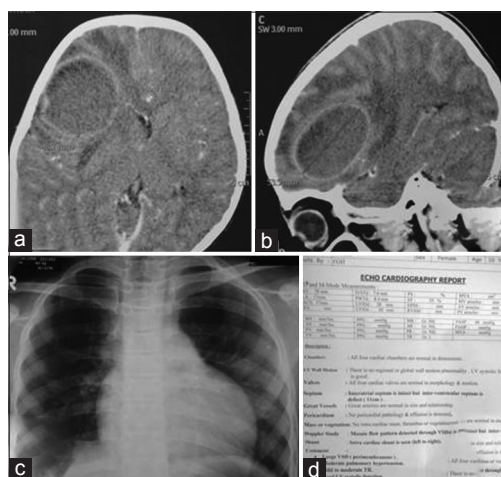


Figure 1: Preoperative contrast computed tomography scan of brain (a: axial section; b: sagittal section) showing right-sided posterior frontal brain abscess in child with tetralogy of Fallot; (c) X-ray chest P/A view showing "boot shaped" heart shadow; (d) echocardiogram report

distribution, numbers of abscess and laboratory findings of patients are shown in Table 5.

In acute cases common clinical features were headache (89.3%), fever (67.5%), vomiting (38%), focal deficit (31%) and seizure (22.6%) focal and secondary generalized). Among the chronic pyogenic cases, there was a history of acute febrile illness in 15 cases (out of 29; 51.7%). In all chronic abscesses, common

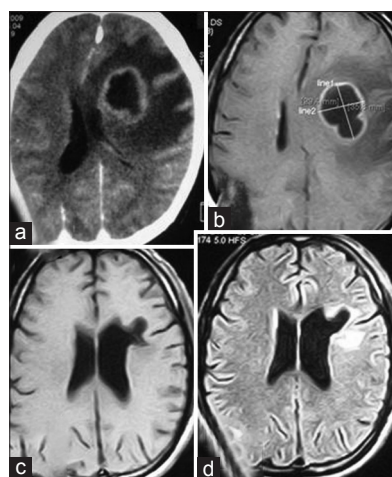


Figure 2: (a) Preoperative contrast CT axial section and (b) preoperative contrast MRI showing left sided paraventricular abscess with mass effect and edema; (c) posttreatment MRI of brain in T1W axial section and (d) posttreatment MRI axial section in fluid-attenuated inversion recovery showing complete resolution of abscess with some gliosis and cerebromalacia. CT: computed tomography; MRI: magnetic resonance imaging

clinical features were mild to moderate headache and progressive focal deficit. In tubercular abscess, clinical features were low-grade fever, weight loss and anorexia in addition to headache. Two patients with tubercular abscess in temporal lobe presented with temporal lobe epilepsy and superior orbital fissure syndrome. Concurrent tuberculosis in another system was found only in 3 out of 14 cases of tubercular abscess. In

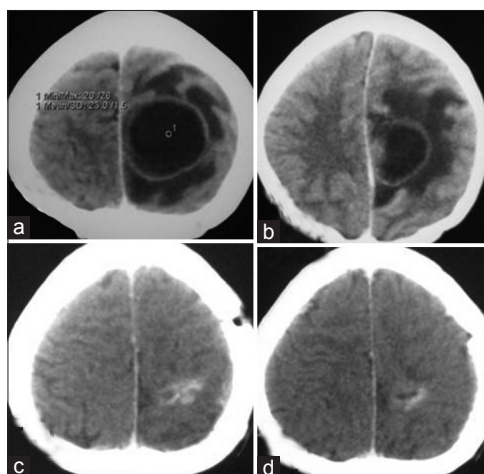


Figure 3: (a and b) Contrast CT scan of brain axial section showing left sided parasagittal fronto-parietal abscess; (c and d) contrast CT scan of brain after completion of treatment resolution of abscess with some residual calcification. CT: computed tomography

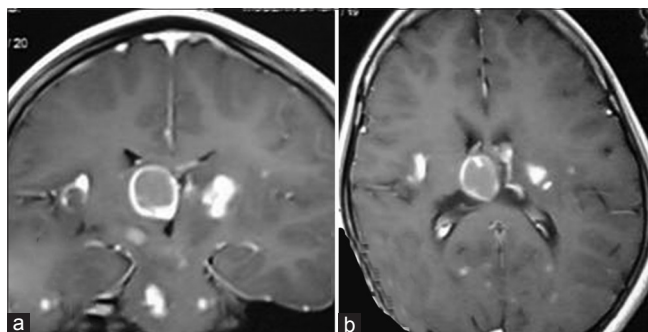


Figure 5: (a) Coronal section and (b) axial section showing tubercular abscess in right lateral ventricular subcallosal region with multiple satellite tubercular lesion throughout the brain

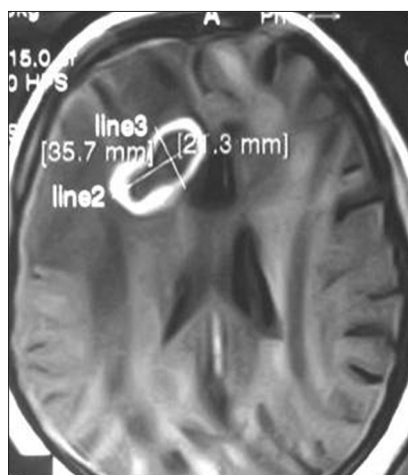


Figure 7: Contrast magnetic resonance imaging of brain axial section showing ring enhancing right frontal aspergillus abscess (proved by postoperative culture of pus and histopathology) with perilesional edema

aspergillus abscess, 1 patient was with renal transplant and 2 were SLE patients [Figure 7]. No primary site for malignancy was found in those 3 brain abscesses in metastasis.

There was hemiparesis in 52 cases, hemiplegia in 23 cases, monoplegia in 12 cases, monoparesis in

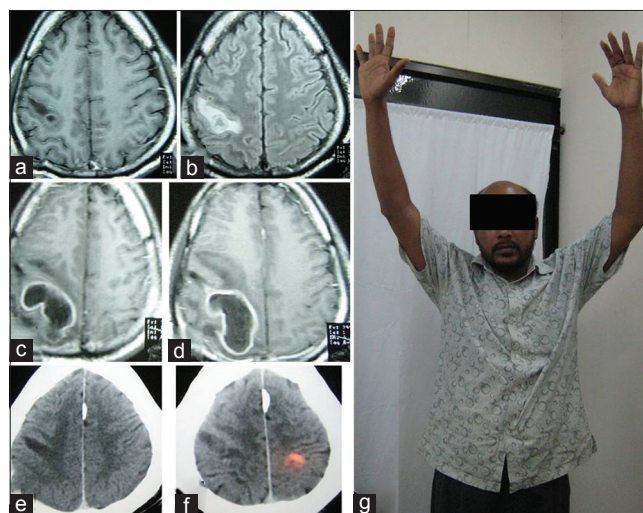


Figure 4: (a and b) MRI of brain axial sections, contrast and fluid-attenuated inversion recovery images respectively was done after sudden development of left sided hemiplegia showing noncontrast enhancing hypointense lesion in precentral gyrus; (c and d) contrast MRI of brain axial sections, 16 days after initial presentation showing ring enhancing lesion (abscess); (e and f) (contrast CT scan axial section 3 months after operation) showing resolution of abscess with some gliosis; (g) patient-3 months after operation (with full neurological recovery). CT: computed tomography; MRI: magnetic resonance imaging

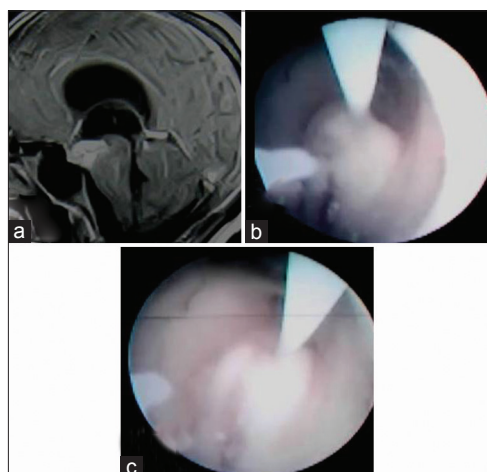


Figure 6: (a) Preoperative contrast magnetic resonance imaging of brain in sagittal section showing contrast enhancing lesion (tubercular abscess) in third ventricular floor and interpeduncular fossa; (b and c) per operative picture during endoscopic third ventriculoscopic interventions showing emergence of tubercular pus from the lesion

19 cases, motor aphasia in 14 cases, dysphasia in 13 cases, and sensory aphasia in 17 cases. Visual disturbances were found in 11 cases (especially in occipital lobe abscess). There was short-term memory loss in 5 cases, bowel and bladder incontinence in 3 cases, frontal lobe syndrome in 4 cases, temporal lobe epilepsy in 21 cases, and gait disturbances in 19 cases. There was coarse hemi tremor in 1 case. In 2 patients, presentations were like that of acute stroke [Figure 4].

Seventy-three (45.06%) patients had adjacent localized cranial infection, chronic suppurative otitis media (CSOM) or paranasal sinusitis. The most common predisposing factors included postneurosurgery (8 cases), postpenetrating injury to

brain (11 cases), CSOM (22 cases), and congenital heart disease (in 10 patients including 4 cases of Tetralogy of Fallot-TOF), infective endocarditis (3 cases), frontal sinusitis (12 cases), ethmoidal sinusitis (4 cases), and 3 patients were immunosuppressed or immunocompromised.

Frontal lobe involved in 49 (30.2%) cases of brain abscess, temporal lobe is next to involved in 37 (22.8%) cases. Parietal, occipital, cerebellar and gangliothalamic zone in 22 (13.6%), 24 (14.8%), 21 (21.96%) and 9 (5.5%) cases respectively. Site distributions of brain abscess were shown in Table 3.

Operations used in brain abscess surgery were single time burr hole aspiration in 111 (68.5%) cases, two or more times burr hole aspiration in 34 (21%) cases, excision of abscess by craniotomy in 16 (9.87%) cases and third ventriculoscopic (endoscopic) tubercular abscess drained with endoscopic third ventriculostomy) in third ventricular floor tubercular abscess in one (0.62%) cases [Figure 6]. Types of operations, residual neuro-deficit, mortality and outcome are illustrated in Table 4. Pus culture indicated negative results in 145 (89.5%) cases. Anaerobic culture and culture for Mycobacterium failed to yield any bacterial growth. Organisms isolated from pus culture are shown in Table 6.

Total number of death was 22 (13.58%) cases. Complete resolution of an abscess with complete recovery of

preoperative neuro-deficit was observed in 131 (80.86%) cases [Figures 2-4]. Complete resolution of an abscess with residual preoperative major neuro-deficit was detected in 9 (5.55%) cases. Persistent major neuro-deficit was hemiparesis 1, motor dysphasia 1, hand weakness 1, foot drop 1, monoparesis 2, sensory dysphasia 1, nominal dysphasia and visual field defect 1. Coarse hemi-tremor resolved postoperatively along with abscess resolution. Mortality and morbidity with GCS at admission and GOS on last follow-up are shown in Table 7. Patients GCS on admission had a significant effect on mortality in brain abscess as shown in Table 8. Six patients with congenital heart diseases underwent cardiac surgery; sinus surgery was performed in 12 patients and 5 patients underwent mastoidectomy in a different sitting within 1 year after brain abscess surgery without any mortality.

DISCUSSION

Brain abscess is an intraparenchymal collection of pus. The incidence of brain abscesses is about 8% of intracranial masses in developing countries, whereas, in Western countries, the incidence is about 1-2%.^[1,4-6] Though potentially curable, there was still a diagnostic and therapeutic challenge. In the last two decades, there is a major advance in the diagnosis and management of brain abscesses, with a corresponding improvement in the survival rate. In the development of brain abscess, inoculation of an organism is required into the brain parenchyma in an area of devitalized brain tissue or in a region with poor microcirculation, and the lesion evolves from an early cerebritis stage to the stage of organization and capsule formation.^[7] Histologically, there are four stages in brain abscess formation: early cerebritis (day 1-3), late cerebritis (day 4-9), early encapsulation (day 10-13) and late capsule stage (day 14 onward). About 2 weeks are required for encapsulation, which is usually less complete on medial or ventricular side due to poor vascular supply.^[8,9] The mode of entry of organisms could be by contiguous spread, hematogenous dissemination, or following trauma.^[4] The common predisposing factors of a brain abscess are CSOM, congenital cyanotic heart disease, and paranasal sinusitis.^[1,5,10-12]

Immunosuppression due to disease or therapy is emerging as an important risk factor for the development of brain abscess.^[4] Here, we found predisposing factors of brain abscesses were similar.

The most common organism isolated from a brain abscess was *Staphylococcus aureus* in the preantibiotic era.^[5] Now, *Streptococcus* spp. have replaced *Staphylococcus* spp. as the most common organisms.^[5,13] Based on the site of origin, the organisms would be different. Streptococci

Table 5: Gender distribution, number of abscess and laboratory findings of patients

Demographic variables	Number (%)
Gender	
Male	125 (77.16)
Female	37 (22.84)
Raised lab parameters	
ESR	41 (25.35)
CRP	84 (51.85)
WBC	78 (48.14)
Number of abscess	
Single	126 (77.7)
Multiple	36 (22.3)

ESR: erythrocyte sedimentation rate; CRP: C-reactive protein; WBC: white blood cell

Table 6: Culture-positive bacterial-fungal isolates from brain abscesses

Bacteria-fungus	Number of patients	Percentage
<i>Streptococcus intermedius</i>	2	10.5%
<i>Ps. Aeruginosa</i>	3	
<i>Staphylococcus aureus</i>	4	
<i>Streptococcus epidermidis</i>	1	
<i>Streptococcus pyogenes</i>	3	
<i>Streptococcus pneumoniae</i>	1	
<i>Mycobacterium</i>	0	
Anarobic	0	
Fungal	3	
No growth	145	89.5%

Ps. eruginosa: *Pseudomonas aeruginosa*

Table 7: Mortality and morbidity with GCS at admission and GOS on last follow-up					
GCS on admission	Number	Mortality	Major residualneurodeficit	GOS on last follow-up	Number (%)
3-7	11 (6.8%)	7	1	1 (death)	13 (8.02)
8-12	29 (17.9%)	5	1	2 (vegetative)	0 (0)
13-15	122 (75.3%)	10	7	3 (severe disability)	0 (0)
				4 (moderate disability)	9 (5.55)
Total	162	22 (13.58%)	9 (5.55%)	5 (good recovery)	131 (80.86)

GCS: Glasgow Coma Scale; GOS: Glasgow Outcome Scale

Table 8: Relationship between mortality and GCS score on admission in brain abscess				
GCS on admission	Total number of patient	Total mortality	Total survivality	Chi-square test
3-12	40	12	28	12.2 (at 5% level of significance calculated Chi-square test value 12.2 is greater than table value 3.84)
13-15	122	10	112	
Total	162	22	140	

GCS: Glasgow Coma Scale

were isolated from abscesses of all types and at all sites, whereas *Enterobacteriaceae* and *Bacteroides* spp. were isolated from otogenic temporal lobe abscesses, which had mixed cultures.^[13] *Streptococcus* spp. have been most commonly isolated from cardiogenic abscesses.^[14] In neonates, the most common organisms are *Proteus* and *Citrobacter* spp. Anaerobes are one of the most common causative organisms in a brain abscess.^[15] Polymicrobial infections are common, indicating the importance of using both aerobic and anaerobic cultures in diagnosis.^[15,16] Cultures for acid-fast bacilli and fungi should be conducted in all cases as occasionally, intracranial tuberculosis as well as fungal infections can present as an abscess.^[17-20] In our series, majority of the culture failed to show positive bacterial growth. More than one-third of otogenic and metastatic abscesses are polymicrobial (aerobic and/or anaerobic). *Bacteroides*, *peptostreptococcus* and *fusobacterium* are common anaerobes and are sensitive to metronidazole.^[21-23] Rhinogenic abscess is generally streptococcal. *Staphylococcus* is common in posttraumatic and postoperative cases. In infants and neonates, postmeningitic abscess is caused by Gram-negative organisms.^[24]

Clinically, brain abscess presents with features of rapidly expanding intracranial mass lesion that is, raised intracranial pressure (ICP) in the form of constant progressive headache refractory to therapy, vomiting, papilledema, focal deficits, convulsions, meningism and altered sensorium. The classical triad of headache, focal neurological deficits and fever is found in 25% cases only.^[5] Brain abscess occurs in the younger age groups usually in the first three decades of life.^[1,6] Seizures have been reported in up to 50% of cases.^[1,6,25] The duration of symptoms is usually < 2 weeks, with rapid onset and progression. Immunocompromised patients may have an insidious onset.^[6] Three patients in this series had bladder and bowel incontinence; one had tubercular abscess in third ventricular floor with hydrocephalus, second

one had one large abscess in frontal lobe and another abscess in cerebritis stage on the opposite frontal lobe and the rest had large paracentral lobule abscess with huge mass effect compressing the opposite side.

A lumbar puncture is contraindicated in patients with a suspected brain abscess because it can result in transtentorial or transforaminal herniation and subsequent death.^[26] CT facilitates early detection, exact localization, accurate characterization, determination of number, size and staging of the abscess. It also detects hydrocephalus, raised ICP, edema and associated infections like subdural empyema and thus helps in treatment planning. It is invaluable in the assessment of the adequacy of treatment and sequential follow-up. An ill-defined area of low density, on plain CT, corresponds to developing necrotic center in the cerebritis stage.^[6] In the early capsule stage, a slightly hyperdense, faint ring is seen surrounding a necrotic hypodense center. With contrast, the ring shows thin regular enhancement of uniform thickness and smooth contour on its inner surface with marked perilesional hypodense area suggestive of edema. In the late capsule stage, the capsule is seen as a ring in plain CT. With contrast, it shows thick enhancement gradually fading in delayed scans. Ring enhancement can be seen in the late cerebritis stage and is not an absolute evidence of encapsulation.^[8,9] Radiological features alone are inadequate to differentiate pyogenic brain abscess from fungal, nocardial or tuberculous abscess, inflammatory granuloma (tuberculoma), neurocysticercosis, toxoplasmosis, metastasis, glioma, resolving haematoma, infarct, hydatid cyst lymphoma and radionecrosis.^[27-30] However, fever, meningism, raised ESR, multilocularity, leptomeningeal or ependymal enhancement, reduction of ring enhancement in delayed scan and finding of gas within the lesion favor a diagnosis of abscess.^[9] Positive labeling in radionuclide imaging with III-Indium labeled leukocytes, C-reactive protein, 99m TC-hexamethylpropylene amine oxime leukocyte scintigraphy, diffusion weighted MRI, Thallium-201

single photon emission CT, and proton MRS are helpful in differentiating abscess from tumor.^[31-38]

Brain abscesses were singular in 77.7% of the subjects and multiple in 22.3%, a result similar to that reported by Landriel *et al.*^[39] The frontal lobe was the most common abscess location in the patients, followed by the temporal and occipital regions. However, in a study carried out by Cavusoglu *et al.*,^[40] the temporoparietal region was the most commonly affected location. Abscesses of unknown cause accounted for 54.94% of the subjects, higher than the values reported for other series.^[12,39,41-43] In most large series of brain abscesses from developing countries, middle ear infection has been reported to be the most common source of intracranial suppuration, a result similar to the current study.^[44]

The basic principle of treatment is the prescription of appropriate antibiotics with or without aspiration, treatment of sequelae that is, hydrocephalus, seizures *etc.*, and eradication of primary source.^[26] According to a number of authors, treatment of brain abscess involves aspiration of the pus or excision of the abscess, followed by parenteral antibiotic therapy.^[1,5,6,12,45] Empirical medical therapy is the best avoided and should be reserved for patients in whom a bacteriological diagnosis has been obtained from a systemic source or who are extremely ill that is, too ill to undergo any forms of intervention.^[45] Small abscesses and lesions in the cerebritis stage respond well to medical therapy alone.^[14] The choice between conservative versus operative treatment is influenced by age, neurological status, location, number, size and stage of abscess formation. Each case must be individualized and treated on its own merits. Conservative treatment can be tried in patients who are alert, clinically stable and have a major risk for surgery and anesthesia. Treatment of sequelae that is, hydrocephalus, seizures, *etc.*, and eradication of primary source should not be neglected. The management should be done by neurosurgeons prepared to operate at the first sign of failure of medical therapy or where immediate neurosurgical help is available. Medical treatment alone should not be applied when the diagnosis is not yet confirmed. Abscess in cerebritis stage, or walled off but smaller than 3 cm diameter could be treated nonsurgically with antibiotics alone.^[27] Serial CT scans are crucial as it may enlarge despite adequate antibiotics therapy.^[46] The complexity of microbial flora in brain abscess necessitates empirical antibiotic therapy against both aerobic and anaerobic organisms. Usually, intravenously administration of “triple high dose” antibiotics for 2 weeks followed by 4 weeks of oral therapy is recommended. Corticosteroid can only be used to reduce edema and administration of

anticonvulsant should be routine in supratentorial abscess, but duration is a matter of debate.^[6]

If serial CT scans show increased size of abscess at any time during conservative treatment with antibiotics or no decrease in size within 4 weeks, a surgical procedure should be performed to confirm the diagnosis, to obtain a sample for culture of identification of specific pathogens and sensitivity to particular antibiotics, and to remove as much purulent material as possible. Walled off abscess larger than 3 cm diameter and a smaller deep-seated white matter abscess are unlikely to respond medical treatment alone. Standard therapy for such lesions should be surgical evacuation followed by appropriate antibiotic.^[47] Instillation of antibiotics inside the abscess cavity can be considered. A surgical drainage allows immediate decompression of mass lesion and reduction of ICP that reduces the duration of antibiotic therapy and hospitalization. It increases the likelihood of cure. Surgery should be performed in case of clinical deterioration, significant mass effect and neurological deficit. Many surgical techniques have been developed, but there is no single best method.^[48] At present, aspiration and excision are two common procedures used. Role of aspiration versus excision is controversial. In choosing between aspiration and excision, various factors including surgical morbidity, success rate and sequelae such as recurrence and seizure disorders also must be considered. Aspiration is a rapid and safe procedure, especially with the use of stereotactic techniques, ultrasound or CT scan guidance. It can be done under local anesthesia, on bedside, even in seriously ill or high-risk patients. Aspiration can be done at any stage of evolution of abscess. If no pus is obtained, biopsy gives positive culture even in early cerebritis stage. A large, superficial, or accessible abscess can be aspirated via appropriately placed burr hole. Real time ultrasound, particularly in infants with open fontanelle and stereotaxy provides precise localization. Free hand needle aspiration can be a very effective life-saving measure in the underdeveloped world where stereotaxy is not available.^[30] More than one aspiration may be required. Repeat aspirations are often necessary for cure.

With free availability of CT scan, role of aspiration has increased, as abscesses can be detected easily and follow up is available immediately. Some authors recommended stereotactic aspiration/biopsy in all patients with suspected brain abscess regardless of size.^[49] Aspiration has a place, both as preparatory to eventual excision (secondary excision) and as a definitive procedure.^[48] Multiloculated abscesses have been treated with stereotactic aspiration of all loculi in single or staged aspiration. Encouraging results with endoscopic stereotactic evacuation of brain abscess has

been shown recently.^[50] Neuroendoscopic treatment, when compared to stereotactic aspiration, has an additional advantage of more complete drainage and lavage.^[51]

Many authors recommended craniotomy and excision for abscesses that enlarge after 2 weeks of antibiotic therapy or that fail to shrink after 3-4 weeks of antibiotics.^[1,6,7,45] Craniotomy is also recommended for multiloculated abscesses and larger lesions with significant mass effect that are superficial and located in noneloquent regions of the brain. A few authors also recommended excision of abscesses in the cerebellum, where recurrent pus collection following aspiration can lead to precipitous neurological worsening.^[26] There are certain advantages to excision of a brain abscess in an otherwise neurologically intact patient. The risk of repeated collection of pus is almost completely eliminated, and hence the expense involved in repeated imaging is saved. The duration of hospitalization is also reduced. Furthermore, in patients with an otogenic brain abscess, the disease in the middle ear can also be surgically treated at the same sitting or soon thereafter.^[18] This also reduces the likelihood of recurrence of the abscess. Abscess resulting from fistulous communication, example, trauma and congenital dermal sinus, require excision of infected granulation tissue and closure of the fistula. Abscess localized to one lobe and contiguous to primary source that is, frontal sinus osteomyelitis, is better treated with excision along with the primary focus. Posttraumatic abscess containing foreign body or contaminated retained bone fragments requires excision to prevent recurrence.^[8,46] Abscesses containing gas are resistant to antibiotics and are better treated with excision.^[52] Large superficial abscesses resistant to multiple aspirations and not showing volume reduction because of adhesions to the dura, due to large brain surface area should be excised for cure. Multiloculated actinomycotic and nocardial abscess may need excision as simple aspiration may prove inadequate.^[53] Excision reduces the incidence of seizures and prevents recurrence. Abscess in cerebritis stage, deep-seated abscesses in eloquent areas and multiple abscesses are the situation where excision should not be considered.^[6]

The mortality rate in our study was 13.58% (22 cases). Sixteen patients died in the immediate postoperative period from brain herniation with very high ICP (15 cases), ARDS (2 cases), septicemia with systemic inflammatory response, multiple organ dysfunction (4 cases) and one patient died from acute pancreatitis after operation. The mortality rate shown here is similar to the rates observed by other authors, which range between 8% and 53%.^[54] Manzar *et al.*^[43] reported that the most important factors influencing

mortality was the neurologic condition of the patient at the time of admission. Here, we found mortality was very high in brain abscess with low GCS score on admission [Tables 7 and 8]. Landriel *et al.*^[39] revealed that age, immunosuppression and hematogenous spread were all associated with poor outcomes.

In conclusion, predisposing factors were seen in nearly half of the cases. In most of the cases, pus culture did not yield causative organisms. From this series, we see that in “chronic abscess group” pyogenic abscess were the commonest followed by tuberculous abscess but possibilities of other causes (i.e. fungal) should not be overlooked. Mortality due to brain abscess was not directly related to surgical intervention but on admission GCS has a significant association with the mortality. Like other diseases, we can state early diagnosis and optimum follow-up, and timely surgical interventions are the keys in the management of brain abscess.

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Cite this article as: Chowdhury FH, Haque MR, Sarkar MH, Chowdhury SM, Hossain Z, Ranjan S. Brain abscess: surgical experiences of 162 cases. *Neuroimmunol Neuroinflammation* 2015;2(3):153-61.

Source of Support: Nil. **Conflict of Interest:** No.

Received: 29-10-2014; **Accepted:** 12-03-2015

Intrathecal dexamethasone and methotrexate treatment of neoplastic meningitis from solid tumors

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ABSTRACT

Aim: Neoplastic meningitis (NM) from solid tumors is an advanced malignancy with poor prognosis. Intrathecal chemotherapy is a reliable treatment, and we have obtained some experiences in the treatment of NM with intrathecal dexamethasone and methotrexate (IT DXM and MTX). **Methods:** Retrospective study of 23 patients with NM from lung cancer ($n = 11$), breast cancer ($n = 3$), gastric cancer ($n = 1$), malignant melanoma ($n = 1$), unknown cancer ($n = 7$) was conducted. Among these patients, eight received IT DXM and MTX treatment, and 15 patients were placed into a palliative care group. Overall survival (OS) was compared, and the patients' characteristics, symptoms, and some laboratory examinations were analyzed to find the risk factors affecting OS. **Results:** OS of IT DXM and MTX group was significantly longer than that of the palliative care group ($P = 0.01$). The median survival (MS) of palliative care group was 7.53 weeks (5.50-9.57; $n = 15$), and of the IT DXM and MTX group, 28.63 weeks (12.50-44.75; $n = 8$); IT DXM and MTX prolonged the OS of NM patients (regression coefficient = -2.923), with odds ratio (OR) being 0.054 (0.09-0.323). Spinal nerves damage decreased the OS (regression coefficient = 1.595), with OR being 4.928 (1.382-17.579). **Conclusion:** IT DXM and MTX have prolonged the patients' MS, which could be used as a fundamental treatment of NM. Time of induction treatment should be flexible and individualized, and induction treatment could restart when central nervous system relapse. Patients with spinal nerves damage are apt to live shorter.

Key words: Clinical experiences, intrathecal dexamethasone and methotrexate, neoplastic meningitis

INTRODUCTION

Neoplastic meningitis (NM) is the leptomeningeal dissemination of metastatic tumors; a devastating complication from solid tumors. The incidence of NM has increased as patients are living longer due to significant improvements in treatment options in the form of large molecule target agents. There are case reports about cancers that don't yet progress into NM, such as ovarian cancer,^[1] prostate cancer,^[2] and renal cancer.^[3] NM is clinically detected in 5-8% of the patients with cancers, while through autopsies NM detected in 19% of the cancerous patients.

Cerebrospinal fluid cytology (CSFC) is the gold standard for determining NM, with the reported sensitivity of

CSFC being 71-94%.^[4-9] The survival of NM ranges from 8 to 16 weeks despite treatment.^[10,11] Patients have a poor Karnofsky Performance Score (KPS), when diagnosed with either bulky central nervous system (CNS) disease, abnormal CSF-flow study, multiple serious neurological deficits, encephalopathy, and extensive systemic cancer without good treatment options have poorer prognosis and need palliative care instead of positive therapy.^[9] For an improved outcome, most patients of NM need a combination of radiation therapy, systemic chemotherapy, and intrathecal chemotherapy. Intrathecal chemotherapy is the main treatment of NM. Methotrexate, cytarabine, thiopeta, liposomal cytarabine are the traditional intrathecal chemotherapy regimens.

Intrathecal methotrexate has a long history of treating NM.^[12] Intrathecal methotrexate is now widely used to treat NM in the patients with those cancers with possible metastasis to the CNS, such as gastric cancer, breast cancer, lymphoma, non-small-cell lung cancer, multiple myeloma, as well as in the patients with cancers rarely spreading to CNS-atypical neurofibroma and with pancreatic cancer.^[13-19] Though many physicians use intrathecal cytotoxic drugs in combination with system

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10.4103/2347-8659.160855

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chemotherapy or target agents,^[15,16,20] it is irreplaceable in the treatment of NM, despite of some reported adverse reactions.^[21,22] We acquire some clinical experience about how to minimize the side effect and how to institute the course of treatment.

METHODS

Inclusion criteria

Subjects were required to present with the clinical signs and symptoms consistent with NM, including headache, confusion, cranial and spinal nerve involvement, nausea and vomit. CSF (200 μ L) was collected from a lumbar puncture and it was centrifuged in (650 rpm) for 4 min using Slide Centrifuge (Shandon Cytospin 4, Thermo). Cell slides were May-Grunwald-Giemsa stained for 5 min then phosphate buffers was added and incubated for 10 min, followed by gentle rinsing with running water. The stained cell slides were observed under the microscope (Oil immersion lens $\times 1000$, Olympus DP72). NM was diagnosed once tumor cells were found by experienced examiners as showed in Figure 1. Patients with cancer cells were allocated to intrathecal dexamethasone and methotrexate (IT DXM and MTX) group and palliative care group according to their families' will.

Subjects were discontinuous cases from 2006 to 2014 who did CSF cytologic exams in the CSF cytological examination laboratory of the Second Hospital Affiliated to Hebei Medical University.

Treatment of neoplastic meningitis

After the NM diagnosis, the patients in IT DXM and MTX group received intrathecal dexamethasone 5 mg and methotrexate 10 mg, two doses a week as an inductive treatment of 4 weeks until the symptom was relieved or tumor cells reduced significantly in CSFC examination. Then the patients underwent treatments with a dehydrating agent, pain killer drugs, benzodiazepines, as well as other supportive treatments in the hospital. Then IT DXM and MTX was given one dose every 2 weeks in the outpatient department until the general condition severely deteriorated and could not sustain one's life. Subjects in the palliative care group received supportive treatments in hospital or at home, according to the families' determination based on the pain of lumbar puncture or economic reasons.

Intrathecal injections were conducted as follows: use of intravenous mannitol 250 mL was 20 min before lumbar puncture and remained throughout the process of lumbar puncture. The infusion apparatuses were readily available in case of use of emergency drugs. Ten milliliter CSF was slowly drained out of the subarachnoid space through a half-clogging needle for CSF examination. The needle was then returned into

the cannulas. Dexamethasone sodium phosphate was diluted from 1 mL (5 mg) to 5 mL with physiological saline and then slowly injected into the subarachnoid space. During the injection, dexamethasone sodium phosphate was mixed with drawing back CSF repeatedly. Methotrexate was diluted to 5 mL and then injected the same way dexamethasone was treated.

Data collection

The patients' characteristics and treatment information at the diagnosis of NM were obtained in the medical record from the Second Hospital of Hebei Medical University. Survival data, subsequent therapeutic schedule, and side effects following discharge were obtained by making the phone calls to ask whether there is paralysis, severe vomiting, headache within 48 h after intrathecal injection, or the symptoms of bone marrow suppression, such as fever, infection, and low blood cell count. Overall survival was calculated from the diagnosis of NM.

RESULTS

The patients' characteristics

Twenty-three subjects were diagnosed as NM according to the positive CSF results as shown in Figure 1. Patient characteristics were summarized in Table 1. Eight patients received IT DXM and MTX treatment as IT DXM and MTX group, and 15 patients as the palliative care group was treated with palliative therapy, such as dehydrant drugs and painkillers.

Among them, 22 subjects showed high intracranial pressure (> 200 mmH₂O), with other common presenting symptoms including inability to walk ($n = 3$), varying degrees of visual loss ($n = 9$), hearing damage ($n = 4$), sphincter disturbances ($n = 4$), seizure ($n = 6$), and confusion ($n = 3$). Furthermore, 1 patient received systemic chemotherapy, 2 received whole brain radiotherapy, and 1 received ventriculoperitoneal shunt (VP shunt) treatment. All patients showed positive results by CSFC exam.

Survival

Overall survival (OS) was assessed from the time of NM diagnosis to death and then Kaplan–Meier

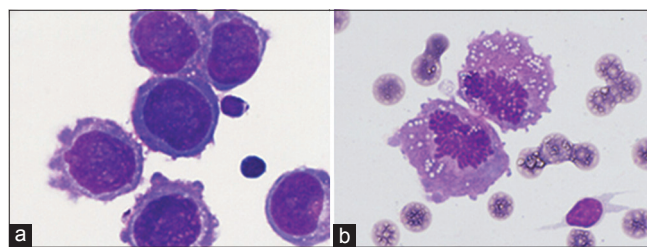


Figure 1: Neoplastic meningitis was diagnosed when irregular-shaped cells with big nucleus (a) or high ratio of mitotic cells (b) in cerebrospinal fluid were deep-stained

analysis (group by treatment as IT DXM and MTX group/palliative care group; log-rank test) was conducted [Table 2 and Figure 2]. The OS of IT DXM and MTX group was significantly longer than that of the palliative care group ($P = 0.01$). The median survival of palliative care group is 7.53 weeks (5.5-9.57; $n = 15$), and have the IT DXM and MTX group, 28.63 weeks (12.50-44.75; $n = 8$), of the total patients, 14.87 weeks (7.93-21.81 weeks; $n = 23$).

We collected the patients' characteristics, symptoms, treatment method, and some laboratory examinations at the initial diagnosis of NM, including IT DXM and MTX, KPS, age, gender, primary tumor, cranial nerves damage, spinal nerves damage, seizure, confusion, and level of hemoglobin, albumin, and globulin. We analyzed the possible survival ratio of these factors using Cox's proportional hazards regression model and the method of forward LR, by entering the factor when $P < 0.05$ and removing it when $P > 0.06$. IT DXM and MTX prolonged the OS of NM (regression coefficient = -2.923), odds ratio (OR) = 0.054 (0.09-0.323). Spinal nerves damage decreased the OS (regression coefficient = 1.595), OR = 4.928 (1.382-17.579). Other factors did not

enter the Cox's model (KPS, $P = 0.935$; age, $P = 0.270$; gender, $P = 0.726$; primary tumor, $P = 0.220$; cranial nerve damage, $P = 0.564$; seizure, $P = 0.605$; confusion, $P = 0.485$; hemoglobin level, $P = 0.434$; albumin level, $P = 0.658$; globulin level, $P = 0.781$).

Bias analysis

There are some innate biases in retrospective studies. Recall bias and confounding bias were the most important biases in our study. Recall bias is innate and uncontrollable, so the conclusion about IT treatment may be not well-grounded. For the latter bias, we analyzed some confounding factors between IT DXM and MTX group and palliative care group. We used KPS as a quantitative index of the subjects' condition. There were no differences in KPS (2 independent samples t -test, $P = 0.733$) and gender (Fisher exact t -test, $P = 0.367$) between the groups, so we can exclude the imbalanced distribution of the KPS and gender and its effects on the different OS between the groups. Age of IT DXM and MTX group is higher than that of palliative group [Mann-Whitney U , $P = 0.043$; 60.5 (56.5, 64.5) vs. 55 (44, 66)]. Aged patients present negative prognostic factors,^[23,24] but IT DXM and MTX group had elder age and longer survival. This is possibly because of the different treatment methods. Given a small number of cases, we just compare the proportion of lung cancer and breast cancer, finding no difference between the groups (Fisher exact t -test, $P = 0.685$; $P = 1.0$, respectively), so we conclude that the primary cancer type was at an equilibrium distribution. Moreover, there were some biases coming from the researchers because this study didn't involve blind method in experimental design.

DISCUSSION

Neoplastic meningitis is a solid tumor at the advanced stage during which patients usually has severe pain and must administrate painkillers frequently. The diagnosis of NM often leads to palliative treatment that is intended to preclude the additional discomfort with aggressive treatment. Meningitis, seizure, vomit or sort of adverse effects were reported in the past studies. In our study, the patients in IT DXM and MTX group show no obvious side effects. This may be caused by a small number of cases, but we think that side effects could be decreased if the drugs were

Table 1: The patients' characteristics

	n (ratio or range)
IT DXM and MTX group/ palliative care	8/15
Male/female	9/14
Age	55 (21-67)
Presenting symptoms	
High intracranial pressure	22
Unable to walk	3
Visual loss	9
Hearing damage	4
Sphincter disturbances	4
Seizure	6
Confusion	3
Cancer type (IT DXM and MTX group/palliative care group)	
Lung cancer	4/6
Breast cancer	1/2
Gastric cancer	0/1
Malignant melanoma	0/1
Unknown	3/5
Concurrent treatment	
IT DXM and MTX	8
Systemic chemo	1
WBRT	2
VP shunt	1

IT DXM and MTX: intrathecal dexamethasone and methotrexate;
VP: ventriculoperitoneal; WBRT: whole brain radiotherapy

Table 2: Overall survival of different treatments

Treatment	n	Median OS weeks	95% CI	P (log-rank)
Palliative care group	15	7.53	5.50-9.57	0.01
IT DXM and MTX	8	28.63	12.50-44.75	
Total	23	14.87	7.93-21.81	

IT DXM and MTX: intrathecal dexamethasone and methotrexate; CI: confidence interval; OS: overall survival

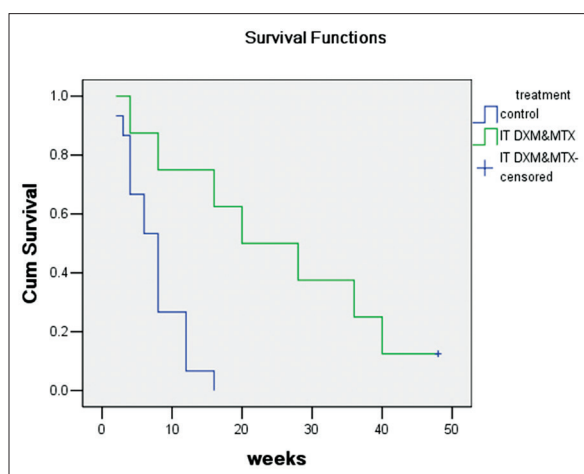


Figure 2: Kaplan-Meier analysis of overall survival in intrathecal dexamethasone and methotrexate group and palliative care group

thoroughly diluted and slowly injected according to the course of treatment. Most of the patients' discomfort was relieved in 3-4 weeks in IT DXM and MTX group with the decreased use of painkillers. IT DXM and MTX is well tolerant despite of the patients' conditions. However, the patients with abnormal flow studies are associated with poor efficacy and intrathecal chemotherapy toxicity.^[25]

Apart from the low drug concentration, we conclude that good tolerance of IT DXM and MTX schedule is related to dexamethasone. Intrathecal steroid therapy can significantly reduce the IL-6 in CSF, a kind of inflammatory factor,^[26] so it may reduce nonspecific inflammatory reaction caused by tumor cells or chemotherapy agents. Dexamethasone has been reported as feasible and well tolerated with concomitant intrathecal liposomal cytarabine in patients with acute lymphoblastic leukemia.^[27] However, no prospective trials in adults with NM prove beneficial to use of intra-CSF glucocorticoids in combination with intra-CSF chemotherapy.

The natural processes of NM are disastrous if not well treated, for most patients will have a quickly deteriorated condition and die within 2 months. Intrathecal methotrexate is not a new therapy, but the random controlled trial is rare. IT DXM and MTX prolonged the patients' survival significantly. The medium survival accords with William R and Theodore's report,^[4,11] and is longer than that reported in Glantz's study,^[28] in which most subjects were dominated by breast cancer. This is different from our study in that lung cancer is the dominating subject in our study that represents shorter survival.^[29]

Among all those factors, IT DXM and MTX prolong the survival, while spinal nerves damage shortens the OS. Other factors cannot be deemed as having no influence

on the OS definitely, although they were not yet fit into the Cox's proportional hazards regression model. Given the small number of patients, these factors cannot be statistically discarded as a Type II error. Just as in Glantz's study, an age > 50, performance status ≤ 70%, primary tumor (lung cancer, malignant melanoma), and lack of cytological response present negative prognostic factors.^[29]

Methotrexate is a folate anti-metabolite and a S-phase specific cytotoxin with a CSF half-life of 4.5-8.0 h.^[24] Therapeutic CSF concentrations obtained in adults and in children of more than 2 years of age are 12 mg IT MTX and 1 μmol/L or more during 48-72 h, respectively.^[30] IT DXM and MTX consists of two injections on a weekly basis for 4 weeks as induction treatment, one injection on a weekly basis for 4 weeks as consolidation treatment, one injection on a monthly basis as maintenance treatment until disease progression.^[13] The patients' responses to IT DXM and MTX are different. Some patients' CSFC remains plenty of tumor cells though induction treatment is accomplished. Other patients' CSFC shows tumor cells lysis, and only single tumor cells 2 weeks after the initiation of induction treatment. Hence, flexible induction time should be discussed, we recommend two injections on a weekly basis for 3 weeks as induction treatment, and continue the treatment one more week if CSFC does not show a decrease in tumor cells. Should CSF relapse as a symbol of IT DXM and MTX termination? The answer is "no" by our experience. Restarting induction treatment could reduce the tumor cells in CSF with relieved symptoms, but randomized controlled trials with more clinical cases should be conducted to confirm this viewpoint. Moreover, new clinical trials of NM based on a tumor-specific histology are needed to establish the role of IT DXM and MTX treatment.

In conclusion, intrathecal dexamethasone and methotrexate are a safe and effective therapy. Although there are diversified intrathecal agents in recent years, other cytotoxic drugs and targeted agents such as trastuzumab^[13] and combined intrathecal chemotherapy^[22] prove efficient in treating NM. Thanks to the uncertain properties of new drugs, combined IT DXM and MTX as a basic treatment may be considered to ensure the therapeutic effect.

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Cite this article as: Lv WJ, He JY, Zou YL, Liu YJ, Zhang QQ, Liu X, Bu H. Intrathecal dexamethasone and methotrexate treatment of neoplastic meningitis from solid tumors. *Neuroimmunol Neuroinflammation* 2015;2(3):162-6.

Source of Support: Nil. **Conflict of Interest:** No.

Received: 21-11-2014; **Accepted:** 08-06-2015

Neurocysticercosis in Nepal: a retrospective clinical analysis

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ABSTRACT

Aim: The prevalence of epilepsy is higher in Nepal. This study was conducted to analyze the clinical manifestations of neurocysticercosis (NCC) among seizure patients admitted to our center. **Methods:** We retrospectively studied all the NCC patients admitted to Neurology Department, Bir Hospital, Kathmandu, Nepal from April 2012 to February 2014. Computer tomography/magnetic resonance imaging (CT/MRI) head, clinical profile, lab investigations and exclusion of other causes were the basis of the NCC diagnosis. Chi-square and Student's *t*-test were used for comparison of variables. **Results:** Out of 131 seizure patients admitted, 21 patients were diagnosed with NCC [mean age: 33.95 ± 16.41 ; male: 15 (71.4%), female: 6 (28.6%)]. Generalized tonic clonic seizure was the most common seizure type in NCC patients (18 patients; 85.7%), two of them had status epilepticus during presentation in Emergency Department. Three patients had focal seizure, one with *epilepsia partialis continua*. Neuroimaging showed multiple NCC lesions in 8 (38.1%) and a single NCC lesion in 13 (61.9%) patients. Seven of them (33.3%) sought traditional healers before being presented to our center. Eight patients (38.1%) were treated with antiepileptics in local health-post without neuroimaging studies done. Calcified stage of NCC was the most frequent CT/MRI findings (12 patients; 57.1%). Phenytoin was preferred both by physicians and patients due to its low cost. **Conclusion:** NCC is a common finding among seizure patients in Nepal. Poor economic status, illiteracy and underdeveloped rural society are the major challenges in prevention and treatment of NCC.

Key words: Cysticercosis, epilepsy, neurocysticercosis, Nepal, south Asia

INTRODUCTION

Neurocysticercosis (NCC) is the most common parasitic infection of the human brain. It is caused by the larval stage of *Taenia solium*, which enters the central nervous system by ingestion of its eggs due to the use of contaminated hand, water or food.^[1] Being a low-income country, the majority of Nepalese population is still illiterate and deal with crops and animal farming. Due to lack of toilets in many Nepalese homes, open field defecation is a common practice. Thus, poor sanitation and improper management of food and meat products are the major causes for higher NCC cases in Nepal.

Seizure is the most common clinical manifestation of NCC, followed by focal neurological deficits and

headache. Diagnosis is often difficult even in developed and non endemic areas, where NCC lesions are confused with tuberculoma or metastatic lesions.^[2,3] Due to unavailability of laboratory tests and radioimaging tools, diagnosis of NCC is often challenging in developing countries. A large treatment gap has been reported from developing countries, 70% in Nepal,^[4] 96% in Nigeria,^[5] 73% in Pakistan^[6] and 90% in India.^[7] Among these, epidemiological studies reported higher prevalence of the treatment gap in rural areas in India and Pakistan.^[6,7]

Since only a few NCC reports were conducted in Nepal, this study aims at describing the clinical manifestations of NCC among seizure patients admitted to our center.

METHODS

We retrospectively analyzed all the available data of NCC patients who were admitted to Neurology Department, Bir Hospital, Kathmandu, Nepal from April 2012 to February 2014. Computer tomography and magnetic resonance imaging (CT and MRI) head were used to classify the various stages of NCC.^[8]

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10.4103/2347-8659.160856

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CT/MRI head, clinical profile, laboratory investigations and exclusion of other causes were the basis of NCC diagnosis. Fundus examination was done in all patients to rule out ocular NCC. Out of 21 NCC patients, MRI Brain was available for 2 patients and CT head plain, and contrast were performed on the remaining patients (19). Serum, anticysticercal antibodies test, was carried out in 2 patients, and serum titer ($> 1:160$) was positive in one of them. NCC was diagnosed according to Del Brutto^[9] revised diagnostic criteria for NCC. These include absolute, major, minor and epidemiological criteria, and the degrees of diagnostic certainty can be further classified under definitive and probable diagnosis. Reports of electroencephalography, complete blood count, chest X-ray, Mantoux test, sputum for acid fast bacilli, renal and liver function test, abdominal ultrasonography and electrocardiography were collected from each patient. The institution ethical board approved the study protocol.

Patients with an intracranial malignancy, head injury, stroke, metabolic disturbance, tuberculosis, brain abscess and HIV were excluded. Patients of pediatric age, that is, below 15 years were not included in our study. Details of patient's personal data, socioeconomic status, clinical profile, drug history and response were recorded.

Data storage and statistical analysis were performed by IBM SPSS Statistics Version 20 for Mac (IBM Corporation, New York, United States). Chi-square and Student's *t*-test were used for comparison of variables; a two-tailed $P < 0.05$ was considered statistically significant.

RESULTS

Out of 131 seizure patients, 46.5% were diagnosed with alcohol withdrawal seizure, followed by idiopathic generalized seizure (17.5%), NCC (16.0%) and others 20%. NCC was the most common cause of secondary epilepsy, followed by brain tuberculoma (3.8%), stroke (3.8%), encephalitis (3.0%), and brain abscess (3.0%) [Table 1].

Neurocysticercosis was diagnosed in 21 patients (16.0%) with mean age of 33.9 ± 16.4 and male:female ratio was 15:6 [Table 2]. According to of the NCC criteria described by Del Brutto,^[9] 10 patients were classified under definitive diagnosis (1 absolute or combination or major, minor and epidemiologic criteria) and 11 patients were diagnosed as probable NCC (combination of major, minor and epidemiologic criteria). About 30% of our NCC patients were local people of Kathmandu. Neuroimaging showed multiple NCC lesions in 8 (38.1%) and a single NCC lesion in 13 (61.9%). Generalized tonic clonic seizure was the

Table 1: Causes of seizure

	Total <i>n</i> (%)	Male <i>n</i> (%)	Female <i>n</i> (%)	<i>P</i>
Alcohol withdrawal syndrome	61 (46.5)	58 (59.8)	3 (8.8)	$< 0.001^b$
Idiopathic generalized seizure	23 (17.5)	8 (8.2)	15 (44.1)	$< 0.001^b$
NCC	21 (16.0)	15 (15.5)	6 (17.6)	0.765
Encephalitis	4 (3.0)	3 (3.1)	1 (2.9)	1.0
Brain abscess	4 (3.0)	1 (1.0)	3 (8.8)	0.053
Stroke				
Hemorrhagic	3 (2.3)	1 (1.0)	2 (5.9)	0.165
Ischemic	2 (1.5)	0	2 (5.9)	^a
Tuberculoma	5 (3.8)	3 (3.1)	2 (5.9)	0.604
Others	8 (6.1)	5 (5.1)	3 (8.8)	0.427
Total	131	97	34	

Data are shown as *n* (%) or mean \pm SD. ^a*P* value not applicable; ^b $P < 0.001$. SD: standard deviation; NCC: neurocysticercosis

Table 2: Characteristics of NCC lesions

	Total <i>n</i> (%)	Male <i>n</i> (%)	Female <i>n</i> (%)	<i>P</i>
Age (years)	33.9 ± 16.4	33.0 ± 16.6	36.3 ± 17.1	0.685
Lesion				
Single	13 (61.9)	9 (60.0)	4 (66.7)	1.0
Multiple	8 (38.1)	6 (40.0)	2 (33.3)	
Seizure				
GTCS	18 (85.7)	14 (93.3)	4 (66.7)	0.184
Partial	3 (14.3)	1 (6.7)	2 (33.3)	
CT/MRI stage				
Multiple	3 (14.3)	2 (13.3)	1 (16.7)	1.0
I	0	0	0	^a
II	5 (23.8)	4 (26.7)	1 (16.7)	1.0
III	1 (4.8)	1 (6.7)	0	^a
IV	12 (57.1)	8 (53.3)	4 (66.7)	0.659

Data are shown as *n* (%) or mean \pm SD. ^a*P* value not applicable. GTCS: generalized tonic clonic seizure; CT: computer tomography; MRI: magnetic resonance imaging; SD: standard deviation; NCC: neurocysticercosis

most common seizure type in NCC patients (18 patients; 85.7%), two of whom had status epilepticus during presentation in an emergency department. Three patients (14.3%) had focal seizure, one of them had epilepsy partialis continua. Headache and vomiting were observed in 7 (33.3%) and 5 (23.8%) patients, respectively [Figure 1]. Calcified stage of NCC was the most frequent CT/MRI findings (12 patients; 57.1%).

Seven patients (33.3%) had sought traditional healers before they were referred to our center. Eight patients (38.1%) were found to be treated with antiepileptics in local health-post without neuroimaging studies done. Phenytoin was preferred both by physicians and patients due to its low cost (12 patients; 57.1%). Valproic acid (5 patients; 23.8%) and carbamazepine (4 patients, 19.04%) were other common first generation antiepileptic drugs (AEDs) reported in our study. Multiple AEDs were administered during treatment of 2 patients.

DISCUSSION

Neurocysticercosis is an endemic disease and a health burden in Nepal. In our study, it is the most common

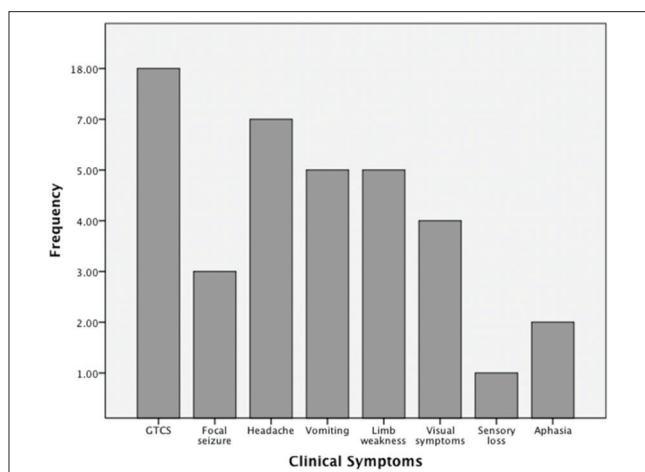


Figure 1: Clinical manifestations in neurocysticercosis patients

acquired cause of the seizure. Low socioeconomic status, poor hygiene and improper management in pigs rearing are the causes of high NCC prevalence in Nepal.^[10] Due to migration from rural areas, NCC is recently increasing in its prevalence also in urban areas. In our study, about 30% of the patients were from Kathmandu valley. Recently, increasing NCC prevalence in nonendemic or developed countries is due to migrant workers and international travellers.^[11] Few studies from China,^[12] Brazil^[13,14] and India^[15] also have shown the higher NCC cases reported in urban areas.

Our study shows NCC is common among young adults. Male patients are more common than female among NCC patients. Female predominance was seen in some studies.^[16,17] Single NCC lesions are found to be more common than multiple NCC lesions [Table 2]. Similar results were reported from various previous studies.^[18-20] However, a pediatric study from Nepal reported higher prevalence of multiple lesions among NCC patients.^[21] No extraparenchymal lesions was observed in our study, in contrast with what reported by Basu *et al.*^[21] showing 20% of extraparenchymal lesions.

Calcified granuloma may act as a seizure focus and may lead to epilepsy.^[22] Calcified lesion with perilesional edema was the most common NCC stage among our patients. About 23.8% patients were classified under colloidal stage, which consisted of active inflammation of the lesion along with scolex degeneration.^[23] Since vesicular stage is usually asymptomatic due to its least inflammatory response to the surrounding brain tissues. Meanwhile, this stage is commonly found to be associated with lesions of other stages, being 14.3% in our study.^[1]

Seizure is the most common presentation in NCC, ranges from 60% to 90%.^[16,18] Almost all NCC patients were presented with seizure, and most of them had focal

neurological deficits (limb weakness, visual disturbance, aphasia, *etc.*). This might be attributed to the fact that only the severe and disabling patient visited our hospital. Furthermore, it is a popular in Nepalese practice that most seizure patients initially visit traditional healers before presenting to hospital.^[4] Similar to the study of Bhattacharjee *et al.*,^[18] the frequency of status epilepticus was low in our patient cohort. Consistent with our observations, generalized seizure was common in some other previous studies.^[20,24] However, most of them reported focal seizure as the major seizure type.^[15,25,26] A Nepalese pediatric study has also reported focal seizure in about 30% of their patients.^[21]

Visiting traditional healers is popular among Nepalese population, especially in remote areas.^[4] Furthermore, lack of personal health, low socioeconomic status, and imaging techniques are the challenges for diagnosis of NCC.

In conclusion, neurocysticercosis is a major cause of acquired epilepsy in Nepal. Low economic status, illiteracy, and underdeveloped rural society are the major obstacles to prevention and treatment of NCC. Due to economic factors and inaccessible AEDs in remote places in Nepal, discontinuation of the drug is common. Proper management of NCC is needed to minimize the prevalence of epilepsy in developing countries.

This is a single center study, and we were unable to recruit the desired number of patients. Due to unavailability and poor economic condition of most of the patients, serum anticysticercal antibodies test was performed in 2 patients only.

ACKNOWLEDGMENTS

We acknowledge with gratitude the efforts of record keeping department and nursing staffs of Neurology Unit, Bir Hospital.

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Cite this article as: Ojha R, Shah DB, Shrestha A, Koirala S, Dahal A, Adhikari K, Bisht A, Wagle P. Neurocysticercosis in Nepal: a retrospective clinical analysis. *Neuroimmunol Neuroinflammation* 2015;2(3):167-70.

Source of Support: Nil. **Conflict of Interest:** No.

Received: 12-11-2014; **Accepted:** 19-02-2015

A case report on subarachnoid and intraventricular neurocysticercosis

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ABSTRACT

Neurocysticercosis is the most common central nervous system helminthic infection in humans. We hereby present a case combining two rare manifestations of neurocysticercosis: the subarachnoid and intraventricular forms. The patient presented with hydrocephalus and neurologic deficits and although endoscopic removal of the cysts and two cycles of postoperative cysticidal drugs resulted in resolution of symptoms, they later recurred. Ventriculoperitoneal shunt placement and a further cycle of albendazole plus dexamethasone led to substantial clinical improvement. Extraparenchymal neurocysticercosis may be challenging to diagnose and treat and is usually associated with a poorer prognosis. Clinicians should be aware of this condition.

Key words: Antiparasitic drug, extraparenchymal neurocysticercosis, intraventricular neurocysticercosis, neuroendoscopic surgery, subarachnoid neurocysticercosis

INTRODUCTION

Neurocysticercosis is caused by a human infestation of larvae of the tapeworm *taenia solium* and is considered the most common helminthic infection of the human central nervous system. The disease presents significant diagnostic and therapeutic challenges. It has heterogeneity in both clinical manifestation and therapeutic response^[1,2] and mortality and morbidity rates remain high.^[3]

Here, we describe a patient with subarachnoid and intraventricular neurocysticercosis who had hydrocephalus and neurologic deficits for several years, received successive surgical interventions, postoperative drug therapies, and finally, the placement of a ventriculoperitoneal (VP) shunt. Shunting proved, in this case, to be an effective treatment, and this may prompt its wider use in the treatment of the mixed form of extraparenchymal neurocysticercosis.

CASE REPORT

A 56-year-old Chinese man was sent to our hospital for further evaluation and treatment. Approximately, 2 years prior to admission, he developed chronic headache with nausea intermittent dizziness, hearing loss, bradyphrenia, and mild lower limb weakness without remarkable findings on neurological examination besides bilateral optic disk edema cerebrospinal fluid (CSF) studies showed intracranial hypertension (opening pressure ≥ 330 mmH₂O) and a CSF leukocytosis of $30 \times 10^6/L$ (90% lymphocytes, 5% monocytes, 3% neutrophils, and 2% eosinophils). Brain magnetic resonance imaging (MRI) showed widened sulci and cisterns bilaterally, as well as enlargement of the subarachnoid spaces. He stated that he used to eat under-cooked meat. Serum immunoblot for the anticysticercal antibodies was equivocal. The assay of CSF anticysticercal antibodies was positive, and a presumptive diagnosis of neurocysticercosis causing significant hydrocephalus was made.

A transfrontal transventricular endoscopic excision and third ventriculostomy were undertaken to relieve the clinical symptoms. Thickening of the arachnoid membrane underneath the third ventricle was found and upon fenestration of the arachnoid to the basic cistern, a multitude of whitish cystic structures with

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thin-walled membrane were seen. These yellow-spotted, irregularly-shaped cysts were intimately attached to the arachnoid or the basilar artery. Their direct visualization led to the confirmation of the diagnosis of subarachnoid neurocysticercosis. Subsequently, cysts were gently grasped and removed for histopathological evaluation, which revealed evidence of degenerative changes and an inflammatory reaction within the walls, mediated by nuclear macrophage and eosinophil infiltration [Figure 1]. The patient then received two cycles of antihelminthic therapies with albendazole and corticosteroid. This resulted in complete resolution of the patient's symptoms and he returned to his normal daily activities.

Approximately 4 months after the surgery, the patient had a recurrence of the same symptoms. Brain MRI again revealed evidence of hydrocephalus. A VP shunt was placed, resulting in no obvious improvement of clinical symptoms.

One year later, the patient reported new symptoms of motor deficiency and urinary incontinence, which led to his admission to our hospital. Physical examination showed he was drowsy, but oriented. He demonstrated full strength in his arms but decreased strength in his legs. The finger-nose and heel-knee-tibia tests lacked accuracy on both sides and Romberg's sign was positive. All the left-sided deep tendon reflexes were pathologically brisk. Babinski's sign was negative on both sides. The patient had no sensory deficits and no obvious meningismus.

Computed tomography (CT) revealed persistent ventricular dilation. A lumbar puncture was performed on this patient, and the opening pressure was now normal (160 mmH₂O). CSF studies also demonstrated a significantly increased number of white blood cells with a predominance of lymphocytes, an elevated protein level (0.81 g/L), and a decreased glucose concentration (0.1 mmol/L). Further evaluations for tuberculosis, bacteremia, fungal infection, and autoimmune processes were negative. Brain MRI indicated multiple small cysts containing a CSF-like

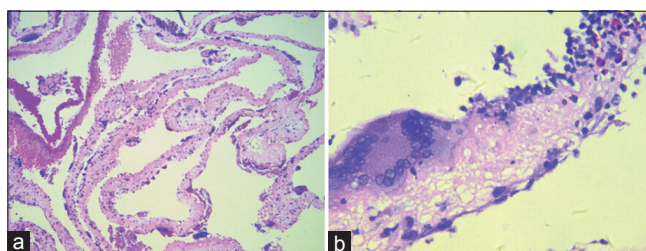


Figure 1: Hematoxylin and eosin stained low power field image (a) cysticercus larva. The multiple cysts of cysticercus larva underwent degenerative changes. High power field image (x100); (b) the wall of cysts infiltrated by multiple nuclear macrophages and eosinophils (x200)

signal within both lateral ventricles that did not enhance with contrast [Figure 2]. A third cycle of albendazole and corticosteroid was administered, which produced an improvement in cognitive status, and lower limb power and coordination.

DISCUSSION

Neurocysticercosis, caused by larvae of the tapeworm *taenia solium*, is the most common form of parasitic brain disease globally.^[1,4] It can occur in intraparenchymal, intraventricular, subarachnoid, or mixed forms.^[5]

A major characteristic of neurocysticercosis is heterogeneity, with the clinical manifestations dependent on the localization, number, and evolutionary stage of the parasites, as well as the intensity of the inflammatory reaction. Patients with neurocysticercosis may be asymptomatic, or present with a wide variety of symptoms.^[1] Typical CSF findings of neurocysticercosis include moderate mononuclear pleocytosis, mainly of lymphocytes and elevated protein concentrations, ranging from 0.5 g/L to 2.0 g/L. In most cases, CSF glucose concentrations are normal or moderately decreased.^[4] These CSF abnormalities are not present in all cases, and so cannot be used as definite diagnostic criteria.^[3] Usually, neuroimaging findings of extraparenchymal cysticerci are subtle: the cystic walls are thin, there is often an absence of pathognomonic scolices, central cysts are isointense to CSF and they do not enhancement after contrast administration.^[3] Detection of specific serum or CSF antibodies plays a helpful role in the diagnosis of cerebral cysticercosis, but it cannot differentiate between viable and degenerated parasites and is unable to confirm CNS localization.^[6] The diagnosis in this case was made with the help of direct endoscopic visualization and histologic demonstration. Since there exist enough

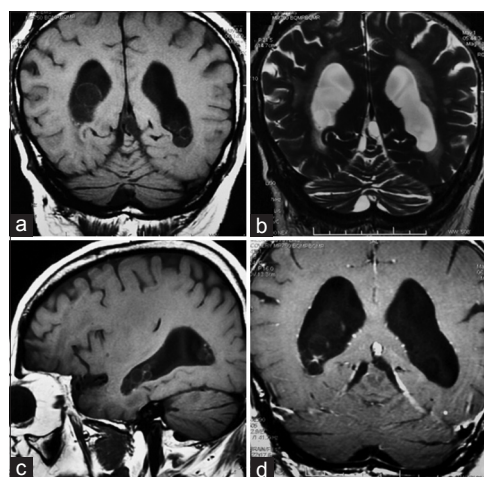


Figure 2: Brain magnetic resonance imaging showed multiple small cysts with cerebrospinal fluid-like signal inside within the lateral ventricles (a and c) T1-weighted, (b) T2-weighted and no enhancement (d) T2-weighted with contrast-enhanced)

space, cysts in nonconfining extraparenchymal areas can grow into interconnected grape-like clusters, known as racemose neurocysticercosis.^[5] The parasite in the extraparenchymal space degenerates owing to the continuous CSF ingress into the vesicles,^[3] forming a hyaline mass. Histopathologically, this is a granulomatous lesion infiltrated by many multinucleate giant macrophages.^[4]

Treatment should be individualized,^[4] particularly for patients with mixed forms of neurocysticercosis. Since extraparenchymal neurocysticercosis is associated with a poorer prognosis, there was a consensus toward more aggressive management.^[7] The surgical option was attractive because drug penetration into the ventricular and subarachnoid spaces is much lower when compared with that into the brain parenchyma. Our patient received endoscopic therapy, which resulted in rapid and safe reduction of the parasite burden. However, given that not all the cysticercus can be removed completely during the surgery, antihelminthic drugs are still required.^[8] Albendazole is the preferred choice because it has a superior penetration of the subarachnoid space, reaching higher concentration in the CSF than alternative agents and shows efficacy in treating both subarachnoid and ventricular cysts.^[9] Between the second and 5th days of antiparasitic therapy, there is usually an exacerbation of neurological symptoms attributed to local inflammation due to the larval death.^[7] For this reason, corticosteroids are given with parasitocidal drugs. Management of elevated intracranial pressure secondary to neurocysticercosis is also a priority. In order to make an improvement to the CSF circulation, this patient underwent endoscopic third ventriculostomy (ETV) and VP shunt replacement. It has been reported ETV could decrease the shunt failure rate from 36% to 8%.^[10]

The optimal duration of anti-parasitic treatment for extraparenchymal neurocysticercosis is not known.^[7]

Further work is required in patients with subarachnoid and ventricular neurocysticercosis to establish the roles of higher doses of albendazole, combined antiparasitic drugs, prolonged drug courses, repeated cycles, surgical interventions, and multidisciplinary collaborative working.^[2,3]

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Cite this article as: Shang C, Guan HZ, Cui LY, Hou B, Feng F, Zhong DR. A case report on subarachnoid and intraventricular neurocysticercosis. *Neuroimmunol Neuroinflammation* 2015;2(3):171-3.

Source of Support: Nil. **Conflict of Interest:** No.

Received: 15-12-2014; **Accepted:** 10-06-2015

A clinically isolated syndrome: butterfly glioma mimic

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ABSTRACT

The report explores a unique and treatable “butterfly”-glioma mimic and the neuroimaging characteristics that help to diagnose this entity. A 35-year-old patient presented with subacute-onset, progressive frontal lobe dysfunction followed by features of raised intracranial pressure. Neuroimaging features were consistent with a “butterfly” lesion that favored the possibility of a gliomatosis cerebri with significant edema and marked corpus callosum and fornix thickening. Contrast-enhanced and perfusion images revealed a confluent tumefactive lesion with a characteristic “broken-ring” pattern of enhancement, mass-effect and low perfusion; features favoring an alternative inflammatory pathology. This was peculiar as calloso-forniceal involvement of this nature has not been previously reported in inflammatory demyelinating mass lesions. This was confirmed as a tumefactive demyelination on histopathology. Following treatment, on clinical and imaging follow-up, significant resolution was evident suggesting a monophasic illness. This case highlights the stringent clinico-radiological-pathological approach required in the evaluation and management of butterfly lesions despite the striking imaging appearances. Tumefactive demyelination in this patient represents a clinically isolated syndromic presentation of an inflammatory pathology that can resemble gliomatosis cerebri. These “butterfly”-glioma mimics are scarcely reported in the literature, are eminently treatable with variable prognosis and prone for relapse.

Key words: Butterfly lesions, glioma, tumefactive demyelination

INTRODUCTION

Confluent “butterfly” lesions involving the corpus callosum often portend a poor prognosis in patients presenting with acute-subacute encephalopathy, with or without focal signs and raised intracranial pressure. Gliomatosis cerebri is often the primary pathological substrate considered in this situation. This report highlights an important mimic of “butterfly”-glioma.

CASE REPORT

A 35-year-old well-educated previously healthy male with no medical co-morbidities presented with complaints of behavioral disturbances for 1 month

prior to the index evaluation. The patient was observed by relatives to be apathetic, occasionally agitated and argumentative and over 15 days developed social dysinhibition, urinary and fecal incontinence. Excessive day time somnolence was also noted. The relatives also noted progressive inattention and memory impairment for a week prior to presentation. There was no history of any fever, headache, vomiting, loss of consciousness, seizures, hallucinations, delusions, focal limb weakness, imbalance nor any history suggestive of cranial nerve involvement.

Clinical examination revealed a conscious oriented patient with normal general physical examination findings. Mini Mental Status Examination score was 26/30 with impaired attention. There was a distinct lack of insight and impaired abstract thinking and judgment, with normal performance on tests of executive function. The rest of the cognitive evaluation was normal. There was no evidence of papilledema, ataxia or extrapyramidal signs. The plantar responses were bilaterally extensor consistent with a bipyramidal dysfunction.

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10.4103/2347-8659.154435

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A clinical impression of a frontal space occupying lesion (SOL) was considered in view of a history suggestive of mesial and orbitofrontal dysfunction with suspicion of white-matter (WM) involvement in view of pyramidal signs. The initial magnetic resonance imaging (MRI) [Figure 1] revealed an ill-defined butterfly shaped intra axial SOL involving bilateral frontal lobes as well as anterior insula with predominant involvement of subcortical deep WM and extension into bilateral caudate nuclei, genu and the anterior 1/3 of body of corpus callosum. Moderate mass-effect over bilateral frontal horns of lateral ventricles was observed. The lesion was hyperintense on T2-weighted and hypointense on T1-weighted images with mild diffusion restriction over the periphery. Peripheral enhancement was noted on intravenous gadolinium administration. There was no MR evidence of calcification or hemorrhage within the lesion. MR spectroscopy (MRS) from the intermediate part of the lesion revealed elevated choline peak, reduced N-acetyl aspartate (NAA) and the presence of lactate; however perfusion values were noted to be low in the lesion.

On admission, the patient was noted to develop rapidly progressive encephalopathy with features of raised intracranial pressure in the form of bradycardia, hypersomnolence and hypertension. With the imaging consideration of an intermediate-high grade SOL and gliomatosis cerebri high on the cards in view of a butterfly-patterned lesion with calloso-forniceal thickening [Figure 1a], a neuronavigation-guided biopsy and as an alternative a frontal decompressive procedure was considered. The frozen-section specimen and histopathology report [Figure 2] was consistent with tumefactive demyelination hence decompression was not performed. Such a presentation mimicking a butterfly glioma is extremely rare. The patient was treated with pulse methyl prednisolone followed by oral prednisolone that was administered in a dose of 1 mg/kg for 8 weeks followed by slow taper and cessation over 1 year. Prior to discharge evoked potentials, and cerebrospinal fluid studies including oligoclonal bands were negative thereby making multiple sclerosis (MS) less likely. Dramatic clinical improvement was noted, and the patient returned to the premorbid personality with normal neuropsychological performance 3 months into treatment. Serial MRI [Figure 3] verified gradual resolution of the WM hyperintensities and contrast enhancement with development of minimal bifrontal atrophy. MRS at 1 year showed reduction in the choline peak with reduced NAA and no evident lactate peak.

DISCUSSION

The neuroimaging characteristics distinctive in the pattern of tumefactive demyelination in the index

case include the butterfly configuration, forniceal thickening and features of a “mass-effect” with a clinical presentation akin to a butterfly glioma; prominent differentiating features being the enhancement and perfusion patterns. The differential diagnosis of acute-subacute acquired “butterfly lesions” involving the corpus callosum as seen in the patient represents a challenge in itself with multimodal imaging playing a crucial role. A host of etiologies can be broadly grouped as: tumors such as glioma, lymphoma and metastasis; inflammatory demyelinating pathologies like tumefactive MS; infections such as progressive multifocal leukoencephalopathy and Whipple’s disease; toxins leading to disseminated necrotizing leukoencephalopathy, e.g. intrathecal or systemic exposure to methotrexate or cytosine arabinoside and acute radiation necrosis.^[1] Imaging characteristics that potentially differentiate these conditions are depicted in Table 1. Clinico-radiological presentation of a non-neoplastic pathology like tumefactive demyelinating lesion (TDL) resembling a butterfly glioma is fraught with chance of a misdiagnosis (due to heterogeneity of imaging characteristics within the lesion itself).

Unlike in our patient, TDL tend to be circumscribed lesions with mild mass-effect or vasogenic edema.^[2] These typically involve the supra-tentorial WM although they may extend to involve the cortical gray matter with gyral edema. In a large series of 168 patients with biopsy confirmed central nervous system inflammatory demyelinating disease, frontal and parietal subcortical regions were most often affected and a butterfly configuration involving the corpus callosum was observed in only 12% of cases.^[3] Forniceal thickening was not described in this series and represents a unique observation in our patient as this deviated the impression towards a neoplastic etiology. Approximately half of TDL have pathological contrast enhancement, usually in the form of ring enhancement.^[2,3] A variety of intracranial pathologies can present as a ring-enhancing lesion (REL) on MRI, including glioma, metastasis, lymphoma, radiation necrosis, infarct, abscess and tumefactive demyelination. Although less common in typical demyelination, REL are more likely to be biopsied in order to exclude these other pathologies that are mandatory from a treatment and prognostication point of view. In a recent series among the most prevalent pathologies associated with ring enhancement, demyelinating lesions of MS constituted a small number (6%) and patterns of T2-weighted hypointensity are useful to differentiate between pathologies.^[4] Figure 1b and c demonstrate this pattern of enhancement in the patient. The enhancing portion of the ring is believed to represent the leading edge of demyelination and thus favors the WM side of

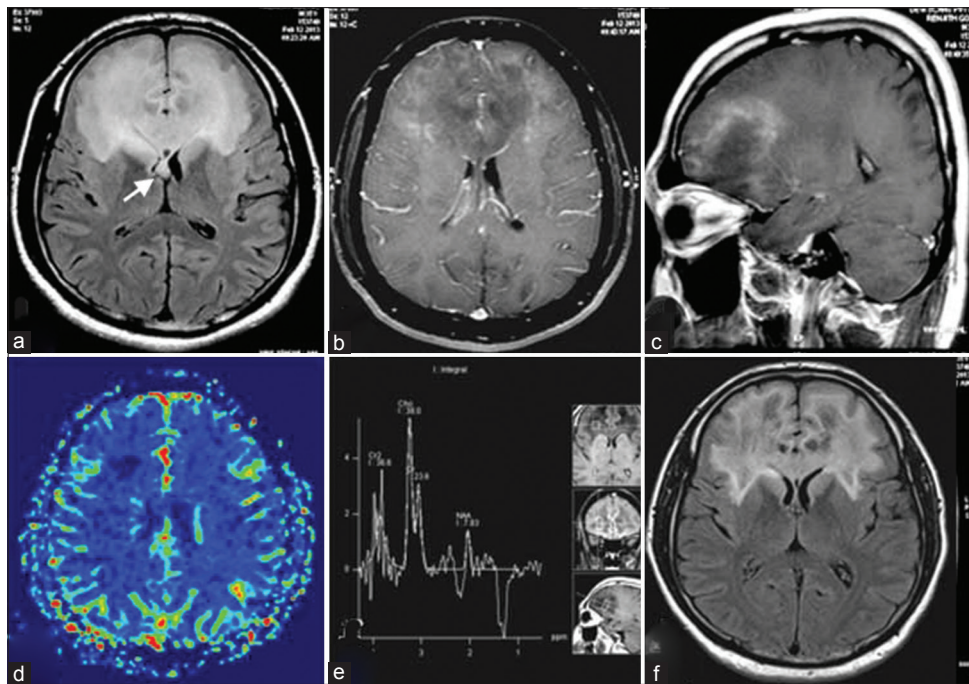


Figure 1: (a) Fluid-attenuated inversion recovery (FLAIR) axial image showing hyperintense lesion along the frontal subcortical white matter with callosal and forniceal involvement (arrow); (b and c) typical open rim pattern of enhancement on axial and sagittal gadolinium T1-weighted images; (d) perfusion imaging indicating low perfusion; (e) magnetic resonance spectroscopy over intermediate zone that demonstrates choline peak with reduction in N-acetyl aspartate; (f) immediate postbiopsy FLAIR image after pulse steroid therapy demonstrating reduction in edema and mass-effect

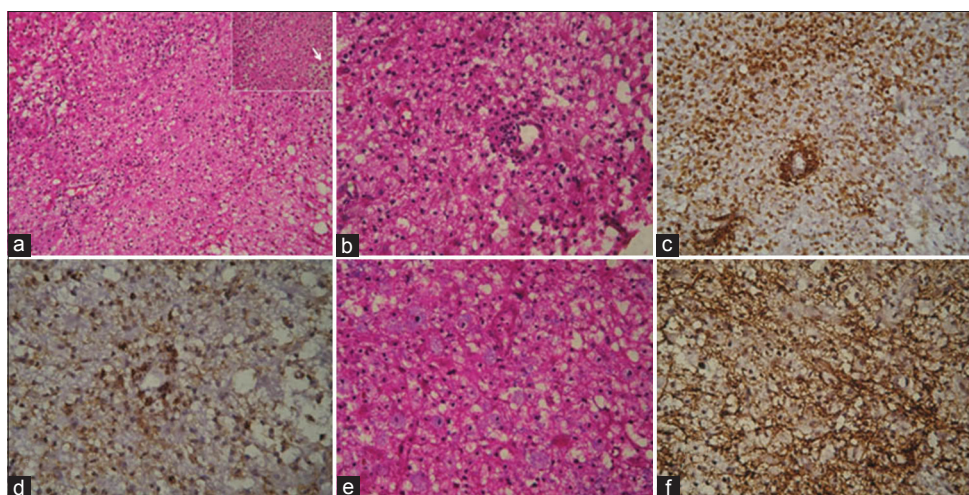


Figure 2: (a) Microscopy shows sheets of foamy macrophages with reactive astrocytes (HE, x400); (inset arrow) sheets of foamy macrophages (HE, x400); (b) perivascular and diffusely scattered lymphocytes (HE, x100); (c) CD68 showing perivascular and diffuse infiltrate of macrophages (x100); (d) CD3 showing perivascular and diffuse T cell infiltrate (x100); (e) luxol fast blue hematoxylin stain shows macrophages with ingested myelin (blue stained, x400); (f) preserved staining of neurofilament protein (x100)

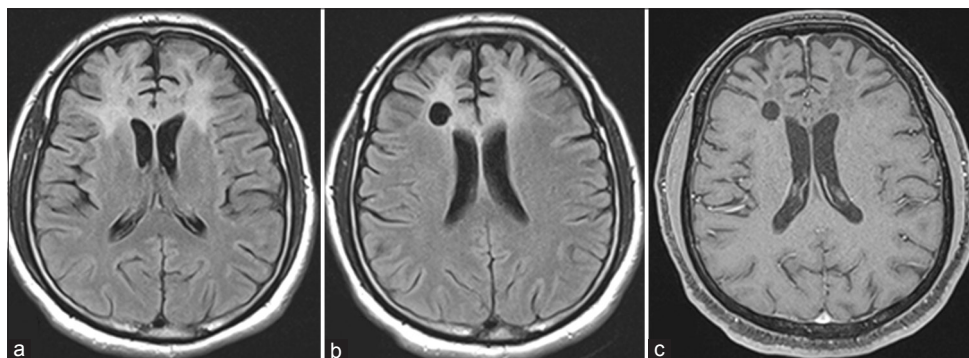


Figure 3: (a) Serial magnetic resonance imaging at 4th month of follow-up showing persistence of fluid-attenuated inversion recovery (FLAIR) hyperintensity with early overlying bifrontal atrophy; (b) FLAIR image at 1 year of follow-up demonstrating marked reduction in hyperintensity and significant atrophy; (c) T1-weighted gadolinium-enhanced image at 1 year showing no contrast enhancement in contrast to Figure 1b and c

Table 1: Approach to differential diagnosis of “butterfly” lesions on MRI

Pathology	T1WI	T2WI	Gadolinium contrast	MR perfusion (rCBV)	Special features
GBM	Iso-/↓	↑/Iso-with solid and cystic components	Heterogenous enhancement of solid portion; REL	↑	MRS: choline, lipid, lactate peaks DWI/ADC: ++ in solid portion GRE: hemorrhagic
Lymphoma	Iso-/↓	Iso-/↓	Homogenous; REL in immunocompromised	N/↓	MRS: same as GBM DWI/ADC: ++
Metastasis	Iso-/hemorrhagic/↓	↑	Variable enhancement patterns (solid, REL, irregular, homogenous, mixed)	N/↑	MRS: choline peak DWI/ADC: variable GRE: variable
PML	↓	↑	Mild peripheral enhancement; ↑ during IRIS	↓	Lesion location at GWM junctions MRS: NAA↓ in WM with U fiber scalloping
Diffuse necrotizing leukoencephalopathy	↓	↑	Rare peripheral enhancement	N/↓	DWI/ADC: ++ Usually evanescent, diffuse/multi-focal; peri-ventricular with sparing of U fibers
Acute radiation necrosis	Iso-/↓	Central-↑ Solid part-↓	REL around necrosis	N/↑	DWI/ADC: ++ GRE: micro-hemorrhages and calcification
Whipple's disease	↓	↑	Punctate, incomplete enhancement	N/↓	Location: thalami, WM and brainstem
Inflammatory demyelination (tumefactive)	↓	↑	“Broken-ring” or closed ring	↓	Variable MRS and DWI/ADC values within lesion

T1WI: T1-weighted imaging; T2WI: T2-weighted imaging; rCBV: regional cerebral blood volume; REL: ring-enhancing lesion; DWI: diffusion weighted imaging; ADC: apparent diffusion coefficient; ↑: hyperintensity; ↓: hypointensity; ++: diffusion restriction; N: normal; MRS: magnetic resonance spectroscopy; GWM: grey-white matter; WM: white matter; GRE: gradient echo imaging; IRIS: immune reconstitution inflammatory syndrome; MRI: magnetic resonance imaging; GBM: glioblastoma multiforme; NAA: N-acetyl aspartate; PML: progressive multifocal leukoencephalopathy

the lesion.^[2] The central non-enhancing core represents a more chronic phase of the inflammatory process. Reduced perfusion may be another suggestive feature of TDL,^[5] however exclusion of a moderate grade butterfly glioma was mandatory in the patient. Demyelination co-existing with primary neoplasms has been reported rarely in the literature but the histopathology and subsequent clinico-radiological follow-up excluded this association.^[6] In comparison with tumors and abscesses, edema in TDL is said to be proportionally minor relative to plaque size contrary to what was seen in our patient.^[7]

Another case series emphasized the unique multimodal imaging characteristics in tumefactive demyelination.^[8] Two or three concentric distinct zones were noted on imaging with distinct metabolic and structural signature in most cases. Increase in the glutamine/glutamate ratio and lactate was noted in tumefactive lesions. On TE 135 ms, the central part showed variable Choline (Cho) and significantly low NAA. The intermediate area showed higher Cho and lower NAA compared to contralateral normal side as was seen in the patient. The outermost layer, which corresponded to the contrast enhancing areas on MRI, showed high Cho, lower NAA, and restricted diffusion. Follow-up imaging, as was seen in this series, showed a reduction in the extent of hyperintensities, however MRS showed persistent abnormalities.

While remission was seen 1 year into follow-up in our patient, it is important to note that only 17%

of cases in Lucchinetti's series remained unifocal during radiological follow-up.^[3] Longitudinal follow-up had revealed eight developed definite MS, and one had isolated demyelinating syndrome by the last follow-up. The unifocal subgroup was more likely to have mass-effect and edema associated with the biopsied lesion on prebiopsy scan, compared with those who developed multifocal lesions. One cannot reliably exclude the fact that with a large multilobar lesion as seen in our case, the area selected for biopsy is more likely due to surgical bias and may not be representative of the true pathology. The serial follow-up makes alternative possibilities remote. Patient age, clinical course prior to biopsy or disability status at last follow-up have not been found to differ between patients with or without a butterfly lesion.^[3]

In a review of 31 cases, Kepes^[9] proposed that TDLs represent an intermediate lesion between those typically seen with MS and acute disseminated encephalomyelitis. Pathologically, these lesions are indistinguishable from typical MS plaques and are characterized by infiltrating foamy macrophages intermingled between reactive astrocytes [Figure 2].^[10] Significant quantities of lipid may accumulate within the plaques as a result of myelin breakdown. The pathologic diagnosis may be challenging based on the initial frozen-section specimen when the primary suspicion is malignancy. In our patient, absence of features such as hyperchromatic nuclear morphology, uneven pattern of distribution of astrocytes, atypical

mitotic figures, necrosis and endothelial proliferation, correlated to the subsequent clinico-radiological profile, glioma co-existing with TDL could be reliably excluded.^[11] Our case highlights the importance of meticulous radiopathological inputs required into analysis of butterfly multifocal lesions that is the key to guide subsequent management of an evidently treatable condition.

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Cite this article as: Menon R, Thomas B, Easwer HV, Sandhyamani S, Nair A, Nair M. A clinically isolated syndrome: butterfly glioma mimic. *Neuroimmunol Neuroinflammation* 2015;2(3):174-8.

Source of Support: Nil. **Conflict of Interest:** No.

Received: 30-10-2014; **Accepted:** 11-02-2015

Complete recovery from paraplegia following total spondylectomy for a primary diffuse B-cell lymphoma of the lumbar spine

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ABSTRACT

Primary non-Hodgkin lymphoma of the spine is very rare and occurs mostly in adults with strong male predominance. Here, we present the case of a 24-year-old girl harboring a primary diffuse B-cell lymphoma of L2 vertebral body, who was admitted in an emergency with cauda equina syndrome and completely recovered after total spondylectomy and adjuvant chemotherapy. Such findings have never been previously reported.

Key words: Bone lymphoma, diffuse large B-cell lymphoma, lumbar vertebra, management, prognosis

INTRODUCTION

Primary lymphoma of bone (PLB) accounts for 2.8-5.9% of non-Hodgkin lymphomas and affects essentially adults with strong male predominance.^[1] Long extremity bones such as the femur represent the most frequent locations,^[2] and single vertebral involvement is observed in only 1.7% of all cases.^[3] At presentation, the disease may resemble traumatic fracture or mimic inflammatory, neuropathic, or infectious conditions.^[4] We present the case of a 24-year-old girl harboring a primary diffuse B-cell lymphoma of L2 (BCL-2) vertebral body, who was admitted in emergency with cauda equina syndrome and completely recovered after total spondylectomy and adjuvant chemotherapy.

CASE REPORT

A 24-year-old girl presented to our emergencies in January 2014 for 2 months' history of severe back pain recently aggravated to incapacity of walking and sphincter disturbances for the past 3 days. Her medical

history was consistent for neither prior pathologies nor prior trauma.

On admission, a physical examination disclosed flaccid paraplegia, with very weak tendon reflexes in both sides and T12 sensory level. Bladder retention was also present. Lymphadenopathy, mass, and organomegaly were not detected, and blood analyses were also normal.

X-rays of the spine showed lytic changes of L2 vertebral body in the vertebra plana shape and magnetic resonance imaging (MRI), realized in emergency, showed a T1-weighted hypo intense, T2-weighted hypo intense and enhanced lesion of L2 vertebral body extending posteriorly into the spinal canal and causing major thecal sac compression [Figure 1].

Surgery was advised in emergency, and a two-stage complete spondylectomy was decided starting with a posterior decompressive approach, and following by a lateral lobotomy approach for the removal of the vertebral body. Stability was ensured by costal rib grafts and posterior spinal instrumentation [Figure 2].

During the surgery, the tumor was grayish and moderately hemorrhagic. L2 posterior arch and the surrounding soft tissues were macroscopically intact.

Pathologic examination made on multiple tissue samples showed infiltration by lymphocytes with relatively large and irregular shaped nuclei [Figure 3].

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10.4103/2347-8659.157962

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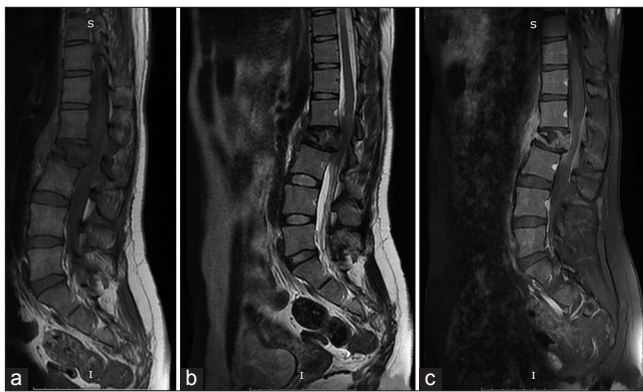


Figure 1: Sagittal T1-weighted (a), T2-weighted (b), and T1-weighted postgadolinium (c) magnetic resonance images showing an hypo intense and enhancing L2 vertebral body tumor extending posteriorly into the spinal canal and causing major thecal sac compression

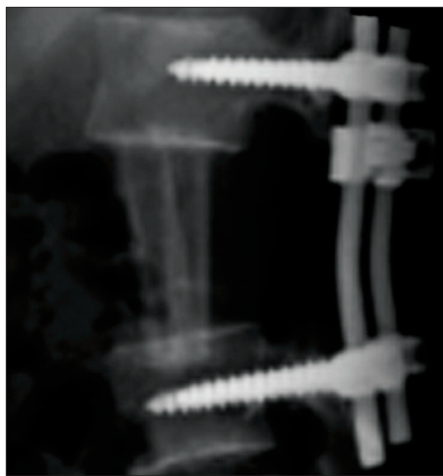


Figure 2: Postoperative lumbar lateral X-rays shows the costal bone grafts replacing the L2 vertebral body and the posterior L1-L3 transpedicular screws fixation

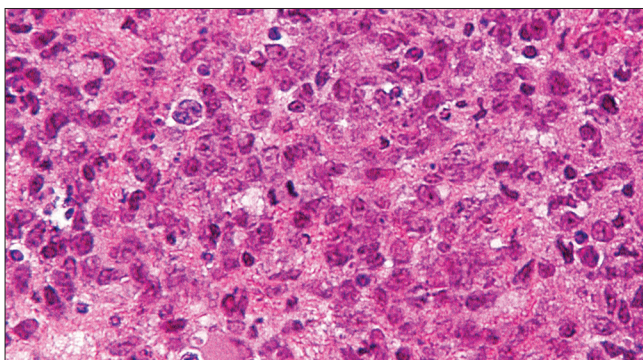


Figure 3: Photomicrograph of the tumor specimen showing typical characteristic of large B-cell lymphoma with a highly cellular tumor composed of large cells with abundant cytoplasm and large round ovoid nucleoli

Immunohistochemical staining was positive for CD79a, CD20, CD45, CD45RO, PCNA, BCL-2, and Vimentin, and negative for CD3, CD30, and CD99, which is typical of a B-cell non-Hodgkin lymphoma.

Laboratory tests including complete blood count with examination of a peripheral smear, chemistries to assess renal and hepatic function, measurement of serum glucose, calcium, albumin, lactate dehydrogenase,

beta2-microglobulin as well as serum protein electrophoresis and HIV serology were realized and all these results were normal. Chest X-ray, computed tomography (CT) scan of the thorax, abdomen, and pelvis and MRI on the whole neuraxes showed no other sites of lymphoma and the final diagnosis was a primary diffuse BCL-2 vertebral body. Adjuvant LMB-89 chemotherapy treatment was started.

Clinically, the patient was enrolled under an intensive care and rehabilitation program. Her condition remarkably improved and complete recovery was reached within 2 weeks postoperatively. She is symptom-free with no clinical or radiological signs of progression at the most recent follow-up examination, 8 months after surgery.

DISCUSSION

Primary lymphoma of bone was first described as a distinct clinicopathological entity by Parker and Jackson in 1939.^[2] Coley *et al.*^[5] in 1950, presented clear diagnostic criteria for PLB: the tumor should primarily focus in one single bone, having a positive histological diagnosis with no evidence of distant soft tissue or distant lymph node involvement. Recently, the WHO classification also recognized multiple bone involvement as a primary bone lymphoma if visceral or lymph node involvement does not exist.^[6]

Primary lymphoma of bone represents a rare clinical entity mostly affecting adult male patients. It is histologically dominated by diffuse large BCL (90% of all cases) and extremity bones such as the femur, tibia or ulna, represent the most common locations.^[2]

The spine is an unusual location, and unique vertebral involvement is exceedingly scarce representing less than 1.7% of all PLB cases.^[3]

The duration of symptoms prior to presentation depends on the aggressiveness of the lesion. Complaints at presentation include mainly night-pain, swelling, mass, fever, weight loss, limp, irritability, and pathological fracture. Neurological signs are rarely seen and often manifest late in the course of the disease.^[2]

In the present case, a previously healthy, 24-year-old girl presented with cauda equina syndrome which is an unusual pattern of presentation, supporting the widely held idea about the unspecific shape of PLB.

Radiologically, PLB has many similarities with metastatic disease although a few differences exists such as patchy sclerosis on CT or bone marrow invasion without a soft tissue mass due to the infiltrative nature of lymphoma on MRI.^[7]

Positron emission tomography can be of important adjunct in detecting multiple or silent lesions, with the tumor appearing as areas of intense hypermetabolic activity.^[8]

In the present case, plain radiograph and emergency MRI were of little help for diagnostic orientation as tumor features were unspecific. Our attitude was consequently more hampered by the emergency than by the tumor nature or radiological features.

It seems clear that the therapy for PLB is still a cause of debate. Its rarity and the associated pathologic heterogeneity do certainly contribute to the lack of clear management guidelines. Several studies suggested that the combination of chemotherapy and radiotherapy was the best treatment option for patients harboring PBL.^[9,10] In fact, Beal *et al.*^[11] eloquently discussed the treatment results and prognostic factors for PLB, and found that the combined approach offers significantly better survival than chemotherapy or radiotherapy administered alone. The place of the surgery in the treatment options remains to be defined. Till now, it has been suggested in case of pathologic fractures, avascular necrosis, spinal cord compression, or the stability compromise.

In the present case, surgery was advised in emergency starting by decompressive laminectomy. Based on the patient age and tumor characteristics, we opted for a total spondylectomy approach and consequently removed L2 vertebral body.

Solitary diffuse large BCL has commonly a favorable prognosis compared to many other malignancies. Spontaneous regression has already been reported and the 5-year event-free survival reaches 75-100%.^[1] But good results are tempered by other dismal progressions like death within 1-year postoperatively following combined therapy.

Age and the presence of immunoblasts represent the most important prognostic factors and were correlated with significantly poorer prognosis.^[12]

For the present case, we believe that our preferential radical surgical approach in association with adjuvant chemotherapy can result in better tumor control and longer disease-free survival for this young patient. Radiotherapy under these considerations will be better administered in case of recurrent disease since its administration will not be possible without irradiating an already suffering conus medullaris.

Finally, lessons must be drawn from the present case and future challenges should be taken into consideration: primary diffuse BCL can localize in the lumbar spine of young female patients and manifest with cauda equina syndrome; radical surgery under these considerations can result in total recovery even after flaccid paraplegia; early identification is of paramount importance and long-term follow-up is mandatory to spot recurrences early.

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Cite this article as: Nsir AB, Boughamouira M, Hadhri R, Mahfoudh M, Hattab N. Complete recovery from paraplegia following total spondylectomy for a primary diffuse B-cell lymphoma of the lumbar spine. *Neuroimmunol Neuroinflammation* 2015;2(3):179-81.

Source of Support: Nil. **Conflict of Interest:** No.

Received: 10-10-2014; **Accepted:** 04-12-2014

Decompressive craniectomy in herpes simplex encephalitis

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ABSTRACT

Intracranial hypertension is a common cause of morbidity in herpes simplex encephalitis (HSE). HSE is the most common form of acute viral encephalitis. Hereby we report a case of HSE in which decompressive craniectomy was performed to treat refractory intracranial hypertension. A 32-year-old male presented with headache, vomiting, fever, and focal seizures involving the right upper limb. Cerebrospinal fluid-meningoencephalitic profile was positive for herpes simplex. Magnetic resonance image of the brain showed swollen and edematous right temporal lobe with increased signal in gray matter and subcortical white matter with loss of gray, white differentiation in T2-weighted sequences. Decompressive craniectomy was performed in view of refractory intracranial hypertension. Decompressive surgery for HSE with refractory hypertension can positively affect patient survival, with good outcomes in terms of cognitive functions.

Key words: Decompressive craniectomy, herpes simplex encephalitis, refractory intracranial hypertension

INTRODUCTION

Herpes simplex encephalitis (HSE) is the most common form of acute viral encephalitis.^[1] HSE is caused by herpes simplex virus types 1 and 2 (HSV-1 and HSV-2), two DNA viruses of the Herpesviridae family. HSV is the most frequent agent of sporadic fatal encephalitis with an annual incidence of 1 in 250,000-500,000.^[2] Untreated HSE has an extremely high mortality rate of 70%.^[3] Early diagnosis and treatment can reduce the mortality rate to 19%.^[4,5] Morbidity in HSE is mainly due to intracranial hypertension.^[4] Therefore, we report a rare case of HSE, which required decompressive craniectomy to treat severe refractory intracranial hypertension.

Patients with HSE usually present with headache, confusion, fever, and seizures.^[6] Failure to diagnose this serious disease early may result in permanent disability or death. The presence of clinical symptoms

and a localized lesion in the temporal lobe usually reflects HSE, but other diseases can also mimic this condition.^[7] Cerebrospinal fluid (CSF) examination is indicated for suspected HSE patients even if the intracranial pressure is increased.^[7]

Herpes simplex virus usually causes a mild disease restricted to the skin and mucosa. Much less commonly, it causes severe encephalitis. While HSV-1 is typically transmitted via the oro-labial route, HSV-2 is transmitted venereally. HSV-1 strains are etiological agents in over 90% of cases of HSE. HSV-2 strains are more commonly isolated in congenitally acquired neonatal HSV meningoencephalitis. After initial replication in skin and mucosa, the HSV-1 virus infects the sensory nerve endings innervating the infected territory and migrates along retrograde axonal flow toward the trigeminal ganglia where it remains latent.

The mechanisms whereby HSV-1 penetrates the nervous system, evades the immune response and causes encephalitis are incompletely understood. HSV could enter into the brain by reactivation of the viral genome in the trigeminal ganglion with axonal spread via the trigeminal nerve into the temporal and frontal lobes. Furthermore, HSV-1 can primarily infect the central nervous system.

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10.4103/2347-8659.158460

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CASE REPORT

A 32-year-old male presented with headache, vomiting, fever, and focal seizures involving the right upper limb of 1-week duration. There was no significant past history. There was no history suggestive of any immunodeficiency. On examination, the patient was hemodynamically stable and conscious but febrile and irritable. He had no focal neurological deficits but had signs of meningeal irritation. The pupils were equal in size and reactive. Magnetic resonance imaging of brain (MRI-brain) [Figure 1] showed swollen and edematous right temporal lobe (a) with increased signal in gray matter and subcortical white matter with loss of gray white differentiation in T2-weighted sequences. There was a signal change in the right insula and sub frontal cortex bilaterally. Restricted diffusion and abnormal leptomeningeal enhancement were also noted. There was mass effect with partial effacement of the body of the right lateral ventricle and midline shift (b). A guarded lumbar puncture was done and CSF study [Table 1] showed protein: 60 mg/dL, sugar: 87 mg/dL, and 200 cells/mm³ with lymphocytic pleocytosis. CSF meningoencephalitic profile was positive for herpes simplex 1 virus.

Cerebrospinal fluid meningoencephalitis profile [Table 1] is a polymerase chain reaction (PCR) equivalent. This is a molecular diagnostic screening technology involving isolation of the genetic material of the causative agent from the given specimen and

Table 1: CSF study

Parameters	Results
CSF protein	60 mg/dL
CSF sugar	87 mg/dL
CSF cells	200 cells/mm ³ with predominantly polymorphs (P64, L28)
CSF meningo-encephalitic profile	Positive for herpes simplex-1

CSF: cerebrospinal fluid

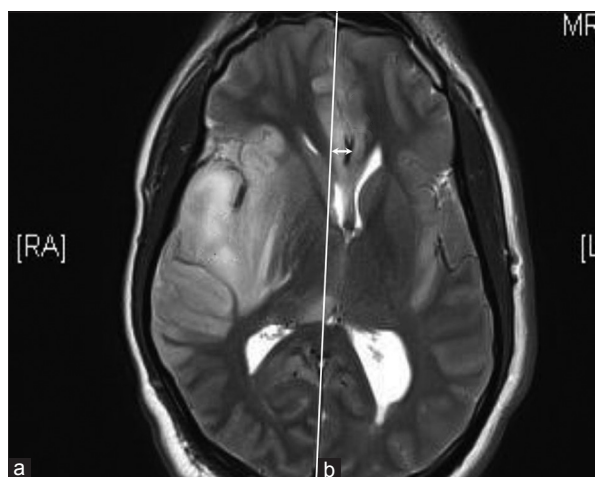


Figure 1: Magnetic resonance imaging of brain: T2-weighted sequence showing swollen and edematous right temporal lobe (a) with mass effect (b) and midline shift as indicated by the arrow

simultaneous amplification of the “syndrome specific signature genes” of all the probable causative agents, followed by “syndrome specific hybridization”.

Based on a presumptive diagnosis of HSE, the patient was started on intravenous acyclovir (500 mg 8th hourly). He was also started on anti-cerebral edema medications, intravenous 20% mannitol 150 mL 6th hourly, and steroids (dexamethasone) 8 mg 8th hourly. His sensorium deteriorated soon after admission, being unresponsive (Glasgow coma scale: 5/15) (eye response 1, verbal response 1, motor response 3) and the right pupil dilated. The patient was emergently intubated and connected to ventilator. Since the patient was already on full-fledged anti-cerebral edema medications, surgical options were then considered as the second measure. A decompressive surgery was immediately performed by a large right frontotemporoparietal craniectomy. The dura was widely opened and lesional tissue of the right temporal was harvested for biopsy. No parenchymal resection was necessary since the brain was adequately decompressed. Dura was closed with an expansive duroplasty. The bone flap was not replaced; instead was preserved in the anterior abdominal wall. Anti-cerebral edema medications were tapered and stopped postoperatively.

Postoperatively, his neurological status stabilized and was weaned off ventilator gradually. Postoperative computed tomography (CT) of brain [Figure 2] after 3 days showed resolution of the mass effect with no midline shift. However, the right temporal lobe remained hypodense. Basal ganglia, thalamus, internal capsule, and caudate nucleus appeared normal.

Electroencephalography (EEG) in the postoperative period showed diffuse right hemispherical slowing in theta to delta range [Figure 3]. Histopathology of the right temporal lesion [Table 2] revealed infiltration

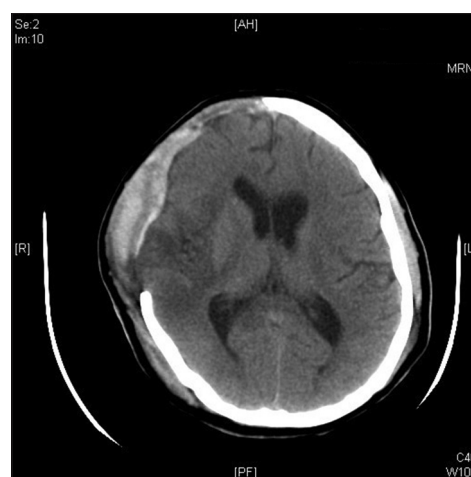


Figure 2: Postoperative computed tomography of brain after 3 days showing resolution of the mass effect with no midline shift

by inflammatory cells and perivascular lymphocytic cuffing [Figure 4] with occasional multinucleated giant cells, consistent with the well-known pathology of HSE. The patient was discharged on the 10th postoperative day in a recovering condition.

Over the next few months, the patient made a remarkable neurological recovery. He regained full consciousness and normal cognition. He did not have further episodes of seizures. He has no residual neurological deficits. Two months after the craniectomy, the bone flap was replaced.

DISCUSSION

In HSE, delay in treatment leads to severe neurological sequelae. Therefore, early diagnosis is of great importance. Diagnostic criteria include clinical symptoms, MRI, EEG, and CSF studies [Table 3]. The sensitivity is increased with the combination of these neurodiagnostic tests.^[12] When HSE is suspected, MRI brain should be done as early as possible as signs of HSE could be detected by MRI earlier than CT scan.^[13] Because MRI is a more sensitive and specific diagnostic tool, it is used instead of CT scans in the majority of patients.^[13-15] The characteristic MRI finding of HSE is hyperintense areas (T2-weighted sequences) in the inferior part of temporal lobes, medial part, and insula. This may also be observed in the frontal and parietal lobes. Bilateral temporal lobe involvement has been reported to be pathognomonic of HSE.^[16] Diffusion limitation observed in T2-FLAIR sequences are also thought to be typical.^[16]

Herpes simplex encephalitis is a medical emergency that requires prompt diagnosis and therapy. However, both are often delayed for several reasons. For instance, the clinical presentation itself is non-specific and may be mistaken for stroke, epilepsy, delirium or a primary psychiatric disorder. CSF cell count is normal in 5-10%

of patients. Neuroimaging may be normal in the early stages. DNA detection may be negative.^[17]

Untreated HSE is progressive and often fatal in 7-14 days. A landmark study by Whitley *et al.*^[18] in 1977 revealed a 70% mortality in untreated patients and severe neurologic deficits in most of the survivors. Mortality in patients treated with acyclovir was 19% in the trials that established its superiority to vidarabine. Subsequent trials reported lower mortalities (6-11%), perhaps because they included patients who were diagnosed by PCR rather than brain biopsy and who

Table 2: Histopathology of the right temporal lesion

Parameters	Results
Microscopy	Sections show pieces of cerebral cortex and subcortical white matter in which there is edema, focal hemorrhage and infarction with infiltration by inflammatory cells Perivascular lymphocytic cuffing and occasional multinucleated giant cells noted No viral inclusions/granuloma seen There is no evidence of malignancy
Diagnosis	Brain biopsy: consistent with herpes simplex encephalitis

**Table 3: Diagnostic criteria for HSE. HSE is diagnosed in a febrile patient with an altered level of consciousness
*in the presence of any 3 of the following diagnostic tests**

Criteria
EEG showing background slowing and frequent PLEDs over the temporal lobe ^[8]
MRI-Brain showing gyral edema in T1-weighted sequences and high signal intensities over the medial temporal lobe and the cingulate gyrus in T2, FLAIR and diffusion-weighted sequences, often with foci of hemorrhage ^[9]
CSF showing lymphocytic pleocytosis, elevated protein and elevated CSF opening pressure ^[10]
Detection of herpes simplex virus DNA in the CSF by polymerase chain reaction: gold standard for diagnosis ^[11]

*With or without focal neurological deficits. EEG: electroencephalography; PLEDs: periodic lateralized epileptiform discharges; MRI-Brain: magnetic resonance imaging of brain; CSF: cerebrospinal fluid; HSE: herpes simplex encephalitis; FLAIR: fluid-attenuated inversion recovery; DNA: deoxyribonucleic acid

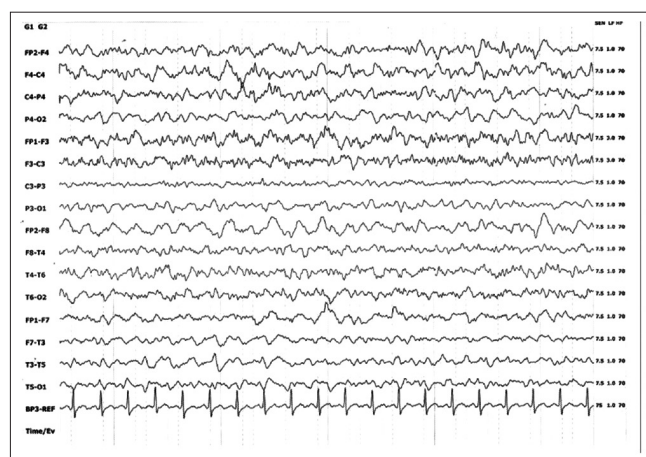


Figure 3: Electroencephalography showing diffuse right hemispherical slowing in theta to delta range

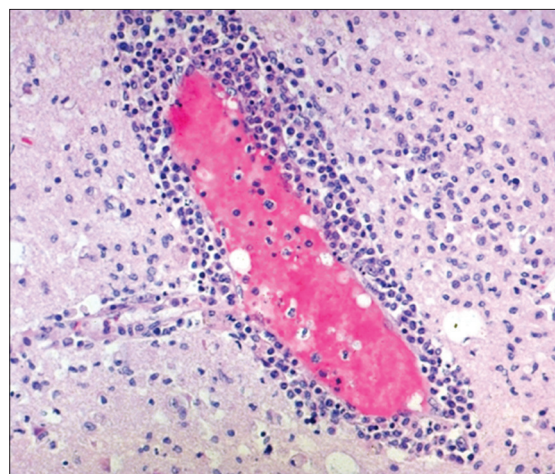


Figure 4: Histopathology of the right temporal lesion showing infiltration by inflammatory cells and perivascular lymphocytic cuffing, which is consistent with herpes simplex encephalitis

Table 4: Diagnosis of HSE in the present case

Diagnostic tests	Characteristic findings	Present case
EEG ^[8]	Background slowing and frequent PLEDs over the temporal lobe Usually, present from day 2 to day 14 after disease onset	Showed diffuse right hemispherical slowing in theta to delta range
Neuroimaging ^[9]	MRI is more sensitive and specific than CT Early findings include gyral edema in T1-weighted sequences and high signal intensities over the medial temporal lobe and the cingulate gyrus in T2, FLAIR and diffusion-weighted sequences, often with foci of hemorrhage Bilateral assymetrical temporal lobe and cingulate gyrus involvement is nearly pathognomonic of HSE but is a late development	T2-weighted sequence showed swollen and edematous right temporal lobe. Restricted diffusion and abnormal leptomeningeal enhancement were also noted
CSF study ^[10]	Elevated CSF opening pressure Lymphocytic CSF pleocytosis Elevated proteins Normal glucose	CSF protein: 60 mg/dL CSF sugar: 87 mg/dL CSF cells: 200 cells/mm ³ with predominantly lymphocytes (P28, L64) CSF meningoencephalitis profile: positive for herpes simplex-1
Virological diagnosis ^[11]	Gold standard for diagnosis Detection of herpes simplex virus DNA in the CSF by PCR	Inflammatory cell infiltration and perivascular lymphocytic cuffing consistent with HSE. No herpes inclusions were seen
Brain biopsy ^[27,28]	Microscopically, necrosis is associated with diffuse inflammation and perivascular lymphocytic infiltration. Viral intranuclear inclusions are inconstant, and viral antigens are detectable only at early stages	

HSE: herpes simplex encephalitis; EEG: electroencephalography; PLEDs: periodic lateralized epileptiform discharges; MRI: magnetic resonance imaging; CT: computed tomography; CSF: cerebrospinal fluid; PCR: polymerase chain reaction; FLAIR: fluid-attenuated inversion recovery; DNA: deoxyribonucleic acid

thus may have been identified earlier with milder cases of HSE.^[19]

Sequelae among survivors are significant and depend on the patient's age and neurologic status at the time of diagnosis. Patients who are comatose at diagnosis have a poor prognosis regardless of their age. In non-comatose patients, the prognosis is age related, with better outcomes occurring in patients younger than 30 years. Anterograde memory often is impaired even with successful treatment of HSE. Retrograde memory, executive function, and language ability may also be impaired. A study by Utley *et al.*^[20] showed that patients who had a shorter delay (< 5 days) between presentation and treatment had better cognitive outcomes. Furthermore, Marschitz *et al.*^[21] reported a case of chorea after HSE.

Despite adequate medical treatment, some HSE patients worsen because of refractory intracranial hypertension. A decompressive craniectomy (in which the skull flap is not immediately replaced, allowing the brain to swell, thus reducing intracranial pressure), with or without anterior temporal lobe resection, can be effective in controlling intractable, elevated intracranial pressure in HSE.^[22,23]

Taferner *et al.*^[24] reported the long-term sequelae (1.5-8 years after craniectomy) of four cases with HSE and confirmed its appropriateness, as it led to full cognitive recovery, resocialization, and reintegration into professional life. Ebel *et al.*^[25] suggested that not only a decompressive craniectomy, but also a partial resection of the temporal lobe may be of benefit for patients with tentorial herniation, because both decompression and reduction of infectious material with cystic tissue necrosis can be achieved. Moreover,

the initial neurologic deficit does not affect the long-term clinical outcome if decompression is done in the early stages.^[26]

In conclusion, we were able to diagnose HSE with the help of clinical findings, MRI-brain, analysis of CSF profile showing lymphocytic pleocytosis and detection of HSV DNA, in our patient [Table 4]. EEG showed diffuse right hemispherical slowing in theta to delta range and brain biopsy was consistent with HSE. Initially, the patient was initiated on empirical acyclovir therapy and anti-cerebral edema medications but later on decompressive craniectomy was necessary to treat refractory intracranial hypertension. Following surgery the patient showed remarkable neurological recovery. Hence, we conclude that, for patients with HSE, it is important for the clinician to detect deterioration of consciousness because of the mass effect caused by the disease-associated inflammatory process as early as possible. Timely recognition of refractory intracranial hypertension and surgical decompression in HSE can be life-saving. Increased intracranial pressure during HSE may be so grave that it may cause a shift of intra-cerebral structures, thus increasing the morbidity and mortality. Decompressive surgery for HSE with refractory hypertension can positively affect patient survival, with good outcomes in terms of neurological recovery.

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Cite this article as: Abdul Jalal MJ, Fernandez SJ, Varghese P, Menon MK. Decompressive craniectomy in herpes simplex encephalitis. *Neuroimmunol Neuroinflammation* 2015;2(3):182-6.

Source of Support: Nil. **Conflict of Interest:** No.

Received: 03-03-2015; **Accepted:** 15-04-2015

Gamma-aminobutyric-acid-B receptor antibodies in limbic encephalitis with small cell lung cancer

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ABSTRACT

Encephalitis associated with antibodies to gamma-aminobutyric-acid B (GABA-B) is a subgroup of autoimmune synaptic encephalitis with typical features of limbic encephalitis and small cell lung cancer (SCLC). We report a case of anti-GABA-B receptor encephalitis in a 57-year-old man who presented with seizures, memory loss, and abnormal behavior. He developed partially neurological responses to immunotherapy, but refused comprehensive tumor screening. The symptoms were aggravated again 4 months later. Workup showed antibodies to GABA-B receptors and tumor screening revealed SCLC. It highlights the importance of early screening of underlying tumor and anti-tumor treatment in paraneoplastic cases.

Key words: Gamma-aminobutyric-acid-B receptor antibodies, limbic encephalitis, small cell lung cancer

INTRODUCTION

Encephalitis associated with antibodies to gamma-aminobutyric-acid B (GABA-B) receptors has been recognized as a subgroup of autoimmune synaptic encephalitis recently.^[1] It has been demonstrated that GABA-B receptors were engaged during rhythmic activity in the hippocampus and sculpted this activity.^[2] Correspondingly, anti-GABA-B encephalitis mostly presents with symptoms of limbic encephalitis (LE), which was characterized by memory loss, confusion, hallucinations, personality changes, and seizures. The GABA-B receptors are also distributed in the cerebral cortex, thalamic nuclei, cerebellum, and amygdala.^[3] The initial symptom presentation of cerebellum or brain stem were thus reported in some patients, such as rotational vertigo, ataxia, and opsoclonus.^[4,5]

In nearly half of patients with anti-GABA-B encephalitis, the immune response occurs as a paraneoplastic event, most of which were small cell lung cancer (SCLC). Here, we report a case of a 57-year-old man who presented

with typical symptoms of LE and was finally found SCLC 5 months after onset.

CASE REPORT

A 57-year-old man was admitted to our hospital with recurrent generalized tonic-clonic seizure (GTCS), which is also called grand mal seizure, for 4 months and aggressive behavior, memory impairment, confusion for 4 days. Subsequent neurological examination revealed disorientation, lack of concentration and loss of memory, predominantly short-term memory. Past medical history was clean.

Blood cell count, general chemistry, thyroid function and tumor markers, including carcinoembryonic antigen, α -fetoprotein, carbohydrate antigen-199 (CA199), CA125, CA242, cytokeratin-211 and neuron-specific enolase (NSE), were unrevealing. Cerebrospinal fluid (CSF) analysis (on day 3) detected an elevation of protein, count of white blood cells and glucose levels [Table 1]. Brain magnetic resonance imaging (MRI) revealed bilateral increased T2 signal in the medial temporal lobe [Figure 1]. Chest computed tomography (CT) revealed enlarged mediastinal lymph nodes.

The seizure was controlled after the administration of valproic acid (500 mg/day) and immunomodulating therapy with 40 mg of methylprednisolone on

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10.4103/2347-8659.159077

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Table 1: Clinical symptoms and images information before and after treatment				
Stage	Presenting symptoms	CSF	MRI	Tumor screening
First admission	GTCS, aggressive behavior, memory impairment, confusion	WBC 12/μL Protein 60.1 mg/dL Glc 4.30 mmol/L	Bilateral increased T2 signal in the medial temporal lobe	Enlarged mediastinal lymph nodes
After treatment	Mild memory impairment	-	Mild increased T2 signal (1-month outpatient follow-up)	-
Second admission	Aggressive behavior, distinct memory impairment (milder than the first admission)	WBC 4/μL Protein 40.2 mg/dL Glc 3.63 mmol/L	Mild increased T2 signal (severer than the follow-up image)	SCLC

CSF: cerebrospinal fluid, MRI: magnetic resonance imaging, GTCS: generalized tonic-clonic seizure, WBC: white blood cell, Glc: glucose, SCLC: small cell lung cancer

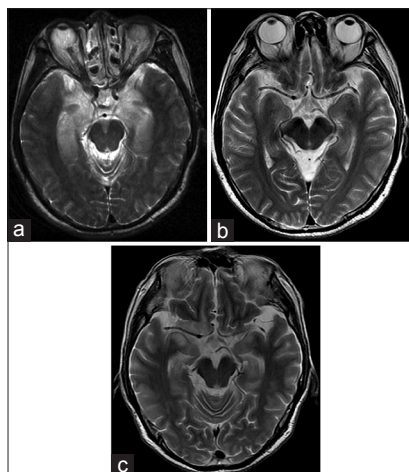


Figure 1: Brain magnetic resonance imaging screens in anti-gamma-aminobutyric-acid B receptor encephalitis: (a) revealed bilateral increased T2 signal in the medial temporal lobe at the first admission; (b) showed milder increased T2 signal 1 months after the first discharge; (c) revealed the lesion worsen again at the second admission

admission. A repeated brain MRI was performed on day 20 and revealed even severer bilateral fluid-attenuated inversion recovery (FLAIR) and T2 hyperintensity in the medial temporal lobe. Therefore, intravenous immunoglobulins (IVIg) (400 mg/kg/day) was administered for 5 days on day 20. The patient developed significant improvement of limbic dysfunction during the IVIg therapy. Although no occupying lesion was found in lung, we suggested further tumor screening including biopsy of mediastinal lymph nodes and fluorodeoxyglucose positron emission tomography. However, the patient refused further extensive screen for underlying tumor and was discharged 25 days after admission, with full recovery of behavior and mental status, and partially improvement of memory impairment. One month after discharge, the follow-up brain MRI showed the lesion in remission [Figure 1].

The patients remained no recurrence of seizure and stable LE symptom during nearly 3 months follow-up. However, he was found collapsed 4 months after discharge, with subsequent recurrence of the abnormal behavior, disorientation, and worsening of memory impairment. Brain MRI revealed distinct lesions again [Figure 1]. CSF analysis revealed normal levels of protein, white blood, and glucose [Table 1]. Results

for central nervous system (CNS) autoantibodies testing revealed CSF antibodies to GABA-B receptor (IgG 1:100). Other tested CNS autoantibodies, including anti-leucine-rich glioma inactivated-1 (LGI1) receptor, anti-contactin-associated protein-like 2 receptor, anti-N-methyl-D-aspartate receptor, anti- α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor, rheumatological autoantibodies, thyroid function tests, anti-thyroglobulin and thyroid peroxidase antibodies were all negative. Tumor marker tests revealed elevated CA125 of 35.2 U/mL and mild elevated of NSE of 25.7 ng/mL. A repeated chest CT showed a mass in right hilus pulmonis, which was finally confirmed to be SCLC by biopsy. Unfortunately, the patient and his carers refused further immunomodulating or antineoplastic treatments. He was discharged with memory impairment, which was persisted after another 3 months follow-up.

DISCUSSION

The misdiagnosis rate of anti-GABA-B receptor encephalitis is high since autoantibodies tests were still not popularized in many countries. Most recently, case serials of patients with anti-GABA-B receptor encephalitis emphasized the typical triad of clinical presentation, including memory alternation, seizures, and SCLC.^[6] There are numbers of SCLC-associated onconeural antibodies, including Hu, amphiphysin, and collapsin response-mediator protein (CV2). Anti-GABA-B receptor antibodies may be detected in patients with LE associated with SCLC who are seronegative for other onconeural antibodies.^[7] The mechanism of SCLC-associated autoimmune responses remains to be elucidated. It may be related to abnormal self-antigen expression in tumor cells, or the antigenic protein might be mutated or modified to those foreign to the immune system. Neurological symptoms often present before the cancer becomes symptomatic, suggesting the importance of early detection and diagnosis of SCLC. The paraneoplastic cases with anti-GABA-B encephalitis, particularly SCLC, presented at an older age range from 53 to 75 years old, and had male predominance.^[1,4] Paraneoplastic cases seemed to have higher titers antibodies, mostly higher than 1:100 (IgG) in CSF.^[1,4,8]

The neuroimaging characteristics of anti-GABA-B encephalitis were nonspecific, nearly half of which might present unilateral or bilateral FLAIR and T2 hyperintensity in the medial temporal lobe, sometimes in cerebellum or brain stem which corresponded to the symptoms. Previous reports found that the severity of clinical symptoms was not associated with the lesion size on brain image, nor was the presence or absence of underlying tumors.^[1]

Regardless of being paraneoplastic or not, most of the patients developed substantial or full neurological responses to immunotherapy, including steroids, IVIg, and plasma exchange. In paraneoplastic cases, however, patients might achieve neurological improvement with successful anti-tumor treatments even without immunotherapy.^[4] In addition, the duration of disease and clinical outcomes varied depending on the presence or absence of SCLC. In our case, the clinical and neuroimaging characteristics recurred without detection and treatment of SCLC, which emphasized the importance of early identification of underlying tumors.

In conclusion, anti-GABA-B receptor encephalitis is a potentially treatable disorder that should be diagnosed by autoantibodies tests. Aggressive immunotherapy is often effective, but anti-tumor therapy is also pivotal for the neurological recovery and long-term outcomes of patients with the paraneoplastic event. This case report joined to the previously reported description of this paraneoplastic syndrome,^[1,4] to delineate the importance of early diagnosis for the prognosis and management.

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Cite this article as: Liu KQ, Yan SQ, Lou M. Gamma-aminobutyric-acid-B receptor antibodies in limbic encephalitis with small cell lung cancer. *Neuroimmunol Neuroinflammation* 2015;2(3):187-9.

Source of Support: Nil. **Conflict of Interest:** No.

Received: 28-02-2015; **Accepted:** 23-04-2015

Isolated palatal palsy: a clinical rarity

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ABSTRACT

Acquired isolated palatal palsy is a rare disease. It is commonly seen in children. It usually presents with acute onset nasal regurgitation of fluids, rhinolalia, and palatal asymmetry. Many causes of this disease, such as infections, trauma, tumor, and brainstem lesions, etc., have been reported. However, the most plausible explanation is immunological/ischemic damage to the affected nerve. After ruling out major potential causes of this disease, the damage is often considered to be idiopathic in nature. This disease has a benign self-limiting course with excellent recovery. In accordance with a hypothesized immunological basis for this condition, treatment with steroids results in significant improvement in its clinical features.

Key words: Idiopathic, palatal palsy, rhinolalia

INTRODUCTION

Unilateral acquired isolated palatal (velopalatopharyngeal) paralysis is a clinical rarity usually seen in children. This isolated palatal palsy is the result of isolated involvement of the pharyngeal branch of the vagus nerve, which supplies motor fibers to muscles of the pharynx and soft palate. It was first described in 1976 by Edin *et al.*,^[1] however its precise etiopathogenesis is still unclear. In the literature, infection-associated cranial mononeuropathy is frequently postulated as a possible cause, although a definite link is still uncertain. A case report of acquired isolated palatal palsy in a young adult is presented below, along with an overview of the available literature.

CASE REPORT

We report a case of a 15-year-old, completely immunized, previously healthy male, admitted with complaints of rhinolalia and nasal regurgitation of fluid for 2 days, which was sudden in onset, nonprogressive, and painless. The oral cavity showed no pseudomembrane. The gag reflex was present bilaterally, but there was less movement of the palate on the right side, and the uvula was deviated toward the left side upon

phonation [Figure 1]. The vocal cords were normal on both sides. All other vital parameters and examinations were normal.

Laboratory tests showed a hemoglobin concentration of 15.2 g/dL, leukocyte count of 7000/mm³, differential leukocyte count of 58/38/2/2, and absolute platelet count of 2.5/mm³. Blood and throat swab cultures were sterile. Cerebrospinal fluid analysis showed a leukocyte count of 4/mm³ consisting entirely of lymphocytes, sugar levels of 54 mg/dL, and protein levels of 29 mg/dL. Viral serology for human immunodeficiency virus, hepatitis B virus (HBV), herpes simplex virus (HSV), and Japanese encephalitis virus was negative. Due to resource constraints, however, other viral causes could not be ruled out. Chest X-ray, electrocardiogram, and diffusion-weighted magnetic resonance imaging (MRI) of the brain revealed no abnormalities [Figure 2].

A short course of prednisolone at 0.5 mg/kg/day was prescribed for 5 days, followed by 0.25 mg/kg/day for another 5 days. On the 7th day after admission, rhinolalia and nasal regurgitation of liquids subsided and the uvula was central upon phonation [Figure 3]. The patient was re-evaluated every 2 weeks for the next 2 months. He remained asymptomatic during follow-up visits.

DISCUSSION

Isolated acquired velopalatopharyngeal hemiparalysis is rare, affecting primarily males (80%) in their first or second decade of life.^[2] Generally, it presents with rhinolalia and ipsilateral nasal escape of fluid with

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10.4103/2347-8659.153981

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Figure 1: Uvula is deviated to the left side on admission

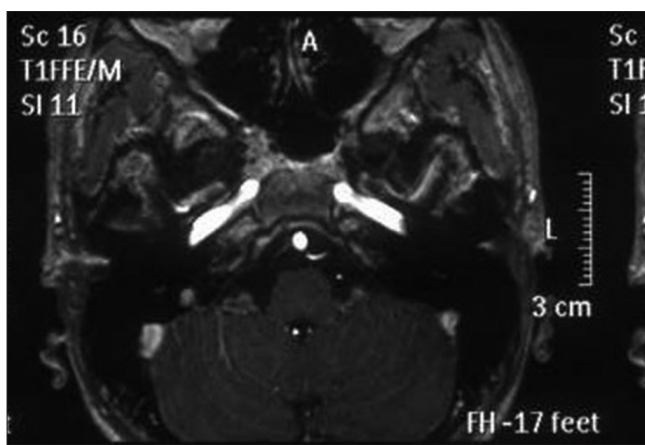


Figure 2: Magnetic resonance imaging of the brain showing no abnormalities

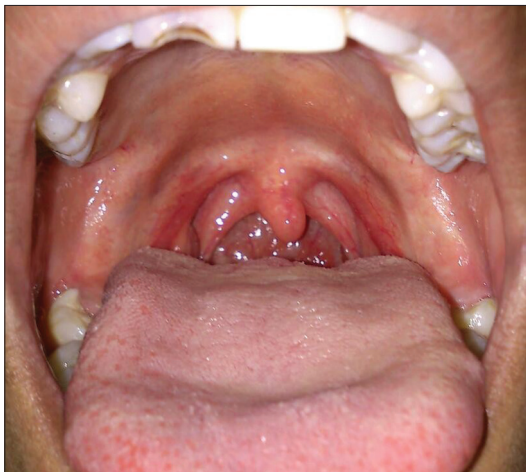


Figure 3: Uvula is central and palatal arches are bilaterally symmetrical on the 7th day after admission

varying degrees of dysphagia, and commonly mimics brainstem lesions.

The etiopathogenesis of this condition is still elusive. Two probable mechanisms have been hypothesized to explain its etiopathogenesis: infectious (mainly viral) and vascular.^[3] The possibility of an infectious/postinfectious origin has been well-documented.^[4,5] For example, acquired isolated palatopharyngeal palsy is a

well-documented postdiphtheritic complication.^[4] The increased prevalence of respiratory and gastrointestinal tract infection and the presence of relatively immature neural tissue in children may lead to more damaging neuroimmune responses to infection, explaining the increased vulnerability during childhood. The vascular hypothesis proposes that ischemia of roots of the glossopharyngeal and vagus nerves due to undetermined causes leads to lower motor neuropathy manifesting as palatopharyngeal incompetence.^[6]

Although isolated palatal palsy is often an idiopathic disease, establishing this idiopathic nature requires the exclusion of other possible factors such as trauma (adenoidectomy or craniofacial trauma), infection (diphtheria, enteric infection, or poliomyelitis), neuromuscular disorders (Guillain-Barré syndrome or motor neuron disease), cranial vessels (internal carotid artery aneurysm or vascular insult), and others (syringobulbia, inflammatory disease affecting various brain stem nuclei and tracts, or tumors, especially of the posterior fossa, which usually have a benign course).^[5,7] Definitive viral etiologies for HSV, Coxsackie, Rubella, HAV, Varicella, and Epstein-Barr virus have also been established.^[4,5,7] Isolated mononeuropathy generally follows infections of the respiratory tract like infectious mononucleosis and parvovirus B-19.^[8,9] Cerebral MRI must also be performed as it allows the exclusion of expansile, ischemic, or demyelinating lesions of the brainstem. Thus, to establish the idiopathic nature of this illness requires exhaustive investigation. Understanding the somatotopic organization of the vagus nerve and associated brain nuclei may help to explain the isolated palatopharyngeal involvement of this condition. In some rare cases, involvement of the cephalad portion of the vagus nerve results in isolated palatopharyngeal palsy.^[5] Laryngoscopy provides direct evidence for sparing of the vocal cords in this condition as concurrent vocal cord palsy has been excluded in cases of isolated acquired velopalatopharyngeal palsy (as in the present case).^[5]

In a systematic review of the literature from 1960 to 2012, only 36 case reports of acquired isolated palatal palsy were found.^[10] The cause of this condition remains undetermined. The disease usually runs a self-limiting course with complete recovery within 2-3 weeks in more than 85% of cases.^[1,2] Prognosis is excellent. Although oral glycerol and steroids have been used empirically for early recovery, no specific treatment is required.^[11] Follow-up is mandatory to observe the further course of the disease. Still, establishing the benign nature of this disorder requires exhaustive investigation in order to differentiate it from other disorders.

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Cite this article as: Singh H, Mathur R, Kaur P. Isolated palatal palsy: a clinical rarity. *Neuroimmunol Neuroinflammation* 2015;2(3):190-2.

Source of Support: Nil. **Conflict of Interest:** No.

Received: 16-10-2014; **Accepted:** 13-01-2015

Neurovascular and neuroinflammatory mechanisms associated with mood disorders

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According to the World Health Organization, mood disorders are a major source of morbidity, disability and mortality worldwide.^[1] In fact, they are the leading cause of suicide.^[2] Consistent data have been collected regarding their epidemiology and clinical, neurobiological and neuropsychological characteristics but their etiology and pathophysiology still remain to be elucidated. The dearth of data limits the possibility of developing new therapeutic strategies aimed at improving patient outcomes.

Recently, an increasing number of studies have focused on neuroinflammation as a potential mechanism involved in the etiopathogenesis of mood disorders.^[3] Before this, a possible relationship between inflammation and psychiatric disorders had been hypothesized. Julius Wagner-Jauregg, the first psychiatrist to be awarded the Nobel Prize in 1927, started from the observations of Hippocrates and Galen and noted that patients diagnosed with psychiatric disorders improved after fever attacks. Thus, he proposed treating them by artificially inducing fever (pyretotherapy).^[4] This treatment strategy implies a primary role of inflammation in the pathophysiology of psychiatric disorders. More recently, this view received new impetus from studies that focused on several inflammatory biomarkers [e.g. interleukin-1 (IL-1), IL-6,

IL-18, tumor necrosis factor- α (TNF- α), interferon- α and brain-derived neurotrophic factor].^[5-8]

The advent of the psychopharmacological era, in general, and of antidepressants, in particular, led clinicians and researchers to concentrate their efforts on the monoaminergic hypothesis of mood disorders. This research line became the standard against which every case had to be measured. In fact, bioamine research in mood disorders attracted the vast majority of resources. Although this research line helped the scientific community gain some insights into the pathophysiology of mood disorders, it did not succeed in dissecting the mystery of mood disorder mechanisms. Some anti-inflammatory properties of anti-depressants^[9] and mood stabilizers^[10] such as serotonin selective reuptake inhibitors, lithium and valproate have been found, but studies are still few and results are inconsistent. Thus, there is a need to explore new pathways to improve our understanding and develop new treatments.

Inflammation is a fundamental physiological homeostatic response of the entire body, which is connected to the stress response, making it part of Selye's "general adaptation syndrome".^[11] It may produce beneficial effects if it is working well and is well-tuned, or it may produce damage and unintended consequences if there is something wrong with its mechanisms, causing it to function inadequately. Undesired consequences may include alterations of mood, sleep, food intake, energy, volition and cognition,

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DOI:
10.4103/2347-8659.167306

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Cite this article as: Spalletta G, Sani G. Neurovascular and neuroinflammatory mechanisms associated with mood disorders. *Neuroimmunol Neuroinflammation* 2015;2:193-4.

Received: 11-04-2015; **Accepted:** 20-04-2015

all of which are part of the classical symptomatology of mood disorders, as well as fever. Cerebrovascular accidents are often associated with the onset of mood disorders, mainly depression. As recently noted by Spalletta *et al.*,^[8] the inflammatory process plays a central role in the onset of poststroke depression because several proinflammatory cytokines, produced during stroke, such as IL-1, IL-6, TNF- α or IL-18 may lead to its amplification, particularly in limbic areas. This may cause widespread activation of indoleamine 2,3-dioxygenase and subsequently serotonin depletion in paralimbic regions, such as the ventral lateral frontal cortex, the polar temporal cortex and the basal ganglia.

In this special issue of *Neuroimmunology and Neuroinflammation*, entitled “neurovascular and neuroinflammatory mechanisms associated with mood disorders”, the authors focus on different perspectives. Kotzalidis *et al.*^[12] and Panaccione *et al.*^[13] review the important role of neuroinflammatory mechanisms in bipolar disorders and manic symptoms. They highlight how critical the imbalance between pro-inflammatory and anti-inflammatory cytokines and more generally, the perturbation of the inflammatory system, are in order to identify their causes and phases. Quaranta *et al.*^[14] hypothesize a possible role of autoimmunity, which may be more relevant in psychotic than in nonpsychotic mood disorders. They review the literature on the relationship between dysregulation of immune homeostasis and psychiatric disorders and suggest that cellular damage due to immune-mediated mechanisms involving excitotoxicity, oxidative stress and mitochondrial dysfunction may be present in medical disorders and severe mood and psychotic disorders, thus potentially reflecting common underlying vulnerabilities. The role of inflammation in other specific clinical populations is also investigated. Serra *et al.*^[15] show preliminary data which suggest there is a relationship between the inflammatory process and juvenile bipolar disorder. Marangoni *et al.*^[16] point out the high prevalence of bipolar disorder in patients with multiple sclerosis; they highlight the need for long-term observational studies to determine whether common mechanisms underlie the two disorders. Serafini *et al.*^[17] propose to use the detection of white matter abnormalities as biological markers of poor outcome, often characterized by suicidal behavior, in patients with major depressive disorders. Lastly, Liguori *et al.*^[18] extensively review the role of gamma-aminobutyric acid in mood disorders and the involvement of different inflammatory mechanisms in the development of excitatory symptoms.

What remains unanswered is the following question: are psychiatric disorders in general, and mood disorders in particular, merely an expression of different abnormalities of inflammation at the system level and in the brain, which are also called neuroinflammation? This provocative question perfectly summarizes the key outstanding issue. Taken together, these reviews represent current research in the field of mood disorders.

By improving our understanding of the mechanisms underlying such disorders, there is hope that in the near future the development of novel therapeutic approaches will result in better outcome.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

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Bipolar disorder preceding the onset of multiple sclerosis

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ABSTRACT

Multiple sclerosis (MS) is the most common inflammatory demyelinating brain disease. The occurrence of psychiatric disorders, especially for major depression, in the course of MS is high. Reports concerning bipolar disorder (BD) remain rather scarce although early descriptions were found in the old neurological literature. The purpose of this article is to provide a critical review of the epidemiology, comorbidity, and treatment findings regarding BD preceding the onset of MS.

Key words: Bipolar disorder, comorbidity, multiple sclerosis, onset

INTRODUCTION

Multiple sclerosis (MS) is the most common autoimmune disease of the central nervous system associated with inflammatory demyelination. In the United States, the prevalence of MS is 0.1%. MS affects more women than men, with an estimated female-to-male ratio of about 2:1. The median and mean ages of MS onset are 23.5 and 30 years of age, respectively. The peak age of onset is about 5 years earlier for women, but the onset of MS can rarely occur as late as the 7th decade.

Although the etiology of MS is unknown, the most widely accepted theory is that MS begins as an inflammatory autoimmune disorder mediated by autoreactive lymphocytes. Later, the disease is dominated by microglial activation and chronic neurodegeneration. Inflammation, demyelination, and axon degeneration are the major pathologic mechanisms that cause the clinical manifestations.

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Bipolar disorder (BD) is a major psychiatric illness with an estimated lifetime prevalence of 2.1% among United States adults. In regard to gender, BD type I occurs with equal frequency in both men and women, but BD type II is more common in women, accounting for up to 30-40% of previously diagnosed unipolar depression.^[1] The age of onset of BD is most commonly around 20 years of age (peaking at ages 15-25 years). BD men appear to have 4-5 years earlier onset than BD women. First onset mania is very rare among elderly people.

The pathophysiology of BD is heterogeneous. Recent scientific findings consider BD as a suite of related neurodevelopmental conditions with interconnected functional abnormalities, early onset, and worsening over time. In addition to accelerating loss of volume in brain regions known to be essential for mood regulation and cognitive function, consistent findings have emerged at a cellular level, providing evidence that BD is reliably associated with dysregulation of glial-neuronal interactions, especially microglia which appear to be overactive. Furthermore, inflammation in the periphery of the body is increased in both depressive and manic phases of the illness, with at least

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Cite this article as: Marangoni C, Nanni MG, Grassi L, Faedda GL. Bipolar disorder preceding the onset of multiple sclerosis. *Neuroimmunol Neuroinflammation* 2015;2:195-9.

Received: 13-03-2015; **Accepted:** 06-08-2015

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Website:
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DOI:
10.4103/2347-8659.167302

some return to normality in the euthymic state. These findings are consistent with changes in the HPA axis, which are known to drive inflammatory activation.^[12]

The occurrence of psychiatric disorders in MS was systematically described as early as in 1872 by Charcot.^[3] Mental changes in MS included pathological laughing and weeping (pseudobulbar affect), euphoria, mania, depression, anxiety, psychosis, and maladaptive personality changes.

The main body of the literature focuses on the comorbid depression found in MS, whereas reports concerning BD remains rather scarce although early descriptions of manic episodes in MS were found in the old neurological literature.^[4-6]

This disproportion reflects the fact that depression is the most frequent among psychiatric disorders in MS, with a lifetime prevalence of 25-50%, a figure two to five times higher than that found in the general population, depending on the country studied.^[7]

The purpose of this article is to provide a critical review of the epidemiology, comorbidity, and treatment findings regarding BD preceding the onset of MS.

EPIDEMIOLOGY OF BD IN MS

Few studies have investigated the prevalence of BD in patients with MS and vice versa. The first epidemiologic study of this kind, conducted on a large sample (more than 700,000 individuals) in Monroe County (NY),^[8] found 10 patients had both MS and BD while epidemiologic data indicated that the expected number of cases would only be 5.4. Joffe *et al.*^[9] conducted a systematic psychiatric evaluation on 100 consecutive MS patients attending a neurology clinic and found a 13% lifetime prevalence of BD. Fis *et al.*^[10] examined the prevalence of BD among hospital service utilizers in Nova Scotia and compared these measures for the MS and non-MS population. The prevalence of BD in hospitalized MS patients was 1.97%, significantly higher than the 0.92% for the non-MS hospital utilizers. In a prospective study in 658 consecutive patients with MS attending an outpatient clinic, Edwards, and Constantinescu^[11] found that MS population had significantly increased rates of BD compared to the general population (odds ratio = 22.02, $P < 0.001$). Finally, Johansson *et al.*^[12] studied comorbidity between MS and BD in a nationwide cohort. The risk of MS was compared in psychiatric patients and in matched unexposed individuals. The risk of MS was increased in patients with BD [hazard ratio (HR) = 1.8, 95% confidence interval (CI): 1.6-2.2, $P < 0.0001$]

and major depression (HR = 1.9, 95% CI: 1.7-2.0, $P < 0.0001$) while the risk of MS in schizophrenia was decreased (HR = 0.6, 95% CI: 0.4-0.9, $P = 0.005$).

BD PRECEDING MS' ONSET

We searched for original articles published in PubMed from inception to 2014 focusing on BD preceding the onset of MS. The search terms used were: (multiple sclerosis) AND (bipolar disorder). All articles identified were English-language, full-text papers. We also searched the reference lists of identified articles for further relevant papers. We selected 16 published articles of case reports where BD preceded the onset of MS [Table 1].

In 26 patients, 20 of which are female, the mean age of onset of BD and MS was 33.42 and 38.46 years, respectively. Nine patients had the onset of BD and MS at the same age. The onset of BD occurred before age 20 years in one patient while 13 patients experienced the onset between age 20 and 29 years, 3 patients between age 30 and 39 years, and 8 patients after age 40 years. The age of onset of MS has a different distribution among these patients: one patient had onset before age 20 years, 6 patients had onset between age 20 and 29 years, 6 patients had onset between age 30 and 39 years and 13 patients had onset after age 40 years.

Twenty-five of these patients were with BD type I (6 with a single manic episode, 6 with recurrent mania, 12 with episodes of both polarity, 1 with rapid cycling) while one had BD type II with rapid cycling. Thirteen patients (4 single manic episode, 3 recurrent mania, and 6 BD type I) had psychotic symptoms in at least one mood episode. Ten patients presented with relapsing/remitting course of MS, 9 with a progressive course, 4 cases presented with pure psychiatric symptomatology, 3 with other course of MS.

Only 3 cases had a positive family history for MS (cases 1, 12, and 22) whereas 6 patients had a psychiatric family history (cases 12-14-22-26 BD, 16 unknown, 19 unipolar).

Regarding the pharmacological treatment for mood episodes, patients received lithium, antipsychotics, antiepileptics, and antidepressants alone or in various combinations. In terms of treatment response according to psychiatric diagnosis, 60% of those with single mania, 66% of those with recurrent mania, 55.5% of those with BD type I presented full or partial response whereas those with rapid cycling had poorer responses to drugs. No cases of mania were induced by treatments.

Table 1: List of patients, identified in case reports, where BD preceded the onset of MS

Case number	Gender	BD onset	BD type	Treatment; response	MS onset	MS presentation and course	Neuroimaging findings	Reference
1	Male	58	Manic episode	Li; full resolution	58	Progressive spastic and ataxic quadriparesis	NR	[13]
2	Female	27	Manic episode	NS	28	R/R cervical cord	MRI: lesions in centrum semiovale, periventricular regions; cerebral and cerebellar atrophy	[14]
3	Female	15	Manic episode	APs; full resolution	15	R/R neuropsychiatric symptoms	MRI: diffuse white matter signal intensity along ventricles extending to the centrum semiovale bilaterally	[15]
4	Female	48	Manic episode	Li + APs; significant improvement	48	Progressive lethargy, restlessness, difficulty concentrating, urinary incontinence, periodic disorientation	MRI: lesions in periventricular and subcortical areas, corpus callosum, right cerebral peduncle, right brainstem	[16]
5	Female	54	Manic episode	AE + AP; poor response	54	Progressive neuropsychiatric symptoms and cognitive impairment	MRI: diffuse and multifocal lesions in cerebral hemispheres	[17]
6	Female	50	Manic episode	AP; poor response	50	Progressive neuropsychiatric symptoms and cognitive impairment	MRI: multiple punctate and patchy periventricular lesions, including Dawson's finger	[17]
7	Male	20	Recurrent mania	Li + APs; full resolution at 1st episode; poor response subsequently	29	Progressive unilateral vision loss, diplopia, paresthesia, ataxia and gait difficulties	CT: diffuse concentric patchy areas	[18]
8	Female	50	Recurrent mania	APs; partial response	56	Progressive ataxic hemiparesis and cognitive deficits	MRI: lesions in centrum semiovale, periventricular regions, thalamus; marked frontal and parietal atrophy	[14]
9	Female	39	Recurrent mania	NS	41	R/R brainstem and thoracic cord	NR	[14]
10	Female	21	Recurrent mania	NS	37	Progressive hemiparesis and ataxia	MRI: lesions in centrum semiovale, periventricular regions	[14]
11	Female	42	Recurrent mania	Li + APs; full resolution	49	Arms paraesthesias, ataxic gait	MRI: multiple hypersignal lesions in periventricular area, cerebellum, corpus callosum, one large and active lesion on right frontal lobe with peripheral oedema; remarkable brain atrophy	[19]
12	Female	27	Recurrent mania	NS	43	Left lower extremity weakness and unstable gait	MRI: lesions in the periventricular and subcortical areas, bilateral centrum semiovale, right pontine and right cerebellar cortex	[20]
13	Female	26	BD-I RC	Li + Aps + AEs; poor response	29	Progressive ataxia, left hemiparesis, intractable nausea vomiting, bowel bladder incontinence, bilateral Babinski	CT: several enhancing lesions in left parietal area, several periventricular areas of low attenuation	[21]
14	Female	46	BD-II RC	Li + ADs; poor response	49	Progressive ataxic gait	CT: normal	[21]
15	Male	23	BD-I	AP; full resolution	27	Progressive ataxic gait, blurry vision, vertical, lateral and intermittent rotary gaze nystagmus	CT: normal	[22]
16	Female	49	BD-I	Li + APs; poor response	49	R/R sensory alterations left leg	NR	[22]
17	Female	25	BD-I	APs + Li; full resolution at first episode; poor response subsequently	30	R/R brainstem and cervical cord	NR	[14]

Contd...

Case number	Gender	BD onset	BD type	Treatment; response	MS onset	MS presentation and course	Neuroimaging findings	Reference
18	Male	21	BD-I	Li + APs; poor response	31	Progressive spastic and ataxic quadriparesis	MRI: lesions in centrum semiovale, periventricular regions, subcortical areas, thalamus, temporal lobes; cerebral atrophy	[14]
19	Female	22	BD-I	NS	27	R/R brainstem and cervical cord	MRI: three lesions in the periventricular area of right hemisphere, one lesion in the left cerebellar hemisphere	[14]
20	Female	33	BD-I	Li + ADs; good response	33	R/R brainstem, lower paraparesis, ataxic gait	MRI: periventricular and temporal horn lesions, one lesion in the right fronto-basal region, bilateral lesions in the splenium of corpus callosum, corona radiata, centra semiovalia, few subcortical lesions; moderate atrophy in supratentorial compartment and trunk of corpus callosum	[23]
21	Female	48	BD-I	NS	48	R/R neuropsychiatric symptoms (+ atonic bladder developed at age 81)	Neuropathology: small, atrophic brain; moderate ventriculomegaly; numerous lesions in periventricular location, right superior frontal convolution, cingulate gyrus, centrum semiovale, corpus callosum, left cerebellar folia	[24]
22	Male	20	BD-I	NS	40	Mild pyramidal signs on the left side	MRI: lesions in the periventricular and subcortical areas, bilateral centrum semiovale, corpus callosum	[20]
23	Female	26	BD-I	AP; full resolution	26	R/R brainstem	MRI: one lesion located parasagittally within the left parietal lobe	[25]
24	Female	39	BD-I	APs + AD; full resolution	40	R/R motor deficit left side, gait impairment, urinary incontinence	MRI: lesions in periventricular area and cervical cord	[26]
25	Male	20	BD-I	Li; poor response	31	R/R paresthesia and weakness left side, urinary incontinence	MRI: lesions in periventricular area, corpus callosum, cervical spine at C2 and C3	[27]
26	Female	20	BD-I	Li; good response	32	R/R quadripyramidal syndrome with right kinetic cerebellar syndrome	MRI: multiple lesions in periventricular and semioval areas, cervical spine at C6	[27]

BD: bipolar disorder; MS: multiple sclerosis; Li: lithium; NR: not reported; NS: not specified; R/R: relapsing remitting; MRI: magnetic resonance imaging; AP: antipsychotic; AE: antiepileptics; AD: antidepressant

We did not find any correlation between BD type and MS course type nor between the pattern of white matter lesions and BD type, although lesions were commonly detected in the periventricular and subcortical white matter, the centrum semiovale bilaterally, frontal, parietal and temporal lobes.

CONCLUSION

The survey of the literature found few epidemiological studies and several case reports of BD clearly preceding MS onset. Based on these data, only limited conclusions can be drawn. In the sample analyzed, the mean ages

of onset of both BD and MS were higher than those reported in the literature. Furthermore, the mean difference between BD onset and MS is 5 years although 9 patients (34.6%) had the onset of BD and MS at the same age. The higher age of onset of MS may indicate that only the form with a later age of onset increases the risk or the co-occurrence/comorbidity with BD.

A high percentage of BD presented with manic episodes (single/recurrent), a finding that may indicated differences between BD occurring in MS patients and those in the general population. Similarly, the higher rate of psychotic features and the low rate of positive

family history may also indicate differences between BD in MS and in the general population.

The available data are insufficient to formulate any hypothesis regarding the etiopathological independence of the two conditions or explain the high rates of co-occurrence and differences in clinical features. In addition, the lack of systematic treatment response data does not permit any comparison between BDs with and without MS. As evidence emerges regarding the inflammatory correlates of both, BD and MS, the value of anti-inflammatory treatments in preventing, delay or ameliorating the course of BD requires further investigations. The limited data on case reports of BD preceding MS yielded several interesting observational findings that might provide information for future research and clinical characterization.

Financial support and sponsorship
Nil.

Conflicts of interest
There are no conflicts of interest.

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Microstructural brain abnormalities, affective temperaments, and suicidal behavior in patients with major depression

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ABSTRACT

According to magnetic resonance imaging (MRI) studies, brain white matter (WM) abnormalities have been suggested to play a critical role in the pathogenesis of major depressive disorder (MDD) and related suicidal behavior. However, MRI findings may be limited by low spatial resolution; therefore, an important contribution to the understanding of the role and significance of WM alterations derived by the development of the most recent magnetic resonance techniques, such as diffusion tensor imaging (DTI). Several DTI studies reported an association between altered WM integrity and MDD/suicidal behavior. Microstructural WM abnormalities may be located in neural circuits critically implicated in emotional processes and mood regulation resulting in enhanced vulnerability to psychiatric morbidity. WM abnormalities detected using DTI may contribute to functional deficits and help to clarify the pathophysiological mechanisms underlying MDD as well as suicidal behavior. By a clinical point of view, research also suggested that affective temperaments may play a relevant role in the psychopathological characteristics of mood disorders, clinical trajectory of episodes and polarity, long-term outcome and suicidality. Unfortunately, only few studies investigated the association between affective temperaments and WM abnormalities and discussed their possible implications in patients with MDD and suicidal behavior. Using a comprehensive search of Medline database, the aim of the present study was to critically review the current literature on the association between WM alterations as assessed by MRI and DTI techniques, affective temperaments, MDD and suicidal behavior.

Key words: Affective temperaments, major depression, microstructural white matter lesions, neuroimaging techniques, suicidal behavior

INTRODUCTION

Major depressive disorder (MDD) is a widespread condition associated with functional impairment and significant disability. Patients with MDD usually experience frequent recurrences, incomplete recovery between episodes, residual symptoms, poor psychosocial adjustment and high risk of suicide.^[1,2]

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Suicidal behavior is a complex and multidimensional condition aimed at self-destruction determining a serious distress in the community and among all those who are near the victim; however, as suicide may be considered a rare social event, its prediction at the individual level is very difficult. Untreated unipolar MDD is one of the most relevant risk factors related to completed suicide accounting for 56-87% of the cases.^[2] Importantly, suicide may be considered a state- and severity-dependent phenomenon, and this is also demonstrated by the fact that suicidality significantly decreases after clinical recovery.^[2] Although identifying some clinical, psychological and psychosocial risk

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Cite this article as: Serafini G, Amore M, Rihmer Z. Microstructural brain abnormalities, affective temperaments, and suicidal behavior in patients with major depression. *Neuroimmunol Neuroinflammation* 2015;2:200-14.

Received: 13-08-2015; **Accepted:** 06-04-2015

Access this article online

Quick Response Code:



Website:
www.nnjournal.net

DOI:
10.4103/2347-8659.167301

factors may help to recognize those depressed or nondepressed individuals who are at increased high risk for suicide,^[3] the mechanisms underlying this complex behavior as well as the link with psychopathology are still poorly understood.

White matter hyperintensities (WMHs) appear as hyperintense signals on T2-weighted magnetic resonance imaging (MRI) and represent ependymal loss and differing degrees of myelination in the brain.^[4,5] WMHs, depending on their localization, may be classified as periventricular WMHs and deep WMHs, with the latter usually having a vascular etiology. Degenerative changes in brain white matter (WM) have been associated with both mood disorders and suicidal behavior in children as well as young adults,^[6-9] however they seem to be not specific of patients with first-episode psychotic disorders.^[10]

Diffusion tensor imaging (DTI) studies can identify microstructural WM abnormalities with high spatial resolution enabling the characterization of WM tracts that relate to critical brain regions implicated in mood regulation. According to the current literature, there are many recent DTI studies examining WM network abnormalities in patients with MDD^[11-14] as well as in those with suicidal behavior.^[15-18] Specifically, some DTI studies reported the existence of frontolimbic WM abnormalities in treatment-naïve first-episode MDD individuals^[19-22] and adolescents with MDD^[23-24] whereas other studies investigated the role of WM microstructural alterations in patients with melancholic MDD characteristics^[25,26] or the effect of treatments on WM integrity in subjects with MDD.^[27-30] Further, DTI studies also investigated the impact of WM abnormalities during the different illness phases (e.g. subclinical depression, throughout the acute MDD episode, at the end of therapy and subsequent follow-up).^[31-33]

Taylor *et al.*^[34] hypothesized that patients with WM lesions may be at higher risk for developing mood disorders and suicide due to possible disruption of neuroanatomical pathways. Mood regulation depends on the complex extensive connections between the prefrontal cortex, amygdala-hippocampus complex, thalamus and basal ganglia.^[35] Mood disorders range from subthreshold affective temperamental traits as measured by the Temperament Evaluation of Memphis, Pisa, Paris and San Diego-auto-questionnaire (TEMPS-A)^[36] up to mood disorders (of minor and major severity) and severe affective psychosis.^[37-40] Affective temperaments may significantly influence the psychopathological characteristics of mood disorders, in particular, the clinical trajectory of episodes and polarity, symptomatology, long-term course, suicidality, and medication adherence.^[37,38,40-44]

To date, the association between microstructural WM abnormalities, affective temperaments, and suicidal behavior in patients with MDD is poorly understood. Therefore, this paper aims to critically review the current literature about this association.

Studies investigating the presence and significance of WM abnormalities and their link with affective temperaments in patients with major depression and suicidal behavior were retrieved from Medline databases. The reference lists of these publications were further investigated to find additional relevant studies. An extensive search was carried out for research published between January 1980 and October 2014 using the following search terms: (“major depressive disorder” OR “MDD” OR “Major depression”) AND (“Affective temperaments”) AND (“White matter hyperintensities” OR “microstructural white matter lesions” OR “white matter abnormalities” OR “white matter changes signals”) AND (“MRI” OR “magnetic resonance imaging”) AND (“Diffusion Tensor Imaging”) OR (“DTI”). Only studies published in English were included. We did not analyze in the present overview studies including patients with bipolar depression nor patients with treatment-resistant major depression.

THE ROLE OF MICROSTRUCTURAL WM BRAIN ABNORMALITIES AS ASSESSED BY MRI IN PATIENTS WITH UNIPOLAR MAJOR DEPRESSION

Microstructural brain lesions, especially in the context of WM integrity, may be frequently found in patients with unipolar major depression.^[45] Based on their systematic review and meta-analysis, cross-sectional subgroup analyses showed that deep WMHs resulted significantly associated with major depression whereas periventricular and overall WMHs did not. In addition, according to longitudinal subgroup analyses, overall WMHs were significantly associated with major depression but deep WMHs did not.

Microstructural WM brain alterations are neuropathologically characterized by decreased oligodendrocyte density, molecular changes in intercellular cell adhesion molecule expression levels and possible ischemia.^[46]

In another meta-analysis, Kempton *et al.*^[47] reported that MDD was associated with lateral ventricle enlargement; larger cerebrospinal fluid volume; smaller volumes of the basal ganglia, thalamus, hippocampus, frontal lobe, orbitofrontal cortex, and gyrus rectus compared with the structures of the healthy brain. Furthermore, during depressive episodes patients reported significantly smaller hippocampal volume than patients during remission.

Gender differences in WM disease of patients with MDD in late life have been recently described by Dotson *et al.*^[48] The authors reported that depressive symptoms predicted increased WMHs rates in men but not in women. A higher rate of WMHs was commonly reported in individuals with late-onset depression (LOD) when compared with healthy elderly controls and subjects with early-onset depression (EOD).^[49-52] Salloway *et al.*^[53] reported that both deep WMHs and periventricular WMHs were significantly more severe in the LOD group than EOD group. More recently, Gunning-Dixon *et al.*^[54] found that 22 patients who did not remit following escitalopram treatment had significantly greater WMHs on MRI than 20 remitted patients and 25 elderly controls. In addition, Takahashi *et al.*^[55] reported that patients with LOD showed a higher rate of deep WMHs and more severe pathological changes especially in the bilateral frontal areas and left parieto-occipital area compared with EOD. Compelling evidence suggested that deep WMHs are more severe in LOD than in healthy controls.^[50,56,57]

The frontal lobes have mutual fiber communications with subcortical nuclei, such as the thalamus, basal ganglia, and amygdala via WM projections mediating emotional processing of information and regulation of emotional states. It has been suggested that dysfunctions of the frontal lobes may be caused by subcortical WM lesions triggering the emergence and maintenance of mood disorders.^[58]

There are also studies suggesting that the presence of WM lesions may be associated with clinical severity and treatment responsiveness. In 12-week, randomized clinical trial comparing Sertraline with Nortriptyline, Sneed *et al.*^[59] also tested the hypothesis that remission from geriatric depression may depend on lesion volume by region of interest and found that patients with higher deep WMHs were 7.14 times more likely to not remit after antidepressant treatment compared to patients with lower deep WMHs. The authors also suggested that those having higher periventricular WMHs and total volumes may be 4.17 and 5.00 times more likely to not remit, respectively.

Increased severity of WM lesions is also associated with a more chronic outcome,^[46,60-62] poorer response to antidepressant medications,^[63-65] and long-term disability^[66] in depressed patients. WMHs are also generally associated with cognitive decline in various domains, particularly executive skills, attention, and mental speed.^[63,67-69]

In addition, Sheline *et al.*^[70] suggested the critical and strategic importance of WMHs location in LOD. The authors reported that 83 depressed subjects

showed greater WMHs in the superior longitudinal fasciculus (SLF), fronto-occipital fasciculus, uncinate fasciculus, extreme capsule, and inferior longitudinal fasciculus that were associated with both cognitive (e.g. episodic memory, processing speed, and executive functions) and emotional functions when compared with 32 healthy controls.

Finally, there are also few studies reporting the association between WMHs and suicidal behavior in MDD patients. In 2012, Serafini *et al.*^[71] reported that among 85 adult outpatients with chronic headache, patients with periventricular WMHs were 1.06 times more likely to report fewer depressive symptoms than patients without PWMHs suggesting that WMH lesions were associated with less depression severity. However, no association between WMHs and suicidal behavior was found. Interestingly, in a meta-analysis including four MRI studies, a significantly higher number of suicide attempters was reported to have WMHs compared to non-attempters.^[72] Specifically, unipolar depressed patients who had attempted suicide were 1.9 and 2.1 times, respectively, more likely to have deep WMHs and periventricular WMHs than nonattempters.

In addition, in a cohort of 99 unipolar/bipolar patients, Pompili *et al.*^[73] reported after logistic regression analyses that periventricular WMHs were robustly associated with suicidal behaviors after controlling for age. Importantly, the same research group^[9] had previously found in a smaller ($n = 65$) cohort of unipolar/bipolar inpatients an higher WMHs prevalence in subjects with past suicide attempts than in those without.

In 2005, Ehrlich *et al.*^[8] suggested a higher prevalence of periventricular WMHs in subjects with past suicide attempts among a cohort of 102 young psychiatric inpatients with MDD. Furthermore, a significant association between WMHs and higher prevalence of past suicide attempts in 48 unipolar depression group had been previously reported.^[7] The same research group^[6] found that youths with deep WMHs mainly located in the parietal and frontal lobes have a significantly higher prevalence of reported past suicide attempts.

WMHs have been commonly reported in both patients with MDD and suicidal behavior; however, the clinical significance of these lesions is still poorly understood as they may be also found in non-depressed individuals with cerebrovascular risk factors such as hypertension,^[56,57,67] diabetes, myocardial infarction or coronary artery disease, and smoking.^[57,74]

THE ROLE OF MICROSTRUCTURAL WM BRAIN ABNORMALITIES AS ASSESSED BY DIFFUSION TENSOR IMAGING IN PATIENTS WITH UNIPOLAR MAJOR DEPRESSION

An important contribution to the understanding of the role and significance of WMHs in patients with mood disorders arrived from the most recent magnetic resonance techniques such as DTI. Since 2002, when Alexopoulos *et al.*^[75] reported an association between lower fractional anisotropy (FA) of the right and left frontal WM regions with a low remission rate, several other DTI studies found an association between microstructural WM abnormalities and MDD. These authors identified for the first time the presence of microstructural WM abnormalities lateral to the anterior cingulate and hypothesized that they were associated with a low rate of remission in 13 patients with geriatric MDD.

Very recently, Qin *et al.*^[11] reported that the hubs of WM brain networks consisting of the bilateral dorsolateral part of superior frontal gyrus, left middle frontal gyrus, bilateral middle temporal gyrus, and bilateral inferior temporal gyrus may accurately distinguish patients with MDD from healthy controls. Lai and Wu^[13] suggested that 44 patients with MDD had significantly lower FA values in the left SLF as well as the right anterior thalamic radiation when compared with 27 healthy controls. These patients had also increased radial diffusivity values in the bilateral SLF and decreased axial diffusivity values in the bilateral anterior thalamic radiation. The authors suggested that FA values resulted negatively associated with depression severity in the SLF, and illness duration in the right SLF and anterior thalamic radiation.

Another recent DTI study^[19] found that adolescents with depression had significantly lower FA and higher radial diffusivity in bilateral uncinate fasciculus. According to tract-based spatial statistics findings, lower WM FA values have been associated with the limbic-cortical-striatal-thalamic circuit, corpus callosum, and anterior and superior corona radiata.

In 2007, Li *et al.*^[76] found microstructural alterations in prefrontal WM occurring early in the course of MDD of 19 first-episodes, untreated MDD young adults relative to healthy controls. In a similar cohort (14 first-episode, treatment-naïve young adult MDD subjects), Ma *et al.*^[77] found significantly lower FA values than controls in the WM of the right middle frontal gyrus, left lateral occipitotemporal gyrus, and subgyral and angular gyri of the right parietal lobe. Moreover, Taylor *et al.*^[78] reported significantly lower

FA values in the right superior frontal gyrus WM of depressed patients than controls.

de Kwaasteniet *et al.*^[21] demonstrated that WM integrity of the uncinate fasciculus was reduced and that functional connectivity between the subgenual anterior cingulate cortex and medial temporal lobe was increased in 18 MDD patients when compared with 24 healthy controls. A negative correlation between uncinate fasciculus integrity and subgenual anterior cingulate cortex functional connectivity with the bilateral hippocampus was also observed only in MDD subjects.

Previously, Bae *et al.*^[79] reported significantly lower FA values in WM of the right anterior cingulate cortex, bilateral superior frontal gyri and left middle frontal gyrus of 106 elderly depressed subjects. Furthermore, a significant reduction in FA values of widespread regions of the frontal and temporal lobes in 13 patients with LOD was found by Nobuhara *et al.*^[80] Interestingly, the severity of depression was inversely related to reduce FA values of the inferior frontal region.

Zhang *et al.*^[81] have previously reported that in a cohort of 21 MDD subjects depression severity had a significant negative correlation with normalized number of fibers in the right uncinate fasciculus. Similarly, Steffens *et al.*^[82] reported a significant association between left uncinate fasciculus FA and resting state functional connectivity between the left ventrolateral prefrontal cortex/left amygdala and between the left ventrolateral prefrontal cortex/left hippocampus. A significant negative correlation has also been suggested between left ventromedial prefrontal cortex-caudate resting state functional connectivity and left, but not right, uncinate fasciculus FA in 24 older patients remitted from MDD.

Another DTI study analyzed the integrity of the WM of corpus callosum and its sub-regions in 16 subjects affected by MDD and 16 age- and gender-matched healthy controls.^[23] The authors reported that the corpus callosal area of the genu was smaller in MDD participants than in 16 healthy controls.

Similar findings have been replicated in adult subjects with treatment-responsive MDD by Guo *et al.*^[83] The authors found lower FA values in the bilateral internal capsule, genu of corpus callosum, bilateral anterior corona radiata, and right external capsule of 22 patients compared to 19 healthy subjects.

Recently, Korgaonkar *et al.*^[29] suggested that anterior cingulate-limbic WM may be a useful predictor of antidepressant treatment outcome in 74 MDD patients who completed 8-week antidepressant treatment. The

authors reported that altered connectivity for the cingulum part of the cingulate and stria terminalis tracts significantly predicted remission. Hayakawa *et al.*^[31] reported a positive correlation between Center for Epidemiologic Studies Depression Scale (CES-D) scale score and radial diffusivity in the right anterior cingulum of 21 subclinically depressed women compared with 21 matched controls.

Another recent report^[12] suggested that in 95 MDD outpatients compared to 102 matched control subjects structural connectivity alterations between nodes of the default mode network and frontal-thalamo-caudate areas (having an important role in emotional and cognitive processing) may play a critical role in the pathophysiology of MDD.

Structural connectivity alterations were also observed in the brainstem and the amygdala in 95 MDD patients compared to 34 gender-matched controls^[14] whereas lower FA values in the body of the corpus callosum, increased radial and mean diffusivity were found after whole-brain analysis. In addition, higher FA values in the uncinate fasciculus together with increased axial diffusivity, reduced radial diffusivity were reported after region-of-interest analysis by Aghajani *et al.*^[84]

A preliminary report^[24] on 17 MDD adolescents suggested decreased WM integrity in the anterior cingulum and anterior corona radiata compared to 16 matched controls. Depression severity has been correlated with reduced WM integrity in the genu of corpus callosum, anterior thalamic radiation, anterior cingulum, and sagittal stratum. Similarly, Bessette *et al.*^[20] reported significantly lower FA of the frontal lobe, bilateral anterior/posterior limbs of the internal capsules, left external capsule, right thalamic radiation and left inferior longitudinal fasciculus in 31 MDD adolescents when compared with 31 healthy volunteers. A first DTI study^[85] found altered WM microstructure in frontolimbic neural pathways of 14 MDD adolescents compared with 14 healthy controls.

Furthermore, Tha *et al.*^[22] reported clusters of FA decrease in several brain regions (e.g. the bilateral frontal WM, anterior limbs of internal capsule, cerebellum, left putamen and right thalamus) in a limited cohort of nine patients with MDD who are not taking antidepressant medications.

Interesting findings were also found in melancholic subtype of MDD.^[25] The authors suggested that MDD melancholic patients ($n = 12$) had lower mean FA in the right ventral tegmental area-lateral orbitofrontal cortex and ventral tegmental area-dorsolateral orbitofrontal cortex connections compared to 21 healthy controls.

Depression total scores were negatively correlated with mean-FA of these brain regions.

Structural abnormalities of cortico-cortical WM motor pathways have been also suggested in 2012 by Bracht *et al.*^[26] in a study of 21 MDD patients relative to 21 healthy controls. They observed that patients had lower activity levels and showed increased mean diffusivity in pathways linking left presupplementary motor area and supplementary motor area, and right supplementary motor area and primary motor cortex. Notably, a negative association between activity level and mean diffusivity of the left dorsolateral prefrontal cortex-pre-supplementary motor area connection was observed only in MDD patients with low activity levels.

In addition, Osoba *et al.*^[32] reported significant FA deficits in the right parietal WM of 20 MDD patients whereas severity of depression has been associated with increased thalamic FA in centromedian tracts and decreased FA in mediodorsal and dorsolateral prefrontal cortex tracts. Lower activity levels in frontal WM regions and posterior cingulum when compared with 21 matched healthy controls were also found by Walther *et al.*^[86] in a cohort of 21 medicated patients with MDD. Depressed subjects also reported negative associations of FA and activity levels below the left primary motor cortex and left parahippocampal gyrus WM.

In 2012, Cole *et al.*^[87] reported that MDD was associated with widespread reductions in WM integrity of the corpus callosum, SLF and anterior corona radiata in 66 patients with recurrent major depression compared to 66 controls. The authors suggested that WM integrity of the corpus callosum was negatively correlated with increasing symptom severity.

Using a 3T scanner DTI, Colloby *et al.*^[88] investigated 68 subjects (30 healthy individuals, and 38 depressed individuals) and found a loss of WM integrity within the frontal, temporal and midbrain fibers. Patients with LOD demonstrated significant lower FA compared with older healthy subjects due to the possible disruption of limbic-orbitofrontal networks. However, after conducting regression analyses, the authors did not find a significant association between DTI parameters and current depressive symptoms.

Moreover, Korgaonkar *et al.*^[89] reported the presence of WM integrity deficits in 11 melancholic MDD subjects relative to 39 healthy controls postulating the existence of a pattern of reduced myelination or other degenerative changes whereas Wu *et al.*^[90] reported a significantly lower FA in the right SLF within the frontal lobe, right middle frontal and left parietal WM

when investigating 23 single-episode, medication-naïve MDD subjects relative to 21 healthy controls.

Lower FA values in the bilateral medial frontal gyri, right subgyral frontal and temporal lobes, left middle frontal, and cingulate gyri have been reported by Ouyang *et al.*^[91] in 18 MDD patients compared to 18 controls.

Abnormalities of WM integrity, especially within cortical-subcortical neural circuits, have been suggested to play a key role in the pathophysiology of MDD. Reduced FA values in the left WM anterior limb of the internal capsule (ALIC), right parahippocampal gyrus, and left posterior cingulate cortex in 25 first-episode, treatment-naïve young adult MDD patients compared with 25 healthy controls have been reported by Zhu *et al.*^[92] Interestingly, the severity of depressive symptoms was negatively correlated with the reduced FA values of the left ALIC suggesting a crucial role of this brain region in the pathogenesis of MDD.

Lamar *et al.*^[93] reported an association between WM damage and endorsed depressive symptoms in 79 euthymic older adults whereas Zou *et al.*^[94] observed a significant decrease in FA of the left hemisphere, including the left ALIC and inferior parietal portion of the SLF in 45 patients with MDD compared with 45 healthy controls. Finally, lower FA of the rostral/dorsal anterior cingulate, dorsolateral prefrontal cortex, genu of the corpus callosum, WM adjacent to the hippocampus, multiple posterior cingulate cortex regions, and insular WM, neostriatum and midbrain together with temporal and parietal regions were found in 23 individuals who failed to remit compared to 25 patients who recovered from depression.^[95]

Meta-analytic evidence of DTI studies^[96] also confirmed the association between WM integrity abnormalities and MDD. Reduced FA in the WM fascicles connecting the prefrontal cortex within cortical (frontal, temporal and occipital lobes) and subcortical areas (amygdala and hippocampus) supported the involvement of these brain areas in the pathogenesis of MDD. Similar findings were reported in a previous meta-analysis when Murphy and Frodl^[97] found reduced WM FA values in SLF and increased FA values in the fronto-occipital fasciculus in depressed patients compared to controls.

There are also DTI studies demonstrating the positive effect of treatments on WM integrity in patients with MDD. Lyden *et al.*^[27] suggested that WM microstructure abnormalities in frontal and limbic regions of 20 MDD patients are modulated by electroconvulsive therapy (ECT). They found significant FA increases of the dorsal fronto-limbic circuits encompassing

the anterior cingulum, forceps minor, and left SLF in patients receiving ECT compared to healthy controls. The authors also reported significant reductions in radial and mean diffusivity of the anterior thalamic radiation related to therapeutic response in MDD patients. In another DTI study, Taylor *et al.*^[98] suggested that 74 subjects who were responders to Sertraline reported higher FA values in the superior frontal gyri and anterior cingulate cortices bilaterally; however, there is no significant association between apparent diffusion coefficient measures and remission.

Furthermore, Nobuhara *et al.*^[99] reported improvements of WM integrity in frontal brain regions after ECT treatment in 8 late-life depressed patients with significant frontal and temporal FA reductions compared with 12 healthy age-matched controls.

In another report, Wang *et al.*^[33] suggested a significant reduction of the 17-item Hamilton depression rating scale in 21 depressed patients after 4-week guided imagery psychotherapy. Significantly increased FA in the right thalamus was reported in depressed individuals compared to healthy controls. Changes in WM have been also described after left repetitive transcranial magnetic stimulation by Kozel *et al.*^[100] A mean (although not significant) increase was found in the left prefrontal WM in 8 depressed patients.

Recently, Hoogenboom *et al.*^[28] found that failure to achieve remission was associated with lower medial body of the fornix FA among 92 MDD patients (of which 29 were nonremitters, 26 partial-remitters, and 37 full-remitters).

Not all studies supported the existence of WM alterations as assessed using DTI techniques in MDD patients. Choi *et al.*^[30] found no significant differences in FA, radial diffusivity, mean diffusivity, and axonal diffusivity using voxel-based morphometry or tract-based spatial statistics approach in 134 medication-free MDD patients compared to 54 healthy controls.

Moreover, Bezerra *et al.*^[101] reported no significant differences among FA or mean diffusivity values between depressed and non-depressed elderly individuals with or without mild/moderate MDD. Abe *et al.*^[102] also suggested no significant difference between 21 right-handed patients and 42 age- and gender-matched controls for FA and whole WM volume. Only a trend of negative correlation between FA and total days in the right anterior cingulate and left frontal WM was reported in this study.

Similarly, Kieseppä *et al.*^[103] investigated 16 MDD patients and found only a trend for lower values of FA

in the left sagittal stratum, and reduced FA in the right cingulate cortex/posterior body of corpus callosum compared to 20 controls.

WMHs AND AFFECTIVE TEMPERAMENTS: CLINICAL IMPLICATIONS REGARDING MDD AND SUICIDALITY

Few studies investigated the association between affective temperaments and WMHs as well as their possible implications on suicidal risk.

Weber *et al.*^[104] conducted a 2-year follow-up study in a cohort of 28 EOD elderly patients and observed increased neuroticism factor, anxiety facet scores, and reduced warmth and positive emotions facet scores only at baseline compared to 48 controls. Significantly higher depression facet scores at both baseline and after 2 years independently of depressive relapse were also reported in EOD elderly patients than controls. In 2010, the same research group^[105] reported no significant group differences in WMH rates between 38 EOD and 62 controls. Importantly, EOD was associated with a significant increase of neuroticism and decrease of extraversion facet scores as assessed by five-factor personality dimensions.

Serafini *et al.*^[106] found that patients with higher dysthymia and lower hyperthymia scores (as assessed by TEMPS-A) were more likely to have higher suicidal risk, more recent suicide attempts, and more deep WMHs than patients with higher hyperthymia and lower dysthymia scores. In this study, different temperament characteristics are reflected by MRI findings. The mentioned results replicate the findings of prior studies showing that depressive, cyclothymic, irritable and anxious temperaments may be considered risk factors for suicidal behavior whereas hyperthymic temperament is a protective factor, at least for suicide attempters.^[106,107]

There are also DTI studies analyzing the presence of WM abnormalities in healthy individuals. Picerni *et al.*^[108] investigated the relationship between cerebellar macro- and micro-structural variations detected by DTI and temperamental traits assessed using temperament and character inventory (TCI) in 100 healthy individuals. The authors found increased WM FA associated with higher novelty-seeking scores suggesting that macro- and micro-structural characteristics of posterior vermis play a critical role in novelty-seeking behaviors. In a previous report, the same research group suggested that the scores of the four temperamental scales of TCI were positively associated with the volumes of cerebellar WM.^[109] Specifically, it has been suggested that novelty seeking

scores were positively associated with WM volume whereas harm avoidance scores were negatively associated with WM volume in a cohort of 125 healthy participants.

Moreover, Bjørnebekk *et al.*^[110] reported an association between social reward dependency and WM microstructure in 263 healthy volunteers. In detail, the authors found that increased reward dependence was associated with reduced FA in anterior brain areas suggesting that WM fiber tract properties may significantly modulate individual differences in social reward. However, no associations were found between novelty seeking behavior as assessed by TCI and DTI indices.

Westyle *et al.*^[111] showed that increased harm avoidance is associated with abnormalities in WM microstructure in a large cohort ($n = 263$) of healthy adults. Increased harm avoidance was associated with reduced FA whereas increased mean diffusivity/radial diffusivity in specific WM tracts, such as corticolimbic pathways, was implicated in emotional processing and reappraisal. The authors speculated that the associations between WM microstructure and anxiety-related personality traits emerged early in life suggesting that both temperament and personality are closely shaped early and remain stable during the life span.^[111]

There are also two voxel-based morphometry studies analyzing the association between WM abnormalities and temperamental features in healthy subjects.

In the first study, Van Schuerbeek *et al.*^[112] suggested that individual variations in brain morphology may be associated with temperament and character dimensions in 68 young healthy female volunteers. The authors found correlations between temperamental traits and WM volume. Specifically, a link between cooperativeness and WM volume has been observed in the medial frontal and precentral gyrus.

In the second study, Kaasinen *et al.*^[113] investigated whether late adulthood brain structural differences may be related to differences in temperament and character in 42 healthy aged adults.

However, no significant correlations between regional WM volumes and personality traits were reported, and only a trend of correlation between right cerebellar WM volume and self-transcendence was observed.

To our knowledge, no other studies supported the association between affective temperaments, WMHs and suicidal risk. Table 1 summarizes the most relevant evidence for the association between WM abnormalities

Table 1: Most relevant studies about the association between WM abnormalities and affective temperaments in patients with MDD and suicidal behavior

Authors	Study design	Sample	Main results	Limitations	Conclusion
Picerni <i>et al.</i> ^[108]	Cross-sectional	100 healthy individuals	NS scores as assessed by TCI were associated positively with cerebellar gray matter volumes and FA, and negatively with gray matter mean diffusivity. Harm Avoidance, Reward Dependence or Persistence scores were not associated with cerebellar structural measures	The study has been conducted on healthy individuals. The possible causal link between cerebellar structures and novelty-related behaviors has not been investigated. The cross-sectional nature of this study should also be considered a further additional limitation	The cerebellum may be a critical structure implicated in novelty related behaviors
Laricchiuta <i>et al.</i> ^[109]	Cross-sectional	125 healthy adults	Increased bilateral caudate and pallidum volumes have been associated with higher NS scores together with increased MD in the bilateral putamen associated with higher HA scores as assessed by TCI. The biological variance associated with NS or HA personality phenotype may be at least partially explained by macro/microstructural variations in the basal ganglia regions	The study has been conducted on healthy subjects. Finally, the cross-sectional nature of this study should be considered a further additional limitation	Subjects with a micro-structure of putamen characterized by higher MD values may be considered more vulnerable in experiencing negative emotional states, withdrawal, and inhibition
Bjørnebekk <i>et al.</i> ^[110]	Cross-sectional	263 healthy adults	Increased RD was associated with decreased FA in anterior regions including frontostriatal and frontolimbic circuits. Higher RD was associated with decreased microstructural integrity of the brain WM as demonstrated by negative associations between RD and FA. NS and DTI indices were not associated	The study has been conducted on healthy individuals. Altered reward processing should be assessed in patients with major neuropsychiatric disorders. Finally, the cross-sectional nature of this study should be considered a further additional limitation	WM microstructure frontostriatal and frontolimbic circuits may account for variability in RD
Weber <i>et al.</i> ^[104]	2-year follow-up	28 EOD elderly patients and 48 HP	Increased Neuroticism factor, Anxiety facet scores, decreased Warmth and Positive Emotions facet scores have been observed at baseline but reached the level of HP at follow-up. Conversely, significantly higher depression facet scores were found in EOD patients than HP at both baseline and after 2 years independently of depressive relapse	The study was limited by the loss of 24% of the participants at follow-up and the reduced sample size. The number of relapses between baseline and follow-up time points, or separate comparisons of recurrent vs. single episode EOD cases have not been considered. Finally, the study did not provide an additional comparison group of patients with LOD	The long-term evolution of MDD may be characterized by the trait like marker depression-related personality facet
Westlye <i>et al.</i> ^[111]	Cross-sectional	263 healthy adults	Increased HA has been associated with reduced FA and increased MD and RD in WM corticolimbic tracts	The study has been conducted on healthy subjects. Alterations in axonal density and membranes together with architecture of the insulating myelin sheaths have been proposed as candidate mechanisms underlying HA although histological data were not available. Finally, the cross-sectional nature of this study should be considered a further additional limitation	Structural corticolimbic connectivity may mediate anxiety-related aspects of personality. Reduced WM integrity may reflect higher susceptibility to psychiatric disease
Van Schuerbeek <i>et al.</i> ^[112]	Cross-sectional	68 female volunteers	A correlation between temperamental traits as assessed by TCI and WM volume has been reported. Specifically, a link between cooperativeness and WM volume of the medial frontal and precentral gyrus has been found	The study has been conducted on healthy individuals. DTI techniques were not used. Environmental factors such as learning and training that may be influenced by personality have been not controlled. Finally, the cross-sectional nature of this study should be considered a further additional limitation	Individual variations in brain morphology may be associated with temperament and character dimensions

Contd...

Table 1: Contd...

Authors	Study design	Sample	Main results	Limitations	Conclusion
Serafini <i>et al.</i> ^[106]	Cross-sectional	247 subjects with major affective disorders	48% of patients had periventricular WMHs and 39% had deep WMHs. Patients with higher dysthymia and lower hyperthymia were more likely to have hopelessness, more WMHs, and more recent suicide attempts when compared with patients with higher hyperthymia and lower dysthymia	The small sample size did not allow the generalization of the present findings. The association between the lethality/number of suicide attempts and the presence/severity of hyperintensities has not been explored. The cognitive effects of medications were not taken into consideration and represented a limitation. Finally, the cross-sectional nature of this study should be considered a further additional limitation	Differences among temperamental groups measured by the TEMPS-A are associated with differences in MRIs. Different temperamental profiles are associated with differences in the subcortical structures of the brain
Weber <i>et al.</i> ^[105]	Cross-sectional	38 elderly remitted patients with EOD and 62 HP	No significant group differences were found in WMH rates between EOD and HP. Conversely, EOD was associated with significant increase of Neuroticism and decrease of Extraversion facet scores as assessed by Five-Factor personality dimensions	Only EOD patients without psychiatric and physical comorbidities have been selected. The study was limited by its cross-sectional nature	Old EOD patients without major psychiatric remained stable and free from cognitive impairment and structural/vascular alterations
Kaasinen <i>et al.</i> ^[113]	Cross-sectional	42 healthy aged adults	A positive association has been observed between GM volume of the temporal, parietal, and frontal cortices, and self-transcendence, a personality trait reflecting mature creativity and spiritualism	The study has been conducted on healthy subjects. The correlation between cooperativeness and CSF was significant only after correction for age and gender. Moreover, it was possible that the relatively small differences of cooperativeness scores in healthy subjects have little impact on behavior. Finally, the cross-sectional nature of this study should be considered a further additional limitation	High self-transcendence, which has adaptive advantages in the later part of life, is associated with relatively greater temporal cortical GM volumes

CSF: cerebrospinal fluid; EOD: early-onset depression; FA: fractional anisotropy; GM: grey matter; HA: harm avoidance; HP: healthy participants; LOD: late-onset depression; MD: mean diffusivity; NS: novelty seeking; RD: reward dependence; TCI: temperament and character inventory; TEMPS-A: temperament evaluation of memphis; Pisa: Paris and San Diego-auto-questionnaire; WM: white matter; WMHs: white matter hyperintensities

and affective temperaments in patients with MDD and suicidal behavior.

CLINICAL IMPLICATIONS AND MAIN LIMITATIONS

According to the selected findings of the present review derived by MRI studies, WMHs have been commonly reported and associated with a poor outcome and increased suicidality in patients with MDD. This has been independently confirmed by DTI studies showing a robust association between WM microstructural abnormalities, MDD, and suicidal behavior. Neuroimaging techniques have also provided interesting results to test the association between temperaments, personality profiles and WM microstructure abnormalities [Table 1].

Based on our results, the presence of microvascular brain abnormalities and specific affective temperaments such as dysthymic subtype may exert a combined negative role in patients with MDD worsening outcome and triggering suicidality. The presence of WM abnormalities together with a dysthymic temperamental profile may be used for grouping subjects with MDD and this may potentially help clinicians in optimizing treatment strategies.

DTI techniques are undoubtedly able to more deeply investigate the nature of abnormalities in WM integrity among patients with MDD. According to DTI evidence, WM abnormalities have been re-conceptualized as microstructural damage related to vascular processes that affect brain connectivity.^[111]

Atherosclerotic or ischemic lesions, micro infarcts,^[114,115] demyelination,^[5] cerebral edema,^[116] astrocyte proliferation and deposition of brain toxic materials^[5] have been commonly proposed as the underlying mechanisms involved in the development of WM alterations. These brain lesions can be identified using both MRI and DTI techniques, with the latter allowing the detection of location, orientation and anisotropy of brain WM tracts.

Until a few years ago, white matter has been supposed to be a passive tissue, but according to recent evidence it has been suggested as actively implicated in major psychiatric conditions and brain functioning. Notably, the timing of WM growth and degree of completion may influence important human abilities such as affect learning, memory, and self-control ability.

Understanding the nature and origin of WM alterations in MDD is of paramount importance as they may

be associated with a poor clinical course, increased disability, negative psychosocial impairment^[117] and response to treatment as well as functional decline.^[118-121]

Recently, WM abnormalities detected using DTI have also contributed to clarify the pathophysiological mechanisms underlying suicidal behavior. Olvet *et al.*^[15] conducted a DTI study on 13 suicide attempters with MDD, 39 unipolar depressed non-attempters, and 46 healthy participants and found that low FA in the dorsomedial prefrontal cortex (DMPFC) was associated with a suicide attempt history. Similar results have also been reported by Jollant *et al.*^[122] in a functional MRI study. They found reduced activation in the DMPFC of remitted MDD suicide attempters compared with subjects who did not attempt suicide.

Lopez-Larson *et al.*^[16] reported that nineteen veterans with mild traumatic brain injury and a history of suicidal behavior had greater FA measures in bilateral thalamic radiations compared to forty veterans with mild traumatic brain injury without suicidal behavior and healthy controls. Among veterans with mild traumatic brain injury and a history of suicidal behavior, right thalamic volumes negatively correlated with anxiety symptoms whereas total mean FA values for the right anterior thalamic radiations positively correlated with impulsivity.

Furthermore, a positive correlation between current suicidal ideation and FA was reported in the cingulate^[17] of 15 male veterans with traumatic brain injury and 17 matched healthy controls. Interestingly, the authors suggested the existence of a neurobiological vulnerability to suicidal risk related to WM microstructure.

Another DTI study^[123] investigated the effect of past suicide attempts in 63 patients with MDD (23 with and 40 without a history of suicide attempts) and 46 healthy controls. The authors reported that those with a history of suicide attempts had greater abnormalities in the left orbitofrontal cortex and thalamus when compared with those without suicide attempts whereas reduced fiber projections through the ALIC to the left medial frontal cortex, orbitofrontal cortex and thalamus were found in both groups of patients [Table 2].

Further potential support (external validation) to the association between microstructural WM abnormalities and suicidality in patients with MDD may be also provided by the earlier age at illness onset in some MDD patients with higher WM abnormalities as well as the very well replicated finding of early-onset suicidality in patients with mood disorders.^[2]

Many years ago, Hopkinson^[124] reported that the risk of depression in the first-degree relatives of depressed subjects was greater (20%) in the EOD group compared with the LOD group (8.3%) over 50 years of age. Similar findings have been later reported by Schultz^[125] and Post^[126] supporting the hypothesis that genetic factors may show greater effects in EOD compared to late-onset depression. More recently, these findings have been replicated by Takahashi *et al.*^[55] who suggested that early-onset type may be more closely associated with nonvascular factors such as genetic factors.

Komaki *et al.*^[127] also reported that WM lesions were significantly correlated with age at initial onset of depression (45.8 years) in 123 MDD subjects. The authors also found that the rate of suicide in those patients with lacunar infarction (17.9% of the total sample) was significantly higher than that in subjects with no abnormal findings or those with WMHs but no lacunar infarction, suggesting that the prognosis was worse in those with lacunar infarction relative to the other two groups. Unfortunately, not all studies found a relationship between subcortical hyperintensities and age at onset in patients with mood disorders.^[128-130]

There may be many possible causes underlying WM lesions that can occur over time and may be quite progressive or rather static. WM lesions may be also detected in younger adults without typical cardio- and cerebro-vascular risk factors and are occasionally associated with inflammatory/demyelinating diseases.^[131] In this case, it has been suggested that they are presumably genetically determined. Recently, Sprooten *et al.*^[132] suggested that WM integrity was a reliable endophenotype for bipolar disorder with important behavioral associations linked to the etiology of this condition. Specifically, they reported widespread WM integrity reductions in unaffected relatives of bipolar patients and cyclothymic temperament. Although the authors did not investigate patients with a history of suicide and they did not report implications related to suicide risk in the analyzed cohort, their study suggested that impaired WM integrity might be a potential mechanism of genetic predisposition for bipolar disorder. Reduced fronto-temporal and fronto-thalamic WM integrity may represent a structural substrate of mood instability in both healthy control subjects and unaffected relatives at high genetic risk for bipolar disorder. Interestingly, cyclothymia resulted negatively associated with WM integrity of the internal capsules bilaterally and left temporal lobe in both high-risk subjects and controls. The authors supported the assumption that WM abnormalities might have behavioral associations related to the symptomatology of the illness.

Table 2: Most relevant DTI studies about alterations in WM integrity and suicidal behavior

Authors	Study design	Sample	Main results	Limitations	Conclusion
Olvet <i>et al.</i> ^[15]	Cross-sectional	13 suicide attempters with MDD, 39 non-attempters with MDD, and 46 HP	Suicide attempters reported lower FA relative to MDD non-attempters and HP in the DLPFC. A significant cluster within the right DLPFC has been confirmed according to uncorrected TBSS findings. No differences in ADC when comparing the three groups using ROI or TBSS methods were found	The small sample size did not allow the generalization of findings. HP group was younger than the non-attempter group. The most recent suicide attempt ranged from 19 days to 39 years before the DTI scan. The possible effect of medications (antidepressants) should be not excluded. Finally, the cross-sectional nature of this study should be considered a further additional limitation	WM abnormalities may contribute to functional deficits associated with suicidal behavior
Lopez-Larson <i>et al.</i> ^[16]	Cross-sectional	40 veterans with mild TBI and no SB, 19 veterans with mild TBI and a history of SB, and 15 HP	Left and right thalamic volumes were reported as significantly increased in those with TBI and a history of SB compared to the HP, TBI and a history of SB, and the combined group. Veterans with TBI and a history of SB had increased FA bilaterally compared to the HP, HP and TBI with a history of SB group, and the TBI with a history of SB only group. Significant positive associations were found for bilateral ATR and BIS in those with TBI and a history of SB	The small sample size did not allow the generalization of findings. Of note, the study included only male subjects. The cross-sectional nature of this study should be considered a further additional limitation	Thalamic enlargement and increased FA in subjects with TBI and a history of SB suggested that this region may be considered a potential biomarker of suicidal behavior
Yurgelun-Todd <i>et al.</i> ^[17]	Cross-sectional	Fifteen male veterans with TBI and 17 matched HP	A significant reduction in FA values of the left cingulum and left/total genu was observed in the TBI group compared to HP. Subjects with TBI were more likely to have higher impulsivity than HP. A positive correlation between current suicidal ideation, impulsivity, and total and right cingulum FA has been observed	The small sample size did not allow the generalization of findings. The cross-sectional nature of this study should be considered a further additional limitation	A potential neurobiological vulnerability to suicidal risk may be mediated by the significant reduction in FA of frontal WM tracts in veterans with mild TBI associated with both impulsivity and suicidality
Jia <i>et al.</i> ^[123]	Cross-sectional	63 patients with MDD (23 with and 40 without a history of suicide attempts) and 46 HP	Both groups of depressed patients had reduced fiber projections through the ALIC to the left medial frontal cortex, orbitofrontal cortex, and thalamus. Those with a history of suicide attempts were more likely to have alterations in the left orbitofrontal cortex and thalamus than those without a history of suicide attempts	The study may be limited in terms of power to examine variability in brain anatomy concerning any specific method of suicide attempts, specific previous treatments, or number of previous attempts and depressive episodes. Personality has not been evaluated. According to DTI analyses, the ability of the present study to distinguish the directionality of altered fiber tracts is very limited. Furthermore, the degree to which alterations in the ALIC are restricted to the left hemisphere needs to be evaluated. Finally, the present findings may be not able to establish the direction of causality	WM alterations of frontothalamic circuits may contribute to cognitive/affective deficits increasing vulnerability for suicidal behavior in depressed patients

ADC: apparent diffusion coefficient; ALIC: left anterior limb of the internal capsule; ATR: anterior thalamic radiations; BD: bipolar disorder; BIS: Barratt Impulsiveness Scale; DTI: diffusion tensor imaging; DLPFC: dorsomedial prefrontal cortex; FA: fractional anisotropy; HP: healthy participants; MDD: major depressive disorder; ROI: region of interest; SB: suicidal behavior; TBI: traumatic brain injury; TBSS: tract-based spatial statistics; WM: white matter

Some limitations potentially contributing to the lack of consistency of the present findings need to be addressed. First, WMHs in patients with MDD should be interpreted as an extreme consequence of underlying microstructural dysfunctions affecting brain connectivity. Second, most studies did not assess patients for the presence and severity of

possible confounding variables (such as vascular risk factors) together with history of substance abuse/dependence and prior mood episodes as well as the burden of comorbidities. Another important caveat regards the use of psychotropic medications potentially influencing both the presence and severity of WM lesions.

CONCLUSION

White matter abnormalities may be proposed as biological markers of poor outcome in patients with MDD also triggering suicidal behavior. The presence of WMHs may be used for grouping and closely monitoring those patients with more severe illness impairments requiring more targeted interventions. However, further prospective studies are needed to more deeply investigate differential outcome trajectories as well as to develop tailored treatment strategies in patients with MDD. This field is rapidly expanding, and we are only at the beginning of this interesting journey. A better understanding of the biological processes involved in the progression of WM alterations is undoubtedly necessary.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

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Neuroinflammation and excitatory symptoms in bipolar disorder

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ABSTRACT

Neuroinflammation has been proposed as a strong biological factor underlying the development of neuropsychiatric diseases. A role for dysregulation of the immune system was initially suggested in depressive disorders and subsequently extended to other illnesses, including bipolar disorder (BD). Indeed, there is growing evidence confirming the presence of a generalized pro-inflammatory state in BD patients, involving alterations in cytokine, acute-phase proteins, and complement factor secretion, white blood cell differentiation, microglial activation, arachidonic acid signaling pathways, and increased oxidative stress markers. Medications commonly used to treat BD, such as lithium, antiepileptics and antipsychotics, show some immunoregulatory activity both *in vitro* and *in vivo*. The aim of our study was to review the role of different inflammatory mechanisms, specifically in the development of excitatory symptoms, via a systematic PubMed search of the literature. Despite the high variability of results among studies, we found evidence indicating specific alterations of the inflammatory response during manic and mixed states of BD. These findings may help to clarify some of the complex mechanisms underlying the development of excitatory symptoms and suggest a potential role for drugs targeting the inflammatory system as new therapeutic options.

Key words: Anti-inflammatory drugs, bipolar disorder, glia, interleukin, mania, mixed states, neuroinflammation

INTRODUCTION

Amongst the wide constellation of factors thought to be involved in the pathophysiology of mental illness, there's accumulating evidence for a pivotal role of the inflammatory system as a risk factor for neuropsychiatric disease onset and progression.^[1-3] In the early 1970s, several studies showed how the brain is able to modulate the immune system, focusing on the role of stress and associated hypothalamus-pituitary axis mechanisms. These observations have been translated in studies

involving patients diagnosed with the major depressive disorder. Indeed, both the clinical observation of high rates of depressive symptoms in patients affected by immune-related diseases, such as cancer, diabetes, and cardiovascular, inflammatory, and autoimmune diseases, and the results of a majority of studies investigating the role of inflammation in depressive disorders confirmed this hypothesis.^[4,5] The link between depressive symptoms and systemic inflammation is strengthened by the experimental observation that the injection of interleukin (IL)-1 β or tumor necrosis factor (TNF)- α in animals produces a range of behavioral abnormalities known as "sickness behavior". Mice show reductions in locomotor activity, social interaction, novelty seeking behavior, saccharine preference, brain self-stimulation, and food and water intake, as well as impairments in learning and memory.^[6-8]

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10.4103/2347-8659.167304

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Cite this article as: Panaccione I, Spalletta G, Sani G. Neuroinflammation and excitatory symptoms in bipolar disorder. *Neuroimmunol Neuroinflammation* 2015;2:215-27.

Received: 06-04-2015; **Accepted:** 05-05-2015

In humans, treatment with interferon (IFN)- α induces depressive symptoms such as anhedonia, fatigue, suicidal thoughts, cognitive impairments, loss of appetite, and sleep alterations,^[9,10] therefore hinting that IFN- α may contribute to the development of mood disorders.^[11,12] Healthy volunteers injected with *Salmonella abortus equi* endotoxin show increased circulating levels of inflammatory mediators and develop psychiatric symptoms, such as anxiety and depressed mood, as well as mild, transient cognitive impairments.^[13] Depressed patients with no other concomitant medical conditions show alterations in the levels of many pro-inflammatory cytokines and acute-phase proteins, both in peripheral blood and cerebrospinal fluid (CSF).^[14] In particular, variations in the concentration of IL-1, IL-6, TNF- α , and C-reactive protein (CRP) have been observed in patients diagnosed with depressive disorders when compared to healthy controls.^[15-20] Glial abnormalities,^[21-23] increased oxidative stress,^[24-27] macrophage activation,^[28-30] and alterations in the arachidonic acid (AA) signaling cascade^[31-35] have also been described in depressed patients. Taken together, these results confirm the substantial involvement of the immune system in the mechanisms of depressive disorders, possibly via interactions between the immune system and the neuroendocrine and autonomic nervous system.^[36-40]

Starting from these observations, the role of the immune system has been investigated in other neuropsychiatric diseases, including bipolar disorder (BD), schizophrenia, anxiety disorders, and personality disorders. A chronic inflammatory state that exacerbates during symptomatic relapses has been suggested to be involved in the pathophysiology of BD.^[41] Interestingly, therapy with IFN- α has been reported several times to induce manic/hypomanic or mixed states in nonpsychiatric patients.^[42-45] One study highlighted the higher prevalence of mild excitatory symptoms (i.e. irritable mood, racing thoughts, distractibility, agitation, and insomnia) associated with anhedonia and fatigue in IFN- α treated patients, rather than typical major depressive or euphoric symptoms.^[46] Another study found that nonpsychiatric patients are more likely to develop depressive symptoms following a therapy with IFN- α if they experienced lifetime subthreshold manic/hypomanic symptoms.^[47] Similarly to what has been observed in depression, there is a high rate of comorbidity between BD and other inflammation-related medical conditions, such as diabetes, metabolic syndrome, and cardiovascular and cerebrovascular diseases,^[48-50] which led some authors to question if BD could be considered as a multi-system inflammatory illness.^[51]

Many studies conducted on BD patients have demonstrated increased levels of pro-inflammatory

markers, both in the blood and in the brain, that is, abnormal T-cell and monocyte-macrophage activation, alterations in chemokine, cytokine, prostaglandin, and acute-phase protein synthesis, aberrant inflammatory-related gene expression, increased oxidative stress markers, and microglial activation.^[28,52-67] The vast majority of studies focused on assessing peripheral cytokine levels. Despite some mixed results, globally BD patients show higher serum concentrations of TNF- α , soluble TNF-receptor 1 (sTNF-R1), IL-1 β , IL-4, soluble IL-2 receptor (sIL-2R), and sIL-6 receptor (sIL-6R) compared to healthy controls, as well as a nearly-significant trend for IL-6.^[54,68-70] These results refer to both euthymic and symptomatic BD patients and do not emphasize the possible differences in the cytokine expression patterns correlated with the presence of excitatory symptoms. We, therefore, performed an analysis of the literature aimed at highlighting the possible presence of alterations in immune system activity, specifically in relation to excitatory symptoms of BD. We also briefly examined the possible use of anti-inflammatory drugs as add-on therapies to improve clinical outcomes.

We performed a literature review through a careful search of articles using PubMed. We conducted an initial search using keywords such as “affective disorder” or “major depression” or “bipolar dis*” and “inflammat*” to provide context. Subsequent searches were performed using the following key words: “bipolar dis*” and/or “mania” or “manic” or “mixed” or “depressi*”, cross-referenced with a set of inflammation-related terms, such as “cytokine”, “IL”, “glia”, “oxidative stress”, and “AA”. To examine the role on the immune system of drugs commonly used to treat excitatory symptoms, we cross-referenced the above-mentioned set of inflammatory-related keywords with “lithium”, “mood-stabilizing agents”, and “antipsychotics”. Finally, to identify studies on the possible role of anti-inflammatory drugs in the management of affective episodes, we cross-referenced the mood-related keywords mentioned earlier with the terms “anti-inflammatory drugs”, “coxib” and “anti-TNF- α ”.

Reference lists from selected papers were subsequently searched to identify further relevant literature. We limited the search to papers written in English. There were no timeframe limitations in our searches. Data on alterations of the inflammatory system in excitatory phases of BD (mania, hypomania and mixed states) are presented together because most of the studies do not specifically differentiate between the three.

IMMUNE SYSTEM ALTERATIONS AND EXCITATORY SYMPTOMS IN AFFECTIVE DISORDERS

Inflammation in manic, hypomanic and mixed states

Cytokines are low-molecular-weight proteins secreted by immune cells, such as white blood cells and microglia, which play a crucial role in modulating the inflammatory response. In the brain, cytokines exert immune protection by promoting the elimination of damaged neurons and have also been demonstrated to influence neurogenesis and cell survival.^[71] However, dysregulation of cytokine synthesis and activity is known to lead to alterations of synaptic transmission and synaptic plasticity, neurotoxicity, and neuronal death.^[8]

An imbalance between pro- and anti-inflammatory cytokines has been suggested to exist in manic patients versus healthy controls,^[72] underlying a more pronounced shift toward a pro-inflammatory status in acute relapses. There is also evidence of an imbalance between cytokines secreted by type 1 T helper (Th1) lymphocytes and type 2 T helper (Th2) lymphocytes during mania, with an increased Th1/Th2 ratio that normalizes after treatment.^[73] IL-1 β levels are increased in the CSF of euthymic bipolar patients with at least one manic/hypomanic episode in the last year when compared with patients without a recent episode.^[74] Peripheral IL-1 β concentrations also positively correlate with suicide risk in BD patients,^[75] and suicide risk is now known to be much higher when excitatory symptoms are present during relapses.^[76,77] IL-1 receptor antagonist (IL-1Ra) serum concentrations are higher in bipolar patients both in mania and partial remission, whereas they normalize when full remission is achieved.^[78] Several studies investigating serum concentrations of IL-8, as well as IL-2, IL-6 and their soluble receptors: IL-2 receptor (sIL-2R) and IL-6 receptor (sIL-6R), found that levels are higher in manic patients than in healthy controls.^[79-81] In particular, peripheral concentrations of IL-2, IL-6, and their receptors have been found to positively correlate with the severity of symptoms and tend to normalize following treatment and during remission.^[58,72,79,80,82] A recent study by Tsai's group^[83] also showed that concentrations of serum IL-6R reflected illness activity in a BD patient with manic relapses during a 63-week observation time. These findings, though, were only partially confirmed by meta-analyses that demonstrated increased peripheral expression of sIL-2R in manic patients, with just a trend toward the higher expression of IL-6 and sIL-6R.^[69,70,84] Levels of both TNF- α and its receptor, tumor necrosis factor receptor type 1 (sTNF-R1), are increased in manic BD patients compared to healthy controls and euthymic patients.^[84] Elevated TNF- α levels observed in mania

do not normalize after treatment, suggesting that TNF- α may be considered as a trait marker of disease.^[72] This hypothesis is supported by the finding that serum TNF- α concentrations in BD patients are higher than in controls both in early (< 3 years) and late stages (> 10 years) of the disease, and that TNF- α levels are higher in late-stage than in early-stage patients.^[85] However, a study from Guloksuz *et al.*^[86] demonstrated by flow cytometry that measured levels of TNF- α are higher in lithium-treated, but not medication-free euthymic BD patients compared to healthy controls, suggesting that the persistently increased levels of TNF- α might result as an effect of lithium therapy rather than reflect a persistent pro-inflammatory inter-episodic status. It is interesting to note that TNF- α has been demonstrated to modulate inflammation and neurotransmission in brain regions regulating impulse control, like the prefrontal cortex and anterior cingulate cortex (ACC).^[87] The expression of some TNF-related genes correlates with ACC activation and aggression in BD children and adolescents.^[88] Moreover, serum TNF- α concentrations correlate with deficits in executive functioning, that is, inhibitory control, in BD patients;^[89] inhibitory control is much more impaired in manic/mixed than in euthymic or depressed BD patients.^[90] Levels of sTNF-R1 positively correlate with elevated mood, being increased in manic states compared to depression,^[91,92] and are much higher in BD-I than in BD-II patients.^[68] Plasma levels of sTNF-R1 also positively correlate with general disease gravity and psychotic features in BD patients.^[93]

The alterations in the expression of TNF- α in BD are quite intriguing since this factor is involved in many processes, such as synaptic transmission, synaptic plasticity, neurodevelopment, neurotoxicity, and regulation of neuronal survival.^[94,95] Increased expression of TNF- α during acute mood episodes is thought to shift the balance between cellular survival and cellular death toward apoptosis,^[96] therefore playing a role in the neurodegeneration observed in chronic BD patients and possibly in cognitive impairment.^[97] Furthermore, TNF- α induces central recruitment of circulating monocytes during peripheral inflammation by cerebral microglia,^[98] which in turn produces more pro-inflammatory cytokines, sustaining the inflammatory status.^[99]

C-reactive protein is a nonspecific acute-phase protein, synthesized by hepatocytes in response to IL-1 and IL-6 secretion during inflammatory processes. CRP levels in BD patients rise during both mania and depression and remain higher than in controls in partial and full remission of symptoms,^[78,83,100] suggesting a constant, nonspecific activation of immunomodulatory processes. Nevertheless, other studies have found increased levels of CRP only in manic bipolar patients and not in depressed or euthymic patients,^[101] and that

the increase in CRP levels positively correlates with the severity of manic symptoms.^[102,103]

The complement system is a part of the innate immune system. It consists of over 30 different proteins and its activation ultimately leads to a massive amplification of the immune response. Increased levels of complement factors C3, C4, and C6 have been found in BD patients during mania.^[104]

Several studies have demonstrated an abnormal activation of T-cells in bipolar patients, regardless of the phase,^[58] whereas others have found an increase in T-cell proliferation and activation during manic states that normalized after full remission.^[105,106] A recent study demonstrated an increased proportion of circulating monocytes in BD patients,^[107] and a correlation between a subcluster of monocyte proinflammatory gene (i.e. CCL2 and CCL7) expression and excitatory symptoms was found.^[108] Interestingly, total leukocyte counts are higher in mixed states than in pure manic states, and this difference appears to be due mainly to an increased number of neutrophils and monocytes.^[109]

Oxidative stress is defined as the imbalance between oxidant and anti-oxidant agents, provoking macromolecular and cellular damage and ultimately inducing impairments in neuronal survival, plasticity, and signal transmission.^[110] Alterations in the oxidative status have been found to differ within mood states in BD patients. Peripheral levels of nitric oxide (NO), a powerful oxidant agent, are higher in BD patients, especially during mania, and also positively correlate with the number of lifetime manic episodes.^[111,112] When compared with euthymic subjects or healthy controls, manic patients show higher levels of thiobarbituric acid reactive substances (TBARS) and protein carbonyl content (PCC), peripheral markers of lipid peroxidation and oxidative damage to proteins, respectively.^[113] TBARS levels normalize after treatment with mood stabilizers and anti-psychotic drugs.^[114] Levels of superoxide dismutase, a main component of the anti-oxidant defense system, are also higher in BD patients during mania, suggesting a compensatory response to increased oxidative stress.^[115] The imbalance in the oxidative state of manic BD patients is normalized by lithium treatment.^[116]

Arachidonic acid is one of the most abundant fatty acids in the brain and is a precursor in the production of prostaglandins (PG). PG are hormone-like lipid compounds, synthesized from AA by cyclooxygenase (COX) isoenzymes, and play crucial roles in the promotion of systemic inflammation.^[117] Protein and mRNA levels of COX isoform-2 (COX-2) and other AA

signaling pathway enzymes, such as phospholipase A2 (PLA2) and membrane prostaglandin E synthase, are higher in the postmortem frontal cortex of bipolar patients than in healthy controls.^[118] A recent study suggests that some of these alterations might be due to epigenetic mechanisms.^[119] Among other pro-inflammatory stimuli, COX-2 expression is induced by TNF- α ,^[120,121] the production of which has been found to be increased during manic relapses of BD as already described. Lamotrigine and valproic acid, two widely used mood stabilizing agents, decrease COX-2 expression in rat frontal cortex.^[122,123] Unlike lamotrigine,^[124] however, agents known to be effective in treating mania, such as lithium, carbamazepine, valproate, and the anti-psychotic drugs clozapine and olanzapine, also decrease AA turnover in rat brain,^[125-135] eventually modulating dopaminergic and glutamatergic transmission.^[136-138] On the other hand, the antidepressants fluoxetine and imipramine, but not bupropion, increase AA signaling and turnover in the rat brain.^[139-141] These findings are intriguing, considering that antidepressant treatment in bipolar patients often leads to a switch from a depressive to a manic/hypomanic state^[142,143] and that, among antidepressants, bupropion is the drug associated with the lowest risk of inducing switching.^[144,145] Taken together, these findings suggest that manic/hypomanic phases might be associated with a higher rate of AA signaling, an interesting hypothesis that would need more in-depth research.^[146] Novel neuroimaging techniques such as positron emission tomography with ¹¹C-labeled fatty acids might help to better clarify the AA turnover and signaling cascade in the brain of BD patients and its role in the development of symptoms.^[147]

Microglial cells are the resident macrophages of the central nervous system and play critical roles both in physiological and pathological functioning of the brain, as well as during neurodevelopment.^[148] One of the most important activities of microglial cells is to regulate inflammation within the CNS via the production of pro-inflammatory cytokines and free radicals, as well as anti-inflammatory components.^[149] Aberrant microglial cell number and function are involved in the pathophysiology of psychiatric disorders, including BD,^[28,66,150-153] possibly modulating GSK-3 β /Wnt pathway activity through neuroinflammation.^[67] However, to date, little is known about direct correlations between excitatory symptoms and glial activity.^[67] During acute manic states, alterations in neuronal/glial interactions and glutamatergic transmission have been demonstrated by proton magnetic resonance spectroscopy.^[154] Some additional evidence on this issue has been provided by animal experiments.

Mice lacking the alpha-2 isoform of Na⁺/K⁺-ATPase, also known as the sodium pump, show some manic-like behavior (i.e. hyperlocomotion), and hyperlocomotion is prevented by pretreatment with lithium.^[155] Na⁺/K⁺-ATPase inhibitors, such as ouabain, have been extensively used in animal experiments to model BP.^[156] The alpha-2 isoform is expressed exclusively in glial cells^[157] and is reduced in postmortem temporal cortex of BD patients.^[158]

Inflammation and excitatory symptoms in depression

Markers of increased immune-inflammatory activity have been demonstrated in patients diagnosed with the major depressive disorder or unipolar depression (UD). These findings have been further confirmed by meta-analyses,^[159,160] but the studies included in the research, if considered individually, did not present homogeneous results. This might be explained by methodological differences in conducting the studies and/or by a variety of confounding factors, one of which might be the presence of some subthreshold excitatory symptoms in depressed patients such as mood lability, inner tension, irritability, racing and crowded thoughts, talkativeness, sleep disturbances, and psychomotor agitation.^[161-163] These symptoms are often misidentified in clinical practice,^[164-166] despite being present in around 40% of patients diagnosed with depression.^[167,168] In addition, about 20% of subjects initially diagnosed with the major depressive disorder and without lifetime manic symptoms develop excitatory features during the course of their disease.^[169]

A recent study found significantly elevated baseline levels of CRP in patients diagnosed with UD that later developed excitatory symptoms compared to unipolar depressed patients who did not show manic symptoms over two years follow-up.^[170] A similar, but nonsignificant, trend toward higher levels of IL-6 and TNF- α was also observed.^[170] Both the presence of excitatory symptoms during the depression and high levels of serum cytokines before treatment are associated with a more severe course of the disease and poor response to antidepressants.^[171-176] It could be therefore hypothesized that an increased inflammatory status might be responsible, at least in part, for this evidence.

Excitatory symptoms are twice as common in bipolar depression than in UD,^[165] and patients diagnosed with major depression that also show psychomotor agitation are nearly three times more likely to undergo mood-switching than depressed patients without excitatory symptoms.^[177] This is consistent with the theory that agitated depression should be

re-conceptualized as an “attenuated mixed state” belonging to bipolar-spectrum disorders.^[171]

Until date, few studies have specifically focused on immune alterations during the bipolar depression. Higher levels of IL-1 β , IL-2, IL-6, IL-8, IL-10, TNF- α , high-sensitivity CRP (hs-CRP), sIL-2R, sIL-6R, sTNF-R1, and IL-1Ra have been found in serum and/or plasma of depressed BD patients,^[79,81,100,178] although these findings were not completely confirmed in meta-analyses.^[69,70,84] Interestingly, some of these markers (i.e. sIL-R2, TNF- α , and sTNF-R1) appear to be elevated during manic/hypomanic phases as well.^[41] Depressed BD patients also show alterations in oxidative stress markers and glial activity.^[179]

Few studies have compared levels of inflammatory markers between BD and UD patients, and the results were mostly nonsignificant. However, a recent work from Bai *et al.*^[54] found that bipolar patients show higher serum levels of sIL-6R, CRP, sTNF-R1, and monocyte chemotactic protein-1 (MCP1) than patients with different subtypes of UD, hinting that dysregulation of the immune system is more severe in BD than in UD.

Inflammation in BD prodromes

Excitatory symptoms, although quite nonspecific, are also frequent during the prodromal stages of BD in adolescents.^[180] These symptoms include mood swings, hyperactivity, sleep disturbances, irritability and aggressiveness, and anxiety.^[181,182] Cytokines are thought to interact with adrenal and gonadal hormones during adolescence, therefore influencing neurodevelopment and contributing to subsequent onset of psychiatric diseases;^[183] a role for a preexisting pro-inflammatory status in adolescents with high-risk of developing BD has been suggested.^[184] Recently, a prospective study demonstrated alterations in the immune state, such as increased inflammatory gene expression in monocytes during adolescence and increased levels of chemokine ligand 2 (CCL2, also known as MCP1), a marker of monocyte activation and migration, during young adulthood in the offspring of BD patients.^[185]

Inflammation in postpartum psychosis

There's a general consensus that postpartum psychosis may often occur as a first episode of BD.^[186,187] Pregnancy in itself is considered a period of great modifications in the function of the immune system and immune activation has been observed during the postpartum period.^[188,189] A recent study found reduced levels of T-cells, increased levels of monocytes, and increased expression of monocyte genes in patients with first-onset postpartum psychosis.^[190]

EFFECT OF PHARMACOLOGICAL TREATMENT ON INFLAMMATORY MARKERS IN BD

Mood stabilizing agents commonly used in the therapy of BD have been suggested to partially exert their activity via the regulation of the immune system and oxidative stress pathways.^[191] A number of studies have provided evidence supporting anti-inflammatory effects of lithium via different mechanisms.^[192,193] Lithium decreases the synthesis of pro-inflammatory enzymes and molecules (i.e. IL-1 β , TNF- α , PG, NO, iNOS, COX-2 and PLA2), and regulates microglial activity *in vitro*.^[194-199] Similarly, lithium therapy shows some immunoregulatory activity in bipolar patients. It has been demonstrated to decrease the number and the activity of inflammatory cytokine-producing cells in BD patients^[197,200] and to reduce the synthesis of Th1 cytokines.^[201] Lithium also normalizes elevated levels of sIL-2R and sIL-26R in rapid-cycling BD patients.^[202]

As mentioned earlier, valproic acid down-regulates the AA signaling cascade by inhibiting the synthesis of COX-1 and COX-2 in the rat brain.^[203] In addition, valproate and other antiepileptic drugs commonly used as mood-stabilizing agents, namely carbamazepine, lamotrigine, oxcarbazepine, and topiramate, significantly reduces the synthesis of a number of cytokines *in vitro*.^[80,204,205] Anti-psychotic drugs such as clozapine, quetiapine, risperidone, and ziprasidone also show some immunoregulatory effect by modulating the AA signaling cascade, cytokine and acute-phase protein synthesis, and microglial activation both *in vitro* and *in vivo*.^[80,129,131,206-217]

FUTURE DIRECTIONS

Because of the converging evidence pointing to inflammatory dysregulation in the pathophysiology of psychiatric diseases, drugs specifically modulating the inflammatory response, such as acetylsalicylic acid and other nonsteroidal anti-inflammatory drugs (NSAIDs), omega-3 fatty acids, anti-TNF- α agents, minocycline, and N-acetyl cysteine (NAC) have been investigated as new therapeutic options, with still controversial results.^[3,218-221] In particular, a randomized study proved the efficacy of adjunctive therapy with celecoxib, a nonsteroidal anti-inflammatory drug and a selective inhibitor of COX-2, in rapidly improving depressive symptoms in depression and mixed states of BDI and BDII patients.^[222] Another study found elevated levels of gene transcripts for prostaglandin D synthetase and prostaglandin D2 11-ketoreductase during depressive episodes in rapid-cycling BD patients; add-on treatment with celecoxib improved the

severity of both depressive and manic/hypomanic phases, although more pronounced benefits were noted during depression.^[223]

Tumor necrosis factor- α has been suggested to play a major role in mechanisms related to inflammation, neurodegeneration, and possibly neuroprogression of the disease in BD. Another study by Guloksuz *et al.*^[224] demonstrated a correlation between higher levels of TNF- α and a poor response to lithium treatment in BD patients. According to this evidence, TNF- α could be considered as a potential new target for the development of new drugs for BD therapy.^[96,225] Antagonism of IL-6 has also been hypothesized to be a novel therapeutic option to improve clinical outcome in BD.^[226]

CONCLUSION

The literature reviewed provides evidence for a role of the inflammatory system in the pathophysiology of mood disorders. Nevertheless, a high rate of variability is observed among the different studies, especially those focused on evaluating the peripheral expression of inflammatory markers. There is a general consensus that BD patients show higher levels of cytokines in blood samples compared to healthy controls; however, data are inconsistent and comparisons between peripheral levels of inflammatory markers in manic/hypomanic/mixed versus euthymic or depressed BD patients fail to converge to univocal conclusions. This may be explained by a number of reasons. First, studies differ in their methodology; some of them by assessing the expression of cytokines in serum, others in plasma, and yet others by evaluating cytokine production by *in vitro* stimulation of white blood cells from BD patients. Second, peripheral cytokine levels are influenced by several confounding factors, such as smoking status, body mass index, sleep disturbances, physical activity, and medications. Third, BD is highly heterogeneous in its manifestations so that a thorough selection of patients and classification of their mood state might be difficult. Finally, not all studies take into account factors such as age at onset and duration of illness, number of relapses, polarity of the last relapse, and the time intercurring from the last episode, which might be of importance in modifying the inflammatory status.

An enhanced pro-inflammatory status might partially explain the high rate of medical conditions often comorbid with BD, that is, cardiovascular, cerebrovascular, and metabolic diseases. Similarly, smoking, sleep impairment, and alcohol and substance abuse, the prevalence of which is high in BD patients, might contribute to the maintenance of a pro-inflammatory

milieu. It is known that chronic inflammation induces a number of negative consequences in the brain, such as a high rate of tissue damage and structural changes in several areas, which in turn underlie functional impairments in neurotransmission. These alterations underpin some kind of both neuroanatomical and neurophysiological "vulnerability" in BD and represent the biological substrate for further relapses and progression of the illness.

Clinical observations indicate that BD is, in fact, a progressive disease, with many recurrences leading to more and more frequent and severe relapses, and associated with a reduction of inter-episode duration time, cognitive decline, and a worsening of the response to treatment, both pharmacological and psychotherapeutic.^[227-232] This ongoing process is likely to be exacerbated during acute phases of the illness, especially excitatory phases, and might be due, at least in part, to a stronger activation of the inflammatory system.^[233,234] Drugs modulating the immune system, or specifically some of its components, currently represent a promising field of investigation toward the development of add-on therapies aimed to achieve better clinical outcomes in the treatment of BD.

Financial support and sponsorship

This study was part of the FIRB project code RBFR12LD0W_002 and has been funded by a grant of the Italian Ministry of Research.

Conflicts of interest

There are no conflicts of interest.

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Psychotic and nonpsychotic mood disorders in autoimmune encephalitis: diagnostic issues and research implications

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ABSTRACT

Recent research on autoimmune disorders suggests additional links between systemic and central nervous system (CNS) pathophysiology, among which the identification of antibody-induced limbic encephalitis provided the strongest evidence for the potential involvement of autoimmunity in the pathogenesis of severe mood and psychotic symptoms. In these illnesses, psychiatric symptoms predominate in the initial phase of the disorder in up to 70% of the cases, and they often lead patients to early psychiatric evaluation. For this reason, it is very important to increase the limited knowledge among psychiatrists about these autoimmune neuropsychiatric diseases, which can mimic psychiatric syndromes, in particular, those typically presented in severe mood disorders and schizophrenia. On the other hand, similarities in clinical presentation suggest that neuroinflammation and systemic immune dysregulation may play a role in the pathophysiology of severe mood and psychotic disorders. A complex interaction between periphery and immune cells of the CNS may result in cellular damage through mechanisms involving excitotoxicity, oxidative stress, and mitochondrial dysfunction. These pathways are possibly shared between comorbid medical disorders and severe mood and psychotic disorders and may reflect common underlying vulnerability.

Key words: Autoimmune encephalitis, mood disorders, psychosis

INTRODUCTION

The connection between autoimmunity and neuropsychiatric symptoms has long been acknowledged, and William Osler provided a description of psychosis in systemic lupus erythematosus in 1895. The myasthenic syndromes are good examples of how autoantibodies can cause neurological symptoms.^[1] As another example, some paraneoplastic syndromes such as cerebellar degeneration or limbic encephalitis (LE) are associated with highly specific antibodies against intracellular neuronal proteins and aggressive cytotoxic T-cell responses that usually lead to irreversible deficits.^[2]

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In recent times, the discovery of a range of autoantibodies acting on specific synaptic sites in the brain has been an important development for the identification of different forms autoimmune encephalitis, often characterized by the initial psychiatric presentation. The predominance of a psychopathological expression often leads patients to early psychiatric evaluation and treatment.^[3] As a result, in many cases, the correct diagnosis may be delayed because of the limited knowledge among psychiatrists about these autoimmune neuropsychiatric diseases mimicking psychiatric syndromes, in particular, severe mood disorders and schizophrenia.^[4-6] Moreover, the fact that a variety of neuropsychiatric disorders may initially present with

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Cite this article as: Quaranta G, Bucci N, Toni C, Perugi G. Psychotic and nonpsychotic mood disorders in autoimmune encephalitis: diagnostic issues and research implications. *Neuroimmunol Neuroinflammation* 2015;2:228-36.

Received: 12-03-2015; **Accepted:** 13-04-2015

Access this article online

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www.nnjournal.net

DOI:
10.4103/2347-8659.160986

psychiatric symptoms very similar to classical mood and psychotic disorders reinforced the hypothesis that innate inflammation/autoimmunity may be relevant to the pathogenesis of psychiatric symptoms at least in a subset of patients with bipolar disorder^[7] or schizophrenia.^[8]

AUTOIMMUNE ENCEPHALITIS

Since 1974, Mitsuda and Fukuda^[9] described “atypical psychosis” as some acute and transient psychotic disorders, which cannot be easily classified as either schizophrenia or mood disorder with psychotic features. Some important clinical characteristics of atypical psychosis include acute onset, emotional and psychomotor disturbances, alternations of consciousness, high prevalence in women, and well-adjusted premorbid personality. In these conditions, an involvement of neurologic brain changes has been hypothesized.

The identification of autoimmune encephalitis, a new category of neuropsychiatric diseases that occurs with focal or widespread involvement of the nervous system in association with antibodies against extracellular epitopes of neuronal cell-surface or synaptic proteins, have led to changed paradigms for the diagnosis and treatment for some neuropsychiatric disorders, and reclassification of syndromes previously defined as idiopathic or with descriptive terms.^[10] Since 2005, there have been 1-2 discoveries of novel syndromes and associated autoantigens per year, including autoantigens the *N*-methyl-D-aspartate receptor (NMDAR),^[11,12] the subunits Kv1.1 and Kv1.2 of the voltage-gated potassium channels (VGKCs), the α -amino-3-hydroxy-5-methyl-4-isoxazol-pro pionic acid receptor (AMPA),^[13] metabotropic glutamate receptor 5,^[14] or the γ -amino-butyric acid B-receptor (GABA_BR),^[15] leucine-rich glioma inactivated 1 (Lgi1),^[16] contactin associated protein 2 (CASPR2).^[17] The study of these disorders has revealed novel mechanisms of how antibodies might alter memory, behavior, cognition and cause mood disorders, psychosis, seizures or abnormal movements.^[18,19] However, though obtaining serum to test for autoantibodies is extremely convenient and relatively noninvasive, the caveats of using serum antibodies as a diagnostic tool need to be considered. Neuronal surface antibodies are not 100% specific.^[20-25] In particular, lower serum titers should be interpreted with caution, and the role of evaluating cerebrospinal fluid (CSF) neuronal surface antibodies rather than serum titers may increase diagnostic specificity.^[10] The presence of neuronal surface antibodies should always be correlated with the clinical picture.

One important clinical feature of autoimmune encephalitis is the strong association between autoantibody production and the presence of teratoma or other neoplasms. Comorbidities of autoimmune encephalitis with small cell lung carcinoma, neuroblastoma, ovarian carcinoma, breast carcinoma, thymoma, and testicular cancers have also been reported.^[26] These findings suggested that the autoantibody syndrome may be triggered by cross-reaction between antibodies produced in response to tumor presence and antigenically similar synaptic proteins within the central nervous system (CNS). Autoimmune encephalitis can develop, however, with or without an underlying tumor, and in the largest case series to date, no teratoma or other type of tumor was detected in 41% of cases.^[3,27]

Different forms of autoimmune encephalitis can affect patients of all ages although some of them seem to preferentially occur in late childhood and young adulthood.^[28,29] The onset of the symptomatology shows a substantial overlap among the different types. Sometimes associated with headache or mild hyperthermia, the initial clinical picture is characterized by a rapid development of a set of psychiatric and/or neurological symptoms. Mood disorders, usually manic or mixed-manic symptoms, anxiety, behavioral problems, psychotic features, mild to moderate disorders of consciousness, and memory loss occur in most types of autoimmune encephalitis, often associated with seizures. Demographic information (such as gender and race), presence or absence of a underlying tumor, brain magnetic resonance imaging (MRI) findings, CSF examination, and the severity and predominance of some symptoms over others can suggest a specific subtype.

Ultimately, two approaches have been proposed for the diagnosis of autoimmune encephalitis: one based on the laboratory examination as proposed by Lancaster and Dalmau^[10] and the other based on clinical diagnostic criteria as proposed by Zuliani *et al.*^[30] The latter recommend that one should suspect a diagnosis of an autoimmune encephalitis when a patient presents with acute or subacute onset of symptoms, evidence of inflammation supported by CSF examination, imaging, or histopathological investigations, and the exclusion of other infectious, metabolic, and toxic etiologies. Supportive criteria include a history of other autoimmune comorbidities, and preceding infectious prodromes. Using a combination of these criteria as well as test of response to therapy, they suggest a model whereby patients can be classified as having definite, probable or possible neuronal cell surface antibody related pathology.^[30,31]

Anti-NMDAR encephalitis

Anti-NMDAR encephalitis, among all the autoimmune encephalitis, is the best-understood variant and the most frequently associated with almost exclusively psychiatric presentation. The illness was first described as a distinct clinical entity in an observational study in 2005 by Vitaliani *et al.*^[11] in four young women who developed acute psychiatric symptoms, seizures, memory deficits, decreased level of consciousness, autonomic instability, and hypoventilation in association with the presence of an ovarian teratoma. Two years later, a paper by this group described the underlying pathology, mediated by auto-antibodies directed against the NR1 subunit of the NMDAR.^[12] These antibodies cause a decrease in the number of NMDARs in target cells by inducing cross-linking and internalization of NMDARs by autophagy.^[32] Therefore, anti-NMDAR encephalitis represents a state of NMDA-R hypofunction, associated to glutamate dysregulation.^[33] Initially, it was classified as a paraneoplastic syndrome^[12] due to the strong association (upwards of 60%) with a teratoma or other tumor types.

Although many patients have been diagnosed with anti-NMDAR encephalitis to date, the exact prevalence of this disorder is unknown. A study of 100 patients revealed that although most patients are young women, the disorder can occur in men and children.^[34] In fact, with increasing awareness of the syndrome, the number of pediatric cases has steadily grown and appears to represent about 40% of all cases.^[28] The younger the patient, the less likely an underlying tumor will be detected at the time of presentation.^[34]

A stereotypical clinical course with different phases is noted for patients with anti-NMDAR encephalitis.^[35] In 70% of patients, the illness begins with a prodromal phase lasting 5-14 days.^[3,36] This nonspecific flu-like prodrome is characterized by subfebrile temperature, fatigue, malaise, headache, nausea, diarrhea, vomiting. This is followed by other clinical phases, which may vary in sequence, presentation and severity.

After the initial phase, psychiatric manifestations go on to develop, including emotional and behavioral disturbances such as apathy, anxiety, panic attacks, fear, depression, decreased cognitive skills, sleep disorders. In most cases, an excited manic or mixed manic symptomatology develops in a variable lapse of time, from hours to days; mood disorders and agitation are almost invariably associated with psychotic features such as grandiose delusions, Capgras syndrome, paranoid interpretation, and different types of hallucinations. From mild to moderate cognitive disorders are frequently presented.^[18,37] During this

phase, patients are often referred to psychiatric assessment and may receive treatment with psychoactive agents or admission to psychiatric facilities.

This psychotic phase can be followed by physical decompensation involving autonomic instability (less common in children), with hypo- or hypertension, hypo- or hyperthermia, hypoventilation, cardiac arrhythmia, decreased responsiveness, and occasionally short-term memory loss. Some patients may also have seizures, most commonly generalized tonic-clonic, but also partial and/or complex type. In some patients, early treatment with antiepileptic drugs may mask seizures. Dyskinesias, extrapyramidal signs, and stereotyped motor automatisms may also be observed.^[38] During this phase, patients not already admitted to the hospital often present to the emergency department because they no longer follow verbal commands and may appear mute (with language disintegration) and akinetic. Patients may maintain gaze as if in a catatonic state, smile inappropriately, or demonstrate stereotyped athetotic movements.^[34,39]

Since the initial descriptions, further studies have expanded the clinical phenotype of this syndrome. Some patients with anti-NMDAR antibodies have predominant or isolated psychiatric features, dystonia, or epilepsy, without the classic multistage presentation, potentially representing a *forme fruste* of anti-NMDAR encephalitis, mimicking a psychiatric disorder.^[34,40]

The diagnosis of anti-NMDAR encephalitis is confirmed by the detection in serum or CSF of antibodies to the NR1 subunit of the NMDA receptor. After treatment or in advanced stages of the disease, the CSF antibodies usually remain elevated if there is no clinical improvement, whereas serum antibodies may be substantially decreased by treatments. The titer of CSF antibodies appears to correlate more closely with the clinical outcome. Patients with anti-NMDAR encephalitis may have abnormalities of both CSF and MRI. 80% of patients with confirmed anti-NMDAR encephalitis have abnormal CSF with the majority of them exhibiting a lymphocytic pleocytosis, but over a half also show raised proteins. There may also be the presence of isolated oligoclonal bands in the CSF of patients with autoimmune encephalitis (around 60%).^[3] In contrast to the consistency of the clinical picture, MRI findings are less predictable; only 55% of patients had increased fluid-attenuated inversion recovery (FLAIR) or T2 signal in one or several brain regions, without significant correlation with patients' symptoms. MRI can be normal or demonstrate medial temporal involvement or focal areas of hyperintensity in the frontal or parietal cortex. Other studies demonstrated that [18F]-fluorodeoxyglucose positron emission

tomography can show cortical hypermetabolism in acute stages and hypometabolism in more subacute stages of the illness.^[5,41] In over three-quarters of patients, EEG shows generalized or frontotemporal slow or disorganized activity without epileptic discharges.^[28] These findings may overlap with electrographic seizures.

Other autoimmune encephalitis

This group of autoimmune diseases can be defined as LE. LE was first described in the 1960s in patients with severe short-term memory impairment or dementia in association with bronchial carcinoma.^[42] Cardinal symptoms of LE are severe short-term memory impairment with psychiatric symptoms such as personality change, depression, anxiety, hallucinations, confusion, and complex partial-often temporal or classically in LGI1 encephalitis facio-brachial tonic seizures^[43] and generalized seizures.^[44] Another prominent symptom found in 40% of patients and also exhibited in mice lacking LGI1,^[16] is myoclonus.

Anti-AMPA encephalitis

AMPA antibodies (Glutamate receptors - GluR1/2) can be one of the autoimmune causes of LE. Glutamate is one of the main excitatory neurotransmitters with GluR1/2 being the predominant AMPA subtype in the hippocampus (GluR3 is associated with the distinct disorder, Rasmussen's syndrome). About 70% of patients have an underlying tumor in the lung, breast or thymus.^[13,45] These patients develop acute limbic dysfunction that can be associated with prominent psychiatric symptoms. Most patients, commonly middle-aged women, present with the subacute onset of disorientation, confusion, memory loss, and aggressive behavior. CSF findings and brain MRI are similar to those of anti-NMDAR encephalitis. The long-term prognosis depends on the controlling of the tumor and presence of coexisting symptoms related to onconeural antibodies.

Anti-γ-amino-butyric acid beta encephalitis

This encephalitis is caused by disruption of the metabotropic GABABR, and equally affects both sexes. These patients present with LE, prominent seizures, and memory dysfunction.^[46] About half of the patients had an associated tumor, either a small cell lung cancer (SCLC) or a neuroendocrine tumor of the lung.^[15] The MRI and CSF findings are similar to those in other types of LE, with unilateral or bilateral increases in medial temporal lobe FLAIR or T2 signal consistent with LE and CSF lymphocytic pleocytosis.

Anti-LGI1 encephalitis

Although previously termed anti-voltage-gated potassium channel encephalitis (anti-VGKC encephalitis), recent evidence suggests that actually

other autoantigens (LGI1 and CASPR2) are associated with this LE.

Antibody-mediated mutation and disrupted function of LGI1 (a secreted neuronal protein that interacts with presynaptic and postsynaptic receptors) have been associated with the syndrome of autosomal dominant lateral temporal lobe epilepsy,^[5,47] and have been shown to cause increased excitability, which may result in memory disturbances, confusion and seizures, with MRI findings that are usually typical of LE. LGI1 antibodies have been most recently associated with adult-onset brief, but frequent motor events which comprise unilateral upper limb jerking and ipsilateral facial grimacing, termed "faciobrachial dystonic seizures".^[43] Some patients develop hyponatremia (60%) or REM sleep-behavior disorders. Only 11% of cases are associated with a neoplasm, most commonly thymoma or SCLC.^[16] In those who do have a tumor, the encephalitis can precede the identification of the neoplasm in up to three-quarters of patients.^[48,49]

Anti-CASPR2 encephalitis

Contactin associated protein 2 is an axonal protein of the neurexin IV superfamily. Mutations in the human gene encoding CASPR2 have been associated with autism, epilepsy, obsessive-compulsive disorders, and Tourette syndrome.^[10] These patients usually develop symptoms of encephalitis, drug-refractory epilepsy, peripheral nerve hyperexcitability, or both (Morvan's syndrome or neuromyotonia).^[17,50] CNS symptoms include cognitive impairment, memory loss, hallucinations, and seizures.

MOOD AND PSYCHOTIC SYMPTOMS IN AUTOIMMUNE ENCEPHALITIS

Depending on the clinical presentation and the timing of evaluation, the psychiatric manifestations of autoimmune encephalitis can be diagnosed following DSM 5 criteria as mood disorder with psychotic and or catatonic features due to a general medical condition (GMC), but also as psychotic disorder or delirium due to GMC, where the GMC is autoimmune encephalitis. In many patients, there is no previous personal or family history of psychiatric disorders. The psychiatric presentation looks like a delirious mania or acute confusional state that develops over a short period of time, fluctuates over the course of the day and manifests with changes in cognition, affect, behavior, and perception. Initially, the features may be subtle, but over days to weeks, there may be dramatic worsening, culminating in an unresponsive and catatonic state.^[51]

Patients may present labile mood, with a wide range of emotions including anxiety, low or euphoric mood,

irritability, apathy or changes in behavior including agitation/restlessness alternating with episodes of somnolence.

A case of anti-NMDAR encephalitis mimicking bipolar disorder has been described.^[52] More recently Steiner *et al.*^[53] examined patients with schizophrenia ($n = 121$), major depressive disorder ($n = 70$), and borderline personality disorder ($n = 38$) and found that 9.9% of the patients diagnosed with schizophrenia had NMDAR-R antibodies in their serum compared with 2.8% of the depressed patients and 0% of the borderline personality disorder. Interestingly, patients with schizophrenia, who were seropositive, had immunoglobulin G antibodies not only directed against the NR1a subunit of the NMDAR but also against the NR1a/NR2b subunit again raising the question about the level of involvement the immune system might have in the pathogenesis of some forms of psychotic disorders.

Perceptual disturbances are also common, including misinterpretations, illusions or hallucinations (visual and auditory) and delusions (often pertaining to hallucinations). During the unresponsive phase, patients have been referred to as catatonic with features involving purposeless motor activity, extreme negativism, bizarre posturing, grimacing, mutism, echolalia, and echopraxia.

It has been hypothesized that in a proportion of patients diagnosed as bipolar or psychotic disorders, autoantibodies may be present during an earlier developmental period resulting in a gradual and chronic exposure to NMDA-R hypofunction.^[54] Because of the similarities in symptom presentation, the possible role of autoimmunity in bipolar- and psychotic-spectrum disorders seems to represent a promising direction for future research.

AUTOIMMUNE DISEASES AND PSYCHOTIC AND NON PSYCHOTIC MOOD DISORDERS

Many evidences suggest a role of inflammatory mediators and immune dysregulations in the pathogenesis of psychiatric disorders such as BD,^[7,55,56] schizophrenia,^[8] depression,^[57] and Alzheimer's disease.^[58]

Autoimmune diseases, as well as BD and schizophrenia, are multifactorial disorders related to an interaction between gene and environment.^[59] A familial occurrence is commonly found.^[60] Moreover, autoimmune reactions often advance much more slowly than immune reactions to pathogens, suggesting that control mechanisms can continue to work until a threshold has been exceeded leading to a progression from subclinical to clinically significant symptomatology

as in mood and psychotic disorders. In addition, in autoimmune diseases, the mechanisms of control may temporarily restore the antigenic tolerance, leading to a cyclical pattern of exacerbation and remission of the disease. These general pathophysiological mechanisms of autoimmunity lead to common clinical features, including familial occurrence, progression from subclinical to the clinical level of the symptomatology, and the exacerbation-remission periodic course. Interestingly, both affective and nonaffective psychosis have been definitively shown to possess all these clinical features.

In a recent study by our group,^[61] on a clinical sample of 347 BD patients, we found a very high prevalence (48.1%) of AAD. The result is particularly interesting for two reasons: first, for the disproportion with the prevalence observed in the general population (in our country the estimated prevalence in general population is about 3.2% for autoimmune diseases and about 20-30% for allergic conditions);^[62,63] second, for the lack of a difference in gender distribution, because AAD are usually more represented in women.

The association between AAD and BD has rarely been systematically investigated in clinical samples. An increased prevalence of mood symptoms has been found in a variety of inflammatory conditions, including auto-immune diseases, cardiovascular diseases, diabetes, obesity, and metabolic syndrome, as well as in more benign inflammatory conditions such as asthma and allergies.^[64] A large Danish cohort study showed that a history of Guillain-Barre syndrome, Crohn's disease, and autoimmune hepatitis was associated with raised risk of BD.^[56] The authors concluded that autoimmune processes precede the onset of BD. A subsequent study, based on Danish hospital data, reported that a broad range of autoimmune diseases and infections requiring hospitalization increase the risk of developing schizophrenia and mood disorders.^[65] The observed associations support a possible immunological contribution in subgroups of patients with severe mental disorders, such as bipolar and other psychotic disorders. However, whether it is a causal relationship or an epiphenomenon due to other environmental factors or common genetic vulnerability remains to be clarified and deserve further research. Genetically vulnerable individuals might be at a particular risk of developing mood disorders as a consequence of autoimmune reactions and inflammation affecting the brain.

Inflammatory mechanisms can affect the brain through many different pathways that are not necessarily mutually exclusive.^[66-68] Peripheral inflammation can affect the brain without passing the blood-CNS

barrier by proinflammatory cytokines activating the tryptophan-kynurenine pathway, regulating serotonin production together with NMDA and glutamate receptor activity and may also indirectly affect dopamine regulation.^[69] The increased inflammation in autoimmune diseases may also influence the brain through increased permeability of the blood-CNS barriers, making the brain vulnerable to immune components, such as cytokines and auto-antibodies. Furthermore, brain-reactive antibodies can induce a range of psychiatric and neurological symptoms, as observed in association with antibody-induced LE.^[12]

More controversial and less explored by the literature is the association we found between AAD comorbidity, generalized anxiety, and cyclothymic-anxious temperament.^[61] This finding suggests a possible intriguing relationship between autoimmune-mediated inflammatory process, trait emotional reactivity, and stress vulnerability.

Although the link between stress and mood disorders is well-recognized,^[70] there are no specific studies examining the role of stress, immune dysregulation, and autoimmunity in BD. Retrospective studies in autoimmune diseases have found that up to 80% of patients report uncommon emotional stress before disease onset,^[71] and it has been suggested that immune system activation may vary across affective states in BD patients.^[72] In a more theoretical perspective, it is possible to hypothesize a “constitutional” reinforcing loop between emotional/mood reactivity and autoimmune/allergic reactivity that characterizes the “usual” self of these individuals and influence the entire span of their existence.

Interestingly, in our sample, the lifetime prevalence of cancer and neoplastic diseases was very low, involving less than 1% of the patients. Recent data suggested an association between certain diagnosis and a lower-than-expected probability (negative comorbidity) of developing other disorders.^[73] It has been recently suggested that the immune system and other regulatory systems, particularly, the peripheral nervous system, convey signals from tumor cells to the brain that might play a part in tumor progression and metastases, through sympathetic and parasympathetic nerves, and by the modulation of the hypothalamic-pituitary-adrenal axis and adrenal medulla activity.^[74] The majority of studies have focused on cancer and schizophrenia;^[75] there are few data regarding patients with BD.^[76] Although individuals with Schizophrenia and BD are exposed to more environmental noxious agents that contribute to tumor development (e.g. tobacco

and alcohol) than the general population, results of several population-based studies indicate that patients with schizophrenia have a lower risk of prostate, colorectal cancers, and melanoma but a higher risk of breast and lung cancer. The increased risk for certain tumors could be associated with diverse and non mutually exclusive factors such as drug side-effects, unhealthy lifestyle, poor access to health care and differences in socioeconomic status. On the other hand, the reduced risk of other types of cancer may be explained by genetic factors, such as the increased expression of various candidate tumor suppressor genes, or the decreased expression of some oncogenes in genomic regions implicated in schizophrenia or BD susceptibility.^[77,78] An alternative explanation may be related to the possibility of an increased immune reactivity in patients with BD and/or other mental disorders.^[7] During the process of carcinogenesis, in fact, naturally occurring antibody responses to tumor antigens were found to be associated with improved survival and protection against the spread of cancer.^[79] In recent trials, on cancer immunotherapy, clinically significant antitumor responses were often associated with the induction of autoimmune toxicity.^[80] This finding suggests that the same immune mechanisms that elicit autoimmunity may also contribute to the destruction of tumors.

CONCLUSION

Neuroinflammation and peripheral immune dysregulation may play a role in the pathophysiology of severe mood and psychotic disorders. Recent research on autoimmune disorders provides additional links between systemic and CNS pathophysiology.^[7] This involves a complex interaction between immune cells of the CNS and periphery resulting in cellular damage through mechanisms involving excitotoxicity, oxidative stress, and mitochondrial dysfunction.^[7] These pathways are possibly shared between comorbid medical disorders and severe mood and psychotic disorders and may reflect common underlying vulnerabilities.

Some of the strongest evidence for the potential of autoimmunity and immune components to cause psychiatric symptoms comes from the identification of antibody-induced LE, where psychiatric symptoms are often dominant in the initial and the remission phase of the disorder in up to 70% of the cases,^[12,34] and which has been demonstrated to be treatable with immune therapies.^[81] The prevalently psychiatric presentation of some of these autoimmune limbic encephalitis often leads patients to early psychiatric evaluation. For this reason, it is very important to increase the

limited knowledge among psychiatrists about these autoimmune neuropsychiatric diseases mimicking psychiatric syndromes, in particular, severe mood disorders and schizophrenia.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

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The role of anti-glutamic acid decarboxylase autoantibodies in mood disorders

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ABSTRACT

Gamma-aminobutyric acid (GABA) possibly plays a causative role in mood disorders. This hypothesis originated with studies on the beneficial effect of valproate in mania and as a mood stabilizer. Since valproate is known for its action in increasing the level of GABA, it was indirectly suggested that decreasing levels of GABA were responsible for mood alterations. To identify factors causing the decreased levels of GABA, studies have concentrated on the activity of the enzyme L-glutamic acid decarboxylase (GAD), which catalyzes the transformation of glutamate to GABA, as a decreasing function of this enzyme induces lower levels of the neurotransmitter. Moreover, a very limited amount of research investigated the possible role of glutamic acid decarboxylase antibodies (GADA) in determining a decreased enzymatic function of GAD. If these findings are confirmed, it will be possible to improve diagnosis and treatment of mood disorders. In addition, if the presence of GADA is associated with a genetic trait, this would allow and facilitate early diagnoses.

Key words: Autoantibodies, bipolar disorder, gamma-aminobutyric acid, glutamate, L-glutamic-acid decarboxylase antibodies, mood disorders

INTRODUCTION

Mood disorders (MDs) are a relatively heterogeneous spectrum of psychiatric conditions. Differences in clinical course (single or recurrent episodes), severity and frequency of mood episodes, and population prevalence may characterize each syndrome [major depressive disorder (MDD), bipolar disorder (BD), cyclothymic disorder, dysthymia] within this broad nosological definition. These disorders generally have a substantial burden on the life of patients as well as on the public health systems.^[1,2] In fact, they have been increasingly recognized as leading causes of the worldwide burden of disease and disability.^[3]

Despite the recent substantial progress in unraveling the complex biological underpinnings of MDs,^[4] in which several biological pathways have been implicated,^[5-7] the pathophysiological mechanisms underlying these conditions are still unclear. Among these, it has been hypothesized that the gamma-aminobutyric acid (GABA) pathway takes the major role.^[8] Specifically, a low GABAergic function might be associated with the biological disruption leading to clinical symptomatology. Furthermore, specific alterations of the GABAergic molecular pathway might be present in patients manifesting distinct symptoms. One of these possible alterations may involve the role of autoantibodies for the L-glutamic acid decarboxylase (GAD), a key enzyme responsible of the synthesis of GABA.

We reviewed the limited research on the mechanisms responsible for the decreased GABAergic function in

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DOI:
10.4103/2347-8659.167300

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Cite this article as: Liguori M, Manchia M, Tondo L. The role of anti-glutamic acid decarboxylase autoantibodies in mood disorders. *Neuroimmunol Neuroinflammation* 2015;2:237-43.

Received: 12-03-2015; **Accepted:** 26-05-2015

MDs using the following key words: “gamma-amino butyric acid” OR “GABA” OR “L-glutamic-acid decarboxylase” OR “GAD” OR “autoantibodies” OR “GADA” AND “mood disorder” OR “major depressive disorder” OR “bipolar disorder” OR “depression”. We found more than 200 publications and we selected all those with pertaining information on the association of any MD and abnormalities in the GABA pathway.

The aim of this review was to focus on: (1) reviewing briefly on the role of GABAergic pathway in MDs; (2) describing the molecular functions of GAD; (3) discussing the specific role of GAD as an important factor in the pathophysiology of MDs; and (4) providing future directions for the implementation of glutamic acid decarboxylase autoantibodies (GADA) screening in clinical practice.

GABA IN MDs

GABA is an inhibitory neurotransmitter present exclusively in the central nervous system (CNS). In a pivotal case series of 4 patients reported by Emrich *et al.*,^[9] these authors demonstrated a marked mood-stabilizing effect of valproic acid (VPA) in the management of acute manic episodes as well as in the maintenance treatment of other 7 patients with recurrent episodes of manic or manic-schizoaffective psychosis, irresponsive to lithium prophylaxis. The beneficial effect of VPA suggested a role of the GABAergic pathway in MDD since this anticonvulsant leads to increased cerebral concentrations of GABA by inhibiting GABA-transaminase which degrades GABA and by facilitating the reuptake of released GABA into cells. VPA also stimulates synthesis of GABA by increasing the activity of glutamic acid decarboxylase.^[10] Subsequently, Emrich *et al.*^[9] discovered a lack of GABA in the CNS of mood disorder patients, which was restored by VPA, hypothesizing that modifications of this neurobiological pathway may be associated with MDs. A more recent study has suggested that in general, GABAergic anticonvulsants possess antimanic properties and that the specific antimanic effect of lithium is associated with an increased action of GABA.^[11] Furthermore, the same authors^[11] suggested that the increased inhibitory neurotransmission induced by long-term lithium treatment counteracts the increased excitatory neurotransmission resulting from elevated levels of glutamate (GLU) which was detected in postmortem brain tissue of BD patients. Following the same line of investigation, GABA plasma level may represent a biological trait marker for MDD. Indeed, Petty and Schlessler^[12] found significantly decreased GABA plasma levels, compared to healthy controls, in 40% of depressed patients, but higher levels of plasma

GABA were found in manic BD individuals. Moreover, they observed that patients with different types of depressions, particularly those with familial loading, had plasma GABA levels significantly lower than control groups. Instead, GABA plasma levels in patients with reactive or bipolar depression did not differ from those of controls.^[12] As a result, it has been proposed that plasma levels of GABA might be a useful marker to predict the susceptibility to a depressive disorder in people with a family history of MDs. Furthermore, plasma levels of GABA may be specific and predictive of response to treatment,^[13] although GABA plasma level appears to show a low sensitivity as a test for depression.

Several other studies showed a decreased concentration of GABA in cerebrospinal fluid (CSF) of patients with severe depressive disorder.^[14-17] In particular, MDD patients over 40 years of age had significantly lower CSF levels of GABA than younger subjects.^[17] In addition, GABA levels in CSF of patients with depression and schizoaffective disorder are lower than those with schizophrenia or neurological conditions,^[16] Parkinson's disease, Huntington's disease, and dementia, all conditions which present at times depressive features.^[18] Of note, free GABA levels in CSF were lower in depressive disorders than in BD manic patients or healthy subjects.^[16,17] In addition to the association of GABA levels with depressive disorders, low levels of GABA have been also found in anxiety disorders^[19,20] and chronic migraine,^[21] which is often comorbid with MDD. In a recent study, Mann *et al.*^[20] found an inverse correlation between psychic anxiety severity and free GABA levels in CSF, independently of age. Interestingly, benzodiazepines, the most used anti-anxiety agents, increased GABA synthesis in the CNS.^[22]

Other proofs of the association between plasma GABA levels and depressive disorders may derive from the effect of electroconvulsive therapy (ECT) on severe refractory depression, since this treatment has been associated with a down-regulation of the GLU/GABA ratio (i.e. an increase in GABA and a decrease of GLU levels) in the hippocampus of rats.^[23,24] In fact, this measure of the GABAergic tone appears to be more informative than single neurotransmitter levels, given that GLU (a precursor of GABA) and GABA exert their effects in a neuromodulatory conjunction.^[25] Similar findings were observed in humans. GABA concentrations measured with proton magnetic resonance spectroscopy were significantly elevated in the occipital cortex of depressed patients following ECT.^[26] The increased levels of GABA in association with ECT may explain its antidepressant actions. In addition, increased GABA concentrations in the

occipital cortex were also found during treatment of MDD with serotonergic antidepressants.^[13]

Significantly lower levels of GABA were observed in the anterior cingulate cortex (ACC) of adolescents with MDD compared with healthy subjects.^[27] Levels were measured by the means of proton magnetic resonance spectroscopy and expressed as ratios to unsuppressed voxel tissue water (VTW). In this age group, significant differences were found for the ratio GABA/VTW in the ACC of adolescents with and without anhedonia but not in those nonanhedonic compared to healthy controls.^[27]

GABA PATHWAY-FOCUS ON GAD

GABA is synthesized from GLU via decarboxylation by GAD, a pyridoxal phosphate-dependent enzyme.^[28] Studies of GAD began in the early 50s with the work of Roberts and Frankel,^[29,30] and Awapara^[31] who independently discovered that GABA is synthesized in GABAergic neurons in CNS. However, GAD and GABA were also detected in the pancreas where the latter is stored in synaptic-like vesicles in islet beta cells.^[32-34] GAD exists in two isoforms: one of a molecular size of 65 kDa is termed GAD₆₅, whilst the other, of 67 kDa, is termed GAD₆₇. Each of them plays a distinct role: GAD₆₅ is the product of a gene located on chromosome 10 whereas GAD₆₇ gene is on chromosome 2.^[35-37] These two proteins appear to be essential for maintaining homeostasis and viability of complex organisms. Studies carried out in GAD₆₇ knockout mice showed a reduction in the levels of GABA and died at the birth of a severe cleft palate.^[38] Instead GAD₆₅ knockout mice presented normal basal levels of GABA and appear normal at birth but developed fatal seizures and anxiety-like phenotypes.^[39] The two isoforms were localized in different neuronal compartments. GAD₆₅ lies primarily in axon terminals and synthesizes GABA for neuronal transmission. GAD₆₇ is widely distributed throughout the neuron for the synthesis of GABA for general metabolic activity.^[35,36,40]

GAD IN MDs

A substantial amount of evidence has suggested a role of GAD in MDs. Karolewicz *et al.*^[41] measured the levels of GAD₆₅ and GAD₆₇ in postmortem brain samples from the gray matter of left dorsolateral prefrontal cortex (DLPFC, Brodmann's Area 9) of MDD patients, treated and untreated with antidepressants. GAD₆₅ and GAD₆₇ were reduced in antidepressant-free MDD subjects compared to matched controls. This reduction was not present in MDD patients

medicated with antidepressants at the time of death, suggesting that GAD₆₅ and GAD₆₇ might play a role in depressive syndromes.^[41] Fatemi *et al.*^[42] investigated the cerebellar levels of Reelin 410, 330 and 180 kDa, GAD₆₅, and GAD₆₇ in subjects with BD, schizophrenia, MDD, and controls using the well-characterized Stanley Brain Consortium Collection. They found a reduction in levels of GAD₆₅ and GAD₆₇ proteins in all psychiatric subjects. These findings could explain increased blood and CSF GLU and glutamine levels in schizophrenic,^[43,44] and depressed subjects,^[45] probably due to an accumulation of these two precursor compounds of GABA. Indeed, normal production of GAD₆₅ and GAD₆₇, as well as of Reelin 410, 330 and 180 kDa, reflects normal GABAergic cell function in several parts of the brain including cerebellum.^[46] Of note, Reelin, a protein responsible for correct lamination of the CNS during the embryonic period, may be involved in the etiology of schizophrenia, BD, and autism.^[47-54] Remarkably, deficits in CNS levels of Reelin can affect memory processing, learning, synaptic organization, and cognition in the adult brain.^[55]

Interestingly, GAD₆₇ appeared to be a promising biomarker for BD and schizophrenia since it discriminated these illnesses among a number of psychiatric conditions with relatively high specificity and sensitivity.^[56] Moreover, decreased levels of GAD were found in the postmortem left DLPFC of BD patients compared with MDD individuals and controls, giving rise to the possibility of differentiating the brain areas involved in unipolar and bipolar depression. Heckers and colleagues first made the description of the distribution of GAD₆₅ and GAD₆₇ mRNA-positive neurons in the human hippocampus.^[57] They found that abnormalities of hippocampal GAD expression are more prominent in BD than in schizophrenia, whereas another study showed a larger reduction of GAD₆₇ mRNA-containing neurons in BD patients than in those with schizophrenia.^[58] Of interest is the evidence that decreased GAD₆₇ expression leads to a reduction of levels of GABA, with a net effect of a reduced GABAergic inhibitory control over glutamatergic cells. Therefore, it has been hypothesized that GAD₆₇ levels could be a surrogate marker for psychosis liability^[59] and pharmacological agents that raise GAD₆₇ expression levels could represent novel targets for antipsychotic therapy.^[59] Taken together, these findings demonstrate that GAD might have a role in modulating the psychopathological presentation in a distinct subset of patients with MDs, possibly those with more prominent mood-congruent or incongruent psychotic features.

GADA IN MDs

As we have previously described, MDs may be associated with low levels of GABA following a decreased activity of GAD. It would, therefore, be reasonable to assume that its antibody, GADA, can somehow be involved in the pathogenesis of MD inhibiting GABAergic function. Support to this hypothesis came from research on the Stiff Person syndrome (SPS). In 1956, Moersch and Woltman^[60] observed this syndrome in 14 patients over the age of 35 years, characterized by fluctuating rigidity and spasms without pyramidal tract dysfunction, or any other known neurologic disorders that could explain the stiffness. Moreover, Levy *et al.*^[61] observed muscular rigidity and episodic spasms superimposed on the rigidity in 20 consecutive patients. They reported as the hallmark sign the continuous contraction of the agonist and antagonist muscles in the trunk that caused hyperlordosis and respiratory problems. Interestingly, several patients initially received a tentative diagnosis of the psychogenic process because their presentation was dominated by task-specific phobias and their stiffness was precipitated by unexpected noises or mental anticipation. In addition, seizures were observed in 10% of cases. Anxious and depressive symptoms in SPS can be explained by alterations in GABAergic neurotransmission. It was demonstrated that stiffness was caused by a reduction of GABA or glycine, the two main inhibitory neurotransmitters and was improved by drugs increasing brain levels of GABA, such as diazepam or VPA.^[62] It was found later that up to 65% of patients may have antibodies GADA against both GAD₆₅ and GAD₆₇:GAD₆₅ and GAD₆₄.^[62,63] In contrast, patients with type-1 diabetes had anti-GAD antibody titers 50 times lower than those of patients with SPS. The epitope of the GAD antigen may also differ between patients with type-1 diabetes and those with the SPS.^[64] These autoantibodies cause a functional impairment in the synthesis of GABA in persons with SPS; therefore, GADA should be considered to play a pathogenic role in this disease. In this context, it is noteworthy that patients with type-1 diabetes mellitus, particularly those with poor glycemic control, often undergo CNS related changes with low cognitive performance and depression. Short-term treatment of depression in patients with diabetes improves their dysphoria and other signs and symptoms of depression.^[65] In Batten disease, a rare genetic neurodegenerative disorder characterized by severe mental impairment, Chattopadhyay *et al.*^[66] studied a mouse model reporting the presence of an autoantibody to GAD₆₅. These authors hypothesized that an autoimmune response to GAD₆₅ may contribute to a preferential loss of GABAergic neurons associated with Batten disease.^[66] Several groups observed

an increased prevalence of autoimmune disease and/or autoantibodies in patients with BD, including autoimmune thyroiditis and autoimmune atrophic gastritis. More specifically, Padmos *et al.*^[67] studied 239 patients with DSM-IV BD, 74 patients with DSM-IV schizophrenia, and 220 healthy control subjects for detection of GAD₆₅, GAD₆₇, and thyroperoxidase antibodies (TPOA), formerly reported to have an increased prevalence in patients with BD. The presence of GAD₆₅ (and not that of TPOA and H⁺/K⁺ adenosine triphosphatase antibody) tended to be associated with BD.^[67] Psychiatric symptoms, such as depression and anxiety, may be prominent, resulting in an incorrect diagnosis. Culav-Sumic *et al.*^[68] described a case of a woman who initially presented with anxious depression and remained resistant to treatment with different classes of antidepressants and additional therapy with lithium and atypical antipsychotics until the detection of GADA supported the diagnosis of SPS. Even the benefit obtained with immunosuppressive treatment with methylprednisolone might support the findings of anxious and depressive symptoms in SPS following the abnormal GABAergic neurotransmission. Finally, Yarlagadda *et al.*^[69] found elevated, but not statistically significant, levels of GADA in 12 patients with chronic psychotic disorders (schizophrenia and schizoaffective disorder) compared to healthy controls suggesting a link between antibodies to GAD₆₅ and chronic psychotic disorders as well as an autoimmune mechanism in the pathogenesis of these disorders.

FUTURE DIRECTIONS

Existing evidence supports the role of GADA in the pathophysiology of a set of heterogeneous disorders that share clinical manifestations of severe motor, behavioral and mood symptoms. It is of interest that BD patients might present increasing titers of GADA compared to healthy controls. It is conceivable (and remains to be tested) that GADA levels might be one of the causative phenotypic manifestations of BD. Indeed, findings of a decreased GABAergic tone during mania might be explained by the diminished synthesis of this inhibitory neurotransmitter due to the action of GADA. Carefully designed studies targeting subsets of BD patients could clarify this hypothesis. The implications for diagnosis and treatment are significant. Detecting GADA in peripheral tissues is a feasible procedure that may assist the diagnostic assessment and depending on the specificity and sensitivity, could be considered a screening test for BD patients. Moreover, GADA could be tested as a marker of response to treatment, particularly to GABAergic agents, such as VPA. Further works are needed to identify the exact pathophysiological

mechanism through which GADA develop and to clarify whether a genetic liability may play a role. Regarding the latter, it is of interest that a recent pharmacogenomic analysis^[70] in Han Chinese BD patients found a strong association between glutamate decarboxylase-like protein 1 (GADL1) gene and response to lithium. The physiological functions of GADL1 gene are not clear, which may be, however, similar to those of GAD.

CONCLUSION

Several studies found that a decrease of brain GABA levels in mood disorder cases can be associated with manic or depressive states. This apparent incongruity may indicate more a mood-stabilizing role of GABA rather than an action on different mood phases. It remains to be established why GABA levels, both in CNS and peripherally, might also present elevation in specific clinical cases. In an attempt to clarify the mechanisms behind these abnormal levels of GABA in the brain, it has been hypothesized an abnormal function of the enzyme GAD that catalyzes the conversion from GLU to GABA. A weak action of this enzyme would justify decreased levels of the neurotransmitter. The aforementioned lines of evidence suggest that the autoantibody to GAD may be a possible causative factor. If this is confirmed, a relatively simple test to assess the level of GADA may provide a better diagnosis of a mood disorder and to improve treatment. If an abnormal level of this antibody is present as a trait rather than being associated with illness episodes, it would allow an early diagnosis of such prevalent and disabling disorder.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

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The role of neuroinflammation in juvenile bipolar disorder

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ABSTRACT

A pathophysiological relationship has been reported between inflammatory processes, decreased levels of neurotrophins, increased oxidative stress and psychiatric disorders in both juvenile and adult ages. Moreover, this relationship remains unclear in juvenile bipolar disorder (BD). We performed a systematic literature review of studies reporting measurements of inflammatory markers, oxidative stress markers or neurotrophins in juvenile and young adult subjects with BD. Concordant findings showed that inflammatory markers are increased since the earlier stages of BD. A positive correlation between decreased levels of a peripheral brain-derived neurotrophic factor and juvenile BD is controversial suggesting that those changes might occur only during the late stage of BD. No changes in central glutathione levels were reported in young adult age BD indicating that oxidative stress may be an outcome of long illness duration and repeated affective episodes. In conclusion, preliminary findings indicate that a certain relationship exists between inflammatory process and juvenile BD but evidence are insufficient to support a causal relationship. Adequately powered and prospective studies are warranted to clarify the role of inflammation, neurotrophins and oxidative stress in juvenile BD.

Key words: Adolescent, bipolar disorder, brain-derived neurotrophic factor, children, inflammation, oxidative stress, pediatric

INTRODUCTION

During the last 20 years, a growing body of evidences has supported a pathophysiological relationship between inflammatory processes, decreased neurotrophins levels, increased oxidative stress and psychiatric disorders in both juvenile and adult ages.^[1,2]

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Multiple studies analyzing peripheral biomarkers of mood disorders have provided important information on the pathophysiologic process underlying adult bipolar disorder (BD).^[1,3] Concordant and consistent evidences have shown that brain-derived neurotrophic factor (BDNF) decreases during both manic and depressive phases of bipolar illness,^[4-6] increases after the treatment with antidepressant and antimanics^[7-9] and correlate with the illness stage with decreased levels in the late stage of BD.^[10]

Several independent laboratories have found that depressive and manic states are associated with an

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Cite this article as: Serra G, De Chiara L, Marangoni C, Faedda GL. The role of neuroinflammation in juvenile bipolar disorder. *Neuroimmunol Neuroinflammation* 2015;2:244-51.

Received: 22-03-2015; **Accepted:** 20-04-2015

imbalance between peripheral levels of pro- and anti-inflammatory cytokines with proteomic analysis revealing that inflammatory pathways are associated with BD and modified by mood-stabilizing lithium treatment.^[1,11] Furthermore, adult subjects with BD are at higher risk of developing comorbid medical illnesses such as diabetes, metabolic and cardiovascular diseases that are also associated with elevated levels of pro-inflammatory markers.^[12,13] Potentially involved cytokines include tumor necrosis factor (TNF), interleukin-2 (IL-2), IL-6, IL-8, IL-13 and apolipoprotein A1.^[14-18]

Finally, growing evidences are showing that increased levels of oxidative stress may be linked to inflammatory and neuroplasticity pathways^[19] and play a role in the pathophysiology of BD.^[20] A meta-analysis found a significant elevation of oxidative stress biomarkers, such as thiobarbituric acid reactive substances (TBARS) and nitric oxide, during all phases of bipolar illness and preliminary data indicated that oxidative stress may be corrected with pharmacological treatments.^[5,6]

As mood disorders have a relatively young median age of onset,^[21] in the last 30 years pediatric mood disorders have been studied more systematically, especially depression and BD.^[22-24] In addition, studying clinical features of mood disorders at onset in the offspring of adults with depression or BD has become a promising research approach.^[25,26]

Several reports have shown a relationship between: (1) the dysregulation of inflammatory markers (increased levels of IL-6, IL-1 β , IL-2, IL-10, INF- α and TNF); (2) genetic variation in inflammatory genes [C-reactive protein (CRP)-gene polymorphism] and pediatric major depressive disorder;^[16] (3) changes in gene expression among subjects with active mood disorders;^[16] (4) preliminary evidences of an association between inflammation and suicidality in depressed youths (decreased TNF- α levels in suicidal compared to nonsuicidal depressed adolescents^[27]) as well as increased mRNA and protein expression of IL-1 β , IL-6 and TNF- α in Brodmann area 10 of suicide victims relative to controls.^[28]

Among children and adolescents with BD, there is a high prevalence of conditions associated with inflammation, such as asthma, cardiovascular disorders, diabetes and obesity,^[12] often associated with inflammatory markers,^[13] including elevated high-sensitivity-C-reactive protein (hsCRP) and IL-6.^[29] This is even more striking considering that subjects with asthma, allergies, and other inflammatory conditions were routinely excluded from psychiatric samples.

Furthermore, recent studies have also examined the potential psychiatric applications of anti-inflammatory

medications, including aspirin, nonsteroidal anti-inflammatory drugs, TNF- α antagonists, and omega-3 fatty acids, in the treatment of mood disorders.^[30-34]

Given the increasing interest in the field of neuroinflammatory mechanisms and mood disorders, we carried out a systematic review of literature analyzing the potential pathogenic role of inflammatory processes, decreased neurotrophin levels and oxidative stress in the pathogenesis of juvenile BD.

SEARCH ALGORITHM AND INCLUSION CRITERIA

We performed a literature search through PubMed using the following search algorithm: (bipolar disorder OR mania OR bipolar depression) AND (child* OR adolesc* OR youth) AND (neuroinflamm* OR inflamm* OR neurovascular OR neurotrophin* OR oxidative stress). Reports found through cross-references were also reviewed and added if they met established search criteria.

We included only original studies specifically reporting measurements of inflammatory markers or oxidative stress markers or neurotrophins in subjects diagnosed with BD. We used the following inclusion criteria: (1) original research; (2) diagnosis of BD; (3) measurement of at least one inflammatory marker or neurotrophin or oxidative stress marker; (4) subjects' age younger than 35 years; (5) reports in English language.

Two psychiatrists screened the article titles for potential relevance, reviewed the identified abstracts and selected the full-text papers potentially meeting the inclusion criteria. The papers not meeting established criteria were excluded.

The following variables were extracted from the reviewed reports: study sample size, type of study, subject age range, subject diagnosis, type of rating scales and diagnostic interviews, measurement method and type of inflammatory marker investigated and main findings of the report.

MAIN FINDINGS

Nine papers were identified through the initial database search, and three adjunctive reports were found from cross-references leading to a total of 91 screened papers [Figure 1]. Twelve reviews were excluded during the abstract screening. Thirty-four full-text papers were assessed for eligibility and 30 of them were excluded due to either (1) failure to report on inflammatory or oxidative stress markers or neurotrophins ($n = 3$); (2) included subjects with age > 35 years ($n = 19$); or (3) included nonaffected bipolar offspring ($n = 3$). Thus, 9 studies met all our

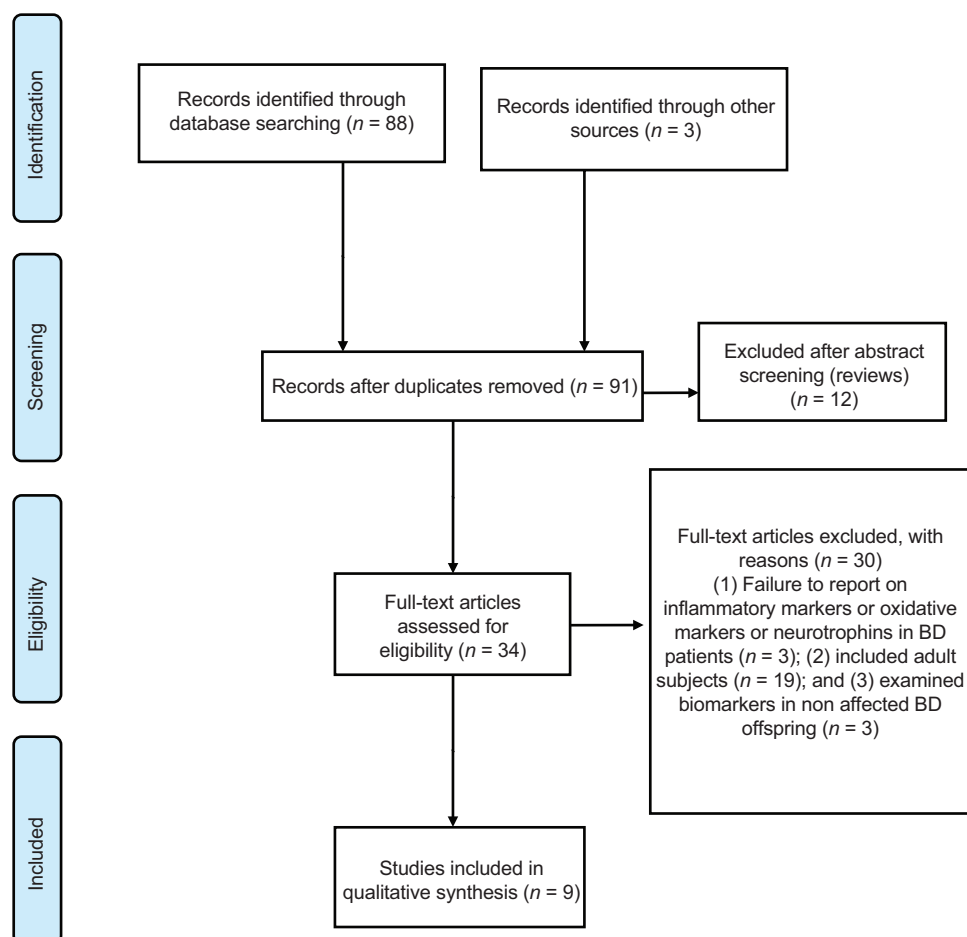


Figure 1: Flow diagram

Table 1: Summary of studies on biological markers in juvenile bipolar disorder						
Study	Type of study	Subjects (Dx)	Age (years)	Psychiatric measures	Biological markers	Main findings
Barzman <i>et al.</i> ^[35]	Cross-sectional	n = 10 (BD-I)	12-17	WASH-U-KSADS; BRACHA	TNF gene	TNF gene expression correlates with both brain activations in amygdala, ACG, and OFC and aggression in adolescents with BD
Birmaher <i>et al.</i> ^[22]	Cross-sectional	n = 30 (18 BD-I; 1 BD-II; 11 BD-NOS)	12-19	K-SADS-PL; MRS; FHS	IL-6; hsCRP; BDNF	Manic symptom severity was significantly associated with hsCRP levels BDNF levels were not correlated with any illness phase
Pandey <i>et al.</i> ^[37]	Longitudinal (8 weeks)	n = 47 (26 BD; 21 HC)	7-17	YMRS; CDRS-R	BDNF	Lymphocyte BDNF mRNA and platelet BDNF levels in drug-free BD subjects were significantly lower than in HCs Lymphocyte BDNF mRNA was significantly increased in medicated BD subjects compared to drug-free BD subjects Lymphocyte BDNF mRNA levels in long-term treated BD subjects was similar to HCs
Chitty <i>et al.</i> ^[39]	Cross-sectional	n = 50 (24 BD-II; 9 BD-spectrum; 17 HC)	BD 18-30; HC 20-29	AUDIT; DASS; Kessler-10	GSH	Decreased GSH in the ACC of high risk drinkers BD subjects No differences in GSH concentration between BD subjects and HCs
Lagopoulos <i>et al.</i> ^[38]	Cross-sectional	n = 104 (13 BD-I; 25 BD-II; 15 BP-spectrum; 51 HC)	16-33	HDRS; BPRS; YMRS; SOFAS; K-10	GSH	No differences in GSH concentration between BD subjects and HCs No significant association between GSH and age of onset or duration of illness No significant correlations between GSH concentration and mania or depressive symptoms

Contd...

Table 1: Contd...

Study	Type of study	Subjects (Dx)	Age (years)	Psychiatric measures	Biological markers	Main findings
Kauer-Sant'Anna, <i>et al.</i> ^[10]	Cross-sectional	<i>n</i> = 120 (30 BD-I early-stage; 30 BD-I late-stage; 60 HC)	Early-stage BD 15-35; late-stage BD 18-65	YMRS; HAM-D-21; GAF	BDNF; TNF- α ; IL-6; IL-10	Decreased BDNF levels in late-stage BD patients compared to HCs Higher TNF- α and IL-6 levels in BD subjects than in HCs during both early and late stage BD Significant negative correlation between length of illness and decreased BDNF levels Positive correlation between TNF- α levels and length of illness
Magalhaes <i>et al.</i> ^[40]	Cross-sectional	<i>n</i> = 231 (33 BD-I; 22 BD-II; 82 MDD; 94 HC)	18-24	SCID	PCC; TBARS	Higher PCC levels BD subjects than in HCs No change in TBARS levels between BD subjects and HCs MDD were not different from control subjects in either PCC or TBARS levels PCC or TBARS levels could not differentiate MDD from BD subjects MDD and BD duration of illness did not correlate with either TBARS or PCC Serum PCC levels were associated with a current manic episode Serum TBARS levels were not associated with mania or depression All depressed groups had serum BDNF levels lower than HCs
Su <i>et al.</i> ^[36]	Cross-sectional	<i>n</i> = 62 (10 bipolar depression; 13 reactive depression; 18 major depression; 21 HC)	18-30	BPRS; HAM-D	BDNF; adiponectin; hsCRP; TNF- α ; IL-6	No differences in BDNF levels between depressive subtypes Plasma adiponectin was lower in BD subjects than in HCs TNF- α was significantly higher in depressed patients than in HCs No differences in TNF- α levels between depressive subtypes No differences in IL-6 and hsCRP concentrations were found between depressed and healthy subjects or between depressive subtypes
Wiener <i>et al.</i> ^[41]	Cross-sectional	<i>n</i> = 231 (82 MDD; 33 BD-I; 22 BD-II; 94 HC)	18-24	HDRS; YMRS; ASSIST	Uric acid; PCC; TBARS	No association between oxidative stress parameters and clinical diagnosis of MDD and BD for women and men

BD: bipolar disorder; NOS: not otherwise specified; HC: healthy control; MDD: major depressive disorder; TNF: tumor necrosis factor; TBARS: thiobarbituric acid reactive substances; PCC: protein carbonyl content; IL: interleukin; hsCRP: high-sensitivity C-reactive protein; BDNF: brain-derived neurotrophic factor; GSH: glutathione; TBARS: thiobarbituric acid reactive substances; ACC: anterior cingulate cortex; mRNA: messenger RNA; ACG: anterior cingulate gyrus; OFC: orbitofrontal cortex

inclusion and exclusion criteria and were included in this review.

Table 1 provides the characteristics of each study, number of included subjects, diagnosis at baseline, age range of the subject sample, considered inflammatory/oxidative stress markers or neurotrophins, and main findings of the considered study.

Pro-inflammatory markers

One study examined serum pro-inflammatory markers IL-6 and hsCRP and serum BDNF among 30 adolescents diagnosed with BD [18 bipolar type I disorder (BD-I), 1 bipolar type II disorder (BD-II) and 11 BD not otherwise specified] from the Course and Outcome Bipolar Youth study.^[29] They found a positive association between manic and hypomanic symptom severity and hsCRP levels. Manic symptom severity was associated with high levels of hsCRP, but not with IL-6 serum levels.

Notably all three subjects with hsCRP levels > 10 μ g/mL had a very high manic symptom score (Mania Rating Scale > 20). Depressive symptom severity was not significantly associated with hsCRP or IL-6 serum levels. Forty percent of participants had levels of hsCRP that are considered at risk for cardiovascular diseases among adults.

Barzman *et al.*^[35] examined the associations between TNF gene expressions, functional brain activation under a frustrative nonreward task and aggression in a sample of 10 adolescents affected by BD-I. They found that gene expression of protein in the TNF pathways correlates with both activation in amygdala, anterior cingulate cortex (ACC) and orbito-frontal cortex and aggression in adolescents with BD suggesting that TNF-related inflammatory genes may play a role in neural activity associated with frustrative nonreward and aggressive behaviors in pediatric BD.

Su *et al.*^[36] investigated pro-inflammatory cytokines levels in a cohort of young males suffering from reactive depression or major depression, or bipolar depression compared to matched sample of healthy control subjects. They found significantly higher levels of TNF- α and significantly lower levels of adiponectin in depressed youths compared to healthy controls, with no difference in both TNF- α and adiponectin levels between depressive subtypes.^[36] No difference was found in IL-6 and hsCRP levels between depressed and healthy subjects and between different subtypes of depression.^[36] Consistently with these findings supporting early changes in pro-inflammatory cytokine levels during the psychopathological development of BD, Kauer-Sant'Anna *et al.*^[10] found that TNF- α and IL-6 levels were already significantly increased in early-stage BD patients compared to healthy controls and continued to be higher in BD subjects than controls also in the late-stage of the disease. Additionally, they found a positive correlation between TNF- α levels and length of illness.^[10] Conversely, the anti-inflammatory IL-10 levels were increased in the early stage of BD but not in the late stage of BD.^[10]

BDNF

Pandey *et al.*^[37] compared gene expression and protein levels of BDNF in a sample of 26 manic or mixed BD adolescents before and after mood-stabilizing treatment with a sample of 21 matched healthy controls. They measured BDNF mRNA levels in lymphocytes of BD subjects before and after treatment and in healthy controls and BDNF protein levels in platelets of drug-free BD and healthy subjects. They found that (1) BDNF mRNA levels in lymphocytes and BDNF protein levels in platelets of drug-free subjects with BD were significantly lower compared to those of healthy controls; (2) long-term treatment with mood-stabilizing drugs significantly increased the levels of BDNF mRNA in the lymphocytes of subjects with BD; and that (3) BDNF mRNA level of BD patients during the 8th week of treatment was comparable to that of healthy control subjects.^[37]

Measurements of BDNF peripheral levels in a sample of young adult males diagnosed with bipolar depression showed that BDNF levels were significantly lower in depressed subjects than in healthy controls.^[36]

These finding were not replicated in a later study^[29] reporting that BDNF levels in a sample of BD adolescents were not correlated with any illness phase (depressive or manic), but was significantly and inversely associated with IL-6 levels. Consistently with this last observation, Kauer-Sant'Anna *et al.*^[10] found that BDNF levels were similar between patients with early stage BD and matched controls but were

significantly decreased in patients with late-stage BD. The decrease in BDNF levels appeared to be proportional to the length of illness and BDNF levels were negatively correlated to the number of mood episodes.^[10]

Oxidative stress

Two studies about the measurement of glutathione (GSH) concentrations in young adult patients with BD compared to healthy subjects suggested that there was no difference in GSH level in the ACC between patients and controls.^[38,39] They reported that GSH levels were not correlated with depressive and manic episode severity^[38] and were not significantly different between unmedicated and medicated subjects.^[39] Also, they found that GSH levels were decreased in bipolar subjects with high levels of alcohol intake.^[39]

Magalhaes *et al.*^[40] suggested that young adults with a lifetime history of hypomania had higher levels of oxidative damage to proteins as measured by the determination of carbonyl groups [protein carbonyl content (PCC)] when compared to healthy young adults. High serum PCC levels were associated with a current manic episode, but not with a current depressive episode. Conversely, the levels of lipid peroxidation as measured using the TBARS method did not significantly differ between mood disorder subjects and healthy controls and did not correlate with manic or depressive mood state.^[40]

A significant gender-related difference in oxidative stress parameters was reported by the same group^[41] showing higher PCC and lower uric acid levels in females when compared to males. No association was found between oxidative stress parameters and bipolar versus major depressive disorder in both genders.^[41]

DISCUSSION

The study of inflammatory factors in chronic psychiatric conditions is a relatively new field of research that has already highlighted several important areas of focus in populations of adult BD patients.^[1,3]

Our review provides a summary of preliminary findings about the link between inflammatory processes, decreased neurotrophins, increased oxidative stress and juvenile or young adult age BD. Two different lines of research have been pursued in this field, one regarding early onset (pediatric) of BD and the other on the effects of course variables (duration of illness, number of episodes, hospitalizations) on changes in inflammatory markers, neurotrophins and markers of oxidative stress. Studying inflammatory mechanisms in pediatric BD

could help to understand the relationship between inflammation and mood episodes. This relationship can be causal (thus preceding and predicting the development of a mood disorder), merely associated with the disease or a consequence of a long lasting illness.

Only three studies^[29,35,37] examined BDNF and inflammatory markers in small populations of pediatric BD, thus the reported findings are mostly preliminary and not replicated. Six additional studies examined the role of oxidative stress, inflammatory cytokines and BDNF in the pathophysiology of early-onset BD during adult age.^[10,36,38-41]

Concordant findings showed that inflammatory markers are increased since the earlier stages of BD with: (1) increased TNF- α gene expression in adolescent BD showing aggressive behaviors;^[35] (2) increased TNF- α levels in young adults with bipolar depression;^[36] (3) increased TNF- α levels since the earlier stages of BD;^[10] and (4) positive correlations between TNF- α levels and length of bipolar illness.^[10] Also, increased levels of hsCRP have been detected in juvenile BD patients during manic and mixed episodes.^[29]

A positive correlation was found between decreased levels of peripheral BDNF and a manic, depressive or mixed episode in juvenile and young adult BD,^[36,37] even though such findings have not been always replicated.^[10,29] In fact, some authors have suggested that changes in peripheral levels of BDNF might occur only during the late stage of BD and might reflect the neurodegeneration of late stage mood disorders.^[10] Indeed, recent preclinical and clinical evidences suggested that the excitotoxicity due to an excessive glutamatergic transmission might play a role in the pathogenesis of the hypothesized neurodegeneration associated with BD.^[42-44] Also, recent studies have shown that TNF- α is a key cytokine stimulating extensive release of glutamate from microglial cells,^[45] whereas the neuroprotective effect of the mood-stabilizing treatments like lithium^[46] and the recently suggested promising memantine^[47,48] is well known.

Finally, findings examining the role of oxidative stress in juvenile BD are substantially controversial as no changes in central GSH levels was measured *in vivo* using magnetic resonance spectroscopy during manic or depressive phase of young adult BD.^[38,39] These findings are inconsistent with studies from other groups finding increased oxidative stress in older samples with illness duration of 10 years on average^[20] indicating that oxidative stress may be an outcome of

long illness duration and repeated affective episodes rather than being a core feature of the pathophysiology of BD at onset.

Reasons of weakness and inconsistency across the studies are diverse and include heterogeneity of the samples (age and considered BD phases, concurrent use of drugs, substance abuse, comorbidity with other medical illnesses, effect of other psychiatry conditions, especially anxiety related disorders), small to modest sample sizes and differences in studied biological pathways. Also, it is worth to underscore that peripheral change in biological markers might not always correspond to comparable changes of the same markers in the central nervous system.

CONCLUSION

There are preliminary findings indicating that a potential relationship exists between inflammatory process and juvenile BD, but evidences are insufficient to support the causality. Adequately powered and prospective studies on high risk population as well as studies examining the relationship between mood-stabilizing treatment and changes in inflammatory, oxidative markers and neurotrophins levels are warranted to understand their role in the pathogenesis of BD.

Financial support and sponsorship

It was supported by the Research Fellowship from Sapienza University of Rome to Dr. Giulia Serra.

Conflicts of interest

There are no conflicts of interest.

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Neuroinflammation in bipolar disorders

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ABSTRACT

Recent literature based on peripheral immunity findings speculated that neuroinflammation, with its connection to microglial activation, is linked to bipolar disorder. The endorsement of the neuroinflammatory hypotheses of bipolar disorder requires the demonstration of causality, which requires longitudinal studies. We aimed to review the evidence for neuroinflammation as a pathogenic mechanism of the bipolar disorder. We carried out a hyper inclusive PubMed search using all appropriate neuroinflammation-related terms and crossed them with bipolar disorder-related terms. The search produced 310 articles and the number rose to 350 after adding articles from other search engines and reference lists. Twenty papers were included that appropriately tackled the issue of the presence (but not of its pathophysiological role) of neuroinflammation in bipolar disorder. Of these, 15 were postmortem and 5 were carried out in living humans. Most articles were consistent with the presence of neuroinflammation in bipolar disorder, but factors such as treatment may mask it. All studies were cross-sectional, preventing causality to be inferred. Thus, no inference can be currently made about the role of neuroinflammation in bipolar disorder, but a link is likely. The issue remains little investigated, despite an excess of reviews on this topic.

Key words: Bipolar disorder, inflammation, neuroinflammation, psychoneuroimmunology, stress

INTRODUCTION

Among the former mood or affective disorders, bipolar disorder is the one that is most intriguing. Its heterogeneity is well-recognized. While traditionally subdivided into bipolar I and bipolar II according to whether there was a history of mania,^[1] some scholars support the existence of more than 10 subtypes.^[2] It is supposed to be pathophysiologically/neurobiologically continuous with other psychiatric disorders such as schizophrenia according to Griesinger's model of *Einheitspsychose*,^[3] and with recurrent major depression.^[4-6]

Mood disorders along with anxiety disorders are considered as "stress disorders."^[7] The organism responds to stress in an integrated manner, involving

the cooperation among the nervous, endocrine, and immune systems,^[8] and stress disorders are believed to be underpinned by derangements in this integration.^[9]

STRESS AND INFLAMMATION

Inflammation is a process described since antiquity, meaning burning in both Greek (φλόγωσις) and Latin (inflammation), and characterized by swelling (tumor), redness (rubor), heating (calor), pain (dolor), and impaired function (functio laesa). It is a general reaction to pathogens in a tissue involving an innate response of cells residing within that tissue. In the acute phase, the process entails the extravasation of immune cells, permeability changes in blood vessels, and the production of chemical mediators, including acute phase proteins, vasoactive amines, eicosanoids, bradykinin and other tachykinins, and chemical attractors. The nonspecific response usually leads to resolution and repair, with restitution to integrity.

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Cite this article as: Kotzalidis GD, Ambrosi E, Simonetti A, Cuomo I, Del Casale A, Caloro M, et al. Neuroinflammation in bipolar disorders. *Neuroimmunol Neuroinflammation* 2015;2:252-62.

Received: 14-06-2015; **Accepted:** 31-07-2015

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10.4103/2347-8659.167309

The inability to restore the previous healthy state may ensue in chronic inflammation, characterized by shifts from the main participating cells toward mononuclear (monocytes, macrophages, lymphocytes, and plasma) cells and fibroblasts and from the main participating molecules toward interferon- γ , interleukins (ILs), growth factors, nitric oxide, and hydrolytic enzymes.^[10]

The stress concept was developed from the work of Selye,^[11,12] who viewed the body in Cannon's frame.^[13] Stress, like inflammation, was proposed to be the body's response to "diverse nocuous agents,"^[11] a general adaptation syndrome characterized by an integrated neuroendocrine and immune response tending to restore homeostasis. Selye^[12] showed that the response also involves the immune system, which constitutes another parallel with the inflammatory response. Currently, the stress response is considered to be evolutionary and likely to amplify an organism's resistance to environmental stressors. Like inflammation, the inability of an organism to adequately address stress may lead to a stress disorder, which in psychiatry is represented by posttraumatic stress disorder, anxiety disorders, and mood disorders such as depression.

In recent years, there has been an increasing recognition of altered immunological parameters in many psychiatric disorders, including depression, bipolar disorder, autism spectrum disorders, and schizophrenia.^[14,15] The rationale is that chronic inflammation, by releasing cytokines, may set brain function into a "sickness mode," thus causing psychiatric disorders. However, how this is carried out is not explained, so it remains an interesting paradigm with no demonstration so far.

WHAT DO WE INTEND BY "NEUROINFLAMMATION?"

Neuroinflammation is defined as inflammation of the central nervous system. It consists of increased glial activation, pro-inflammatory cytokine content, blood-brain-barrier permeability, and leukocyte extravasation. The process is believed to be driven by IL-1 beta (IL-1 β), a cytokine that has been found to be increased in neurodegenerative disorders such as Alzheimer's disease, Parkinson's disease, and multiple sclerosis. IL-1 β stimulates the IL-1 receptor/IL-1 accessory protein complex to increase glia nuclear factor kappa B-dependent transcription of pro-inflammatory cytokines, such as tumor necrosis factor (TNF)- α , IL-6, and interferons, as well as the neutrophil-recruiting chemokines CXCL1 and CXCL2.

The whole process appears, however, to be mediated through stress,^[16] which is a very general term, but it is essential to understand neuroinflammation as part of a whole in the pathophysiology of various disorders, and disease in general.

There has been evidence that neuroinflammation is reflected in changes in peripheral immunity,^[17] but the reverse may not be true, hence, evidence of peripheral immunological alterations cannot be taken to indicate the presence of neuroinflammation. There is no consensus as to whether central and peripheral immunity are specular, and a recent study that adequately addressed the issue showed that brain immune markers were found to be independent from the peripheral activity of the immune system.^[18]

Summarizing, stress may lead to the establishment of a chronic inflammatory reaction, which may occur in the brain in parallel to the periphery, thus setting brain function in a sickness mode that may substitute default activity and perpetuate the disorder. This does not explain the oscillatory mood activity. The presence of neuroinflammation in the brain in patients with bipolar disorder can help to answer whether neuroinflammation is a general way by which manic-depressive symptoms are produced. It is not a sufficient evidence to demonstrate some of these symptoms in people with autoimmune neuroinflammation or other disorders showing both neuroinflammation and cognitive or mood alterations proper of bipolar disorder. Instead, convincing evidence requires the demonstration of neuroinflammation in a population of patients with bipolar disorders who have no other comorbidity. For this reason, we consider studies that point to the existence of neuroinflammation in bipolar disorder only those studies investigating microglial activation or showing increased presence of neuroinflammatory markers in the human brain, in people with bipolar disorder compared to healthy or nonpsychiatric controls. These may be postmortem studies or studies in living humans that involve the cerebrospinal fluid (CSF) or brain imaging.

Having this in mind, we aimed to review the evidence for neuroinflammation in the pathogenesis of bipolar disorder.

OUR SEARCH STRATEGY TO INVESTIGATE NEUROINFLAMMATION IN BIPOLAR DISORDER

We performed a careful PubMed search using the following strategy: (neuroinflammation* or glia* or microglia* or [(CXCL1 or CXCL2 or IL-1b* or IL-6 or

interleukin* or interferon* or tumor necrosis factor or TNF* or NKκB) and (brain or cerebrospinal fluid or CSF)] and (“bipolar disorder” or mania or manic or “recurrent depression”). There were no restrictions as to publication date or language. To be included, the paper had to be an original article and carried out in humans. We did not restrict our search to human studies using the related PubMed function, because in our experience this practice does not exclude complete animal studies but is likely to conceal some relevant human investigations instead. Hence, animal studies were subsequently excluded on the basis of evidence. Furthermore, we excluded reviews and meta-analyses, opinion/speculative papers, animal studies, case reports, as well as papers conducted without respect to the 1964 Declaration of Helsinki principles of human rights, reviews and meta-analyses, opinion/speculative papers, animal studies, as well as case reports. However, their references, especially those of reviews/meta-analyses were searched for possible further includible papers. This review followed the bureaucratic Prisma statement^[19,20] when appropriate. We inverted the order recommended for screening, that is, first exclude duplicates and then the screen. We first classified studies according to their type, then excluded animal studies, reviews and studies of peripheral immunity, and included only human studies with data, including clinical and postmortem, but not *in vitro* experiments on cell lines. We also excluded and classified as unfocused those postmortem studies investigating glia in the brain without telling microglia from other types of glial cells. We excluded all nonpeer reviewed literature, as it could constitute a source of bias. We considered as duplicates only studies that reported the same results in different published reports. When different studies by the same research group progressively report on increasingly larger samples and when the past used sample is used and accrued, we adopt the strategy to disregard the first appearing papers, including only the last study with the larger sample, provided its quality of evidence is high. Papers reporting on the same samples, but on different measures, were considered as different studies and included if appropriate. Contrary to the distinction made by the Prisma statement between records and full-text articles, we considered all papers emerging from our research as papers to obtain in full text and carefully searched for any data that conformed to our aims. Our experience is that you can never say if you only read the abstract, especially for not so recently published papers.

Contrariwise to what Prisma dictates, we did not label our review as “systematic” and did not attempt to carry out a meta-analysis, both because the concepts of

quantitative and qualitative syntheses are quite weak in Prisma, and their definitions fuzzy and because we were subsequently faced with an extreme heterogeneity of study designs and results that cannot be meta-analyzed.

WHAT DID THE SEARCH PRODUCE?

The PubMed search yielded 316 papers as of 11th of July 2015. The search of the reference lists of these papers produced further 40 potentially interesting papers. Of the total output of 356 papers, 100 were completely unfocused and were captured for the presence of spandrels in the search strategy (however, we chose not to modify the strategy by adopting a more specific approach, since this would result in a potential loss of otherwise eligible material), 98 were excluded because they were reviews or meta-analyses, 16 were opinion articles/editorials or speculative with no experimental data, 55 were animal studies, 5 were case studies or case series, 7 did not include patients with bipolar disorder separately or did not investigate neuroinflammation at all, 50 were investigations of peripheral markers, 1 was a duplicate, and 4 were carried out *in vitro* on human glial cells. The flow diagram in Figure 1 shows the search strategies and papers selected for review according to a modified Prisma algorithm. A total final number of 20 papers were found to be eligible and were analyzed. The search output spanned from 1982 to 2015; recalling that the first occurrence in PubMed of the term neuroinflammatory was in 1983 that of neuroinflammation was in 1995, and that microglial activation and related terms appeared during 1973-1975, we may presume that there was a dearth of focused papers in the 1st year, that is, prior to 2000. However, one of the includible papers was dated 1997. This was the case (19 papers up to 1999 and 337 from 2000 on) and the trend has been increasing through years, witnessing an increasing interest of the scientific community in the neuroinflammation issue in psychiatric disorders in general, and in bipolar disorder in particular. Of interest, two of the included papers were not identified by the PubMed search despite the strategy was appropriate for singling them out. The included papers are shown in Tables 1 and 2. They fell into two broad categories, postmortem ($n = 15$) and *in vivo* ($n = 5$), the latter comprising four studies of neuroinflammatory mediators in the CSF and one was an ingenious positron emission tomography study of the binding of a neuroinflammatory marker in the brain. The only study we excluded as a duplicate was Rolstad *et al.*^[21] which focused on the correlation between CSF neuroinflammatory markers and cognitive performance and admittedly reported data previously reported in Jakobsson *et al.*,^[18] despite differences in sample size.

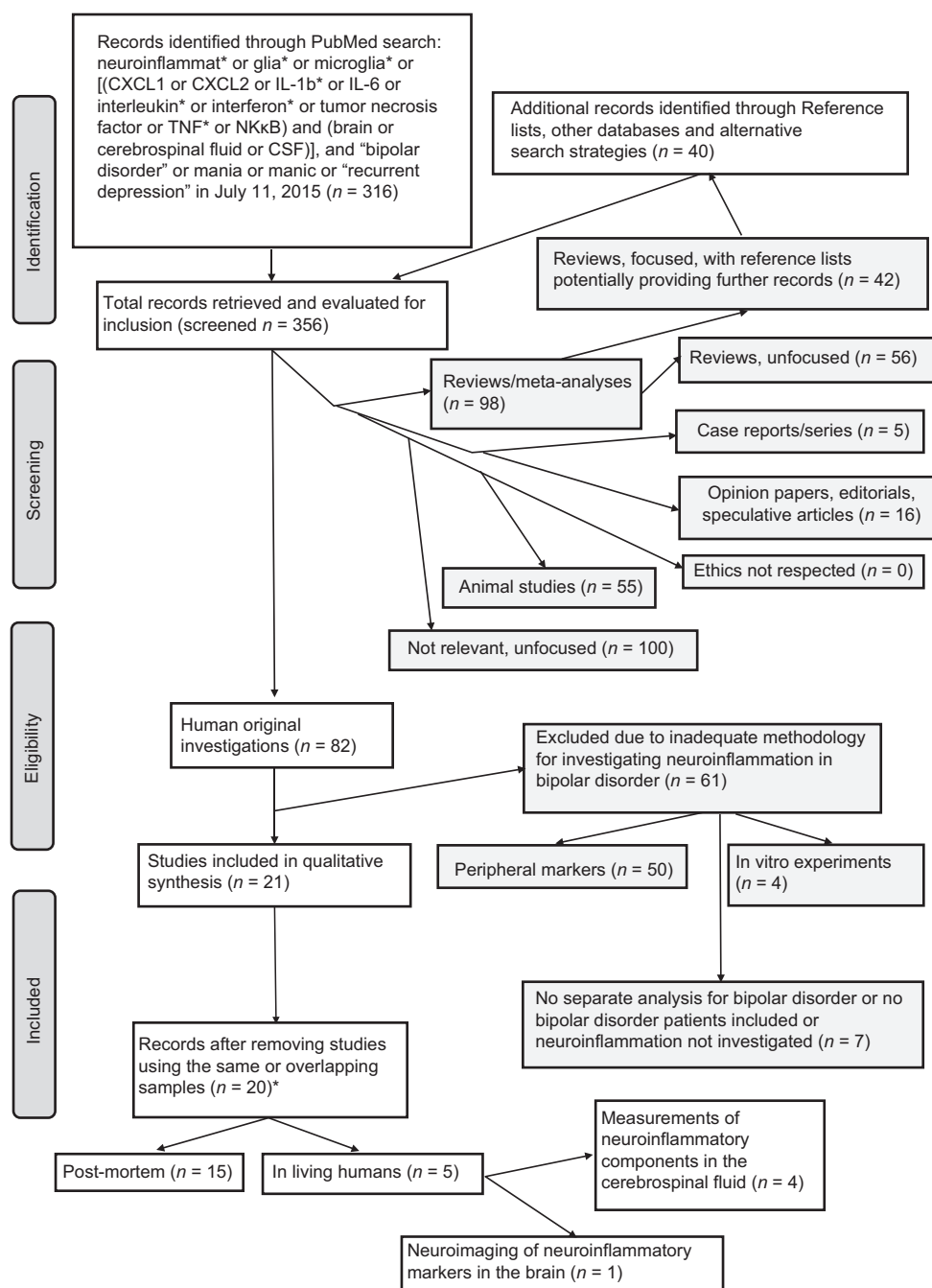


Figure 1: Results for search and inclusion and design typology (*Despite the existence of overlapping samples, most studies except one, were not considered duplicates since they reported on different datasets)

BASED ON THE SEARCH, IS THERE ANY EVIDENCE FOR NEUROINFLAMMATION IN BIPOLAR DISORDER?

This review attempted to answer the question of whether neuroinflammation plays a role in the pathophysiology of bipolar disorder.

We may not speak of conclusive evidence of neuroinflammatory mechanisms in bipolar disorder when the obtained evidence is too indirect. For example, when a molecule like N-acetylcysteine is found to have some therapeutic activity in bipolar disorder, we may not specify whether this is related

to its anti-neuroinflammatory, anti-oxidative stress, or anti-apoptotic effect or to its mitochondrial dysfunction countering or glutamate/dopamine balancing actions,^[44] unless accompanied by evidence of neuroinflammatory markers moving in the desired direction (and the demonstration of their alteration at baseline). Furthermore, the evidence of abnormal peripheral inflammatory reactivity cannot be taken as evidence of neuroinflammation.

Our search yielded a high number of interesting articles, but few of them suited the purpose of this review. This was due to the fact that our search strategy was over-inclusive to avoid missing any suitable article.

Table 1: Postmortem studies included in this review investigating microglia or neuroinflammation in bipolar disorder

Study	Origin	Source	Population	Measure(s)	Results	Observations
Ishizuka <i>et al.</i> ^[22]	Japan (Kumamoto, Fukuoka, Kyushu-Maidashi)	Kumamoto University, Department of Anatomy (postmortem autopsies); Neurosurgery School of Medicine, Fukuoka (brain sampling during brain surgery)	11 neuro-psychiatric patients, 1 bipolar; 2 surgical (brain sampled during neurosurgery for brain tumor)	Northern blot-immunohistochemistry, <i>in situ</i> hybridization to assess expression and distribution of MIP 1 α /LD78 in brain	Increased expression of MIP 1 α /LD78 in the bipolar patient in white matter glial cells	Evidence for increased expression of inflammatory marker in glia in the brain of just one patient with bipolar disorder; four patients with schizophrenia showed neuronal as well as glial distribution abnormalities
Hamidi <i>et al.</i> ^[23]	USA (Washington University, St. Louis, Mo; NIMH, Bethesda, Md)	Harvard Brain Tissue Resource Center	9 bipolar versus 8 major depression versus 10 nonpsychiatric controls	Microglial density (cells/mm ³) in the amygdala	No differences between bipolar disorder and controls	Patients with bipolar disorder had been exposed to valproate or lithium more often than patients with major depression; higher suicide rates in patients versus controls
Frank <i>et al.</i> ^[24]	Germany (Mannheim, Heidelberg, Oberschleissheim)	Stanley Foundation Brain Collection, Bethesda	3 bipolar, 4 schizophrenia, 4 nonpsychiatric controls for comparing retroviral RNA in various brain areas; 35 bipolar, 35 schizophrenia, 35 nonpsychiatric controls for all analyses in BA 46 (dorsolateral prefrontal cortex)	Microarray-based analysis of HERV transcriptional activity in the dorsolateral prefrontal cortex. DNA chip investigated through <i>Env</i> -specific QRT-PCR. An animal retrovirus-specific microarray was performed to test the hypothesis of zoonosis	HML-2 family (HERV-K10) significantly overrepresented in bipolar disorder and schizophrenia, compared to control brains. HERV E4-1 transcription overrepresented in bipolar disorder. <i>Env</i> expression of HERV-W, HERV-FRD, and HML unaffected regardless of the clinical picture. No transcripts of any animal retroviruses detected with pet chip in all 105 human brains	HERV transcription in brain weakly correlates with schizophrenia and related disorders, but may be affected by individual genetic background, brain-infiltrating immune cells, or medical treatment; the higher incidence of HERV-K10 transcripts in schizophrenia and bipolar disorder is a probable consequence of high brain immune reactivity
Dean <i>et al.</i> ^[25]	Australia (University of Melbourne, Parkville, VIC)	Autopsied cases at Victorian Institute of Forensic Medicine	8 bipolar versus 20 schizophrenia versus 20 nonpsychiatric controls	Prefrontal S100 β levels (indirect evidence; astrocyte S100 β and microglial IL-1 β induce one another)	Decreased BA 9 and increased BA 40 S100 β levels in bipolar disorder I versus other groups	Postmortem interval arbitrarily defined in not witnessed deaths; no suicide
Foster <i>et al.</i> ^[26]	England (King's-Maudsley), Canada (University of British Columbia, Vancouver), Norway (Ullevål University, Oslo) and USA (UCSD, San Diego, CA)	Stanley Foundation Brain Collection	15 bipolar, 15 schizophrenia, 15 major depression, and 15 nonpsychiatric controls	Double immune-fluorescence for the neuroinflammation marker calprotectin and microglia in BA 9 (dorsolateral prefrontal cortex)	Higher levels in schizophrenia, lowest in controls, intermediate in major depression, and bipolar disorder in dorsolateral prefrontal microglia	Evidence of neuroinflammation in bipolar disorder, but not so strong as in schizophrenia; post-mortem interval, age, and sex distribution not reported, but said to have not influenced results

Contd..

Table 1: Contd...

Study	Origin	Source	Population	Measure(s)	Results	Observations
Weis <i>et al.</i> ^[27]	USA (Bethesda, Md), Austria (Linz, Oberösterreich) and Switzerland (University of Zürich)	Stanley Neuropathology Consortium Collection	15 bipolar, 15 schizophrenia, 15 major depression, and 15 nonpsychiatric controls	Immunohistochemistry to detect PrP ^c -positive cells in the cingulate gyrus	In the bipolar group, neuroleptics decreased the numerical density of PrP ^c -positive neurons and increased that of PrP ^c -positive white matter microglia	This is a very indirect measure of neuroinflammation, pointing to the possibility that drug treatment has something to do with it. Causes of death not provided
Rao <i>et al.</i> ^[28]	USA (NIH, Bethesda, Md and North Carolina)	Harvard Brain Tissue Resource Center (McLean Hospital, Belmont, MA, USA)	10 bipolar, 10 nonpsychiatric controls	Western blot, total RNA isolation-real time reverse transcriptase PCR and immunohistochemistry of frontal cortex membrane, nuclear, and cytoplasmic extracts	Higher protein and mRNA levels of IL-1 β , IL-1 receptor, MyD88, NF- κ B subunits, and astroglial and microglial markers GFAP, iNOS, <i>c-fos</i> and CD11b in the frontal cortex of patients with bipolar disorder	Excitotoxic markers were also up-regulated; the authors speculated that glutamatergic derangement (as shown by decreased protein and mRNA for NMDA receptors NR-1 and NR-3A) could be responsible for neuroinflammation
Kim <i>et al.</i> ^[29]	USA (NIH, Bethesda, Md)	Harvard Brain Tissue Resource Center (McLean Hospital, Belmont, MA, USA)	10 bipolar, 10 nonpsychiatric controls (same sample as Rao <i>et al.</i> ^[28])	Western blot, total RNA isolation-real time reverse transcriptase PCR and immunohistochemistry of frontal cortex membrane, nuclear, and cytoplasmic extracts	Increased protein and mRNA of AA-selective cPLA2 IVA, secretory sPLA2-IIA, COX-2 and mPGES in bipolar disorder; decreased COX-1 and cPGES compared to control brains	Deranged neuroinflammatory response, which the authors relate to excitotoxicity; tendency to repeat the conclusions of the preceding paper (Rao <i>et al.</i> ^[28]). The arachidonic cascade is not only involved in neuroinflammation but in other processes as well
Steiner <i>et al.</i> ^[30]	Germany (University of Magdeburg)	Magdeburg brain bank (D)	12 suicide victims (5 bipolar, 7 major depression) versus 10 nonpsychiatric controls	Immunohistochemistry for the NMDA agonist quinolinic acid in the microglia of the anterior cingulate gyrus	Increased quinolinic acid-staining cells in the anterior midcingulate cortex and the subgenual, but not in the pregenual cortex, in the major depression, but not bipolar disorder, suicide victims	Drug treatment may have affected quinolinic acid content of microglia (thus masking a possible difference from controls in bipolar disorder); admittedly, microglial immunoreactivity may not be attributed to increased synthesis or decreased metabolic breakdown; relevance to neuroinflammation only indirect
Rao <i>et al.</i> ^[31]	USA (NIH, Bethesda, Md)	Harvard Brain Tissue Resource Center (McLean Hospital, Belmont, MA, USA)	10 bipolar, 10 nonpsychiatric controls (same sample as Rao <i>et al.</i> ^[28]), matched; 10 Alzheimer's disease, 10 nonpsychiatric controls, matched	Genomic DNA isolation, gene-specific and global DNA methylation; total RNA isolation-real time reverse transcriptase PCR for BDNF, NF- κ B p50 and NF- κ B p65, and global histone acetylation and phosphorylation, all from BA 9 (dorsolateral prefrontal cortex)	Increased mRNA and protein levels of neuroinflammatory markers (IL-1 β and TNF- α) and of markers of astrocytic and microglial activation in both bipolar and Alzheimer	Data compatible with altered frontal cortex epigenetic regulation related to neuroinflammation in bipolar disorder

Contd..

Table 1: Contd...

Study	Origin	Source	Population	Measure(s)	Results	Observations
Dean <i>et al.</i> ^[32]	Australia (University of Melbourne, Parkville, VIC)	Victorian Brain Bank Network, Mental Health Research Institute, Parkville, Australia	10 bipolar, 10 major depression, 19 schizophrenia, 30 age- and gender-matched nonpsychiatric controls	Western blotting and PCR for IL-1 β and TNF-related measures in the prefrontal cortex and in the anterior cingulate	Transmembrane, but not soluble TNF- α transcript increases are found in the cingulate (BA 24), but not prefrontal cortex (BA 46); other groups did not show such increases; soluble TNF- α and IL-1 β levels did not differ from controls in any mental group; decreased <i>TNFR2</i> levels in bipolar disorder in BA 46; results not consistent with neuroinflammation	Significantly more patients with bipolar disorder had committed suicide compared to the other groups
Gos <i>et al.</i> ^[33]	Germany (Magdeburg and Leipzig) and Poland (Gdańsk)	Magdeburg brain bank (D)	8 bipolar versus 9 major depression versus 13 nonpsychiatric controls	Hippocampal S100 β levels (indirect evidence; astrocyte S100 β and microglial interleukin-1 β induce one another)*	Numerical density of S100 β immuno-positive astrocytes bilaterally decreased in CA1 pyramidal layer in both major depression and bipolar brains compared to controls; decreased density of S100 β immuno-positive oligodendrocytes in left alveus only in bipolar disorder	No suicide among bipolar disorder patients, but 7 suicides among major depression patients; no evidence of neuroinflammation
Hercher <i>et al.</i> ^[34]	Canada (University of British Columbia, Vancouver, BC)	Stanley Medical Research Institute's brain collection	20 bipolar versus 20 schizophrenia versus 20 nonpsychiatric controls	Prefrontal microglial clustering coefficient and prefrontal microglial density (cells/mm ²)	Not different from controls and patients with schizophrenia	Bipolar patients had committed suicide more often than controls and had more often a heavy drug abuse history
Fillman <i>et al.</i> ^[35]	Australia (Sydney, NSW, Australia) and USA (University of Pennsylvania, Philadelphia, Pa and Stanley Medical Research Institute, Rockville, Md)	Stanley Medical Research Institute (Array Cohort)	34 bipolar, 35 schizophrenia, 35 nonpsychiatric controls	Sample dichotomized to high- ($n = 32$) and low- ($n = 68$) inflammation/stress clusters according to differences in inflammatory and stress-related gene transcripts of RNA extracted from the frontal cortex and assessed through microarray analysis and PCR	Trend for bipolar patients to belong to the high inflammation/stress group ($n = 11$), while patients with schizophrenia were significantly more likely than controls ($n = 15$ vs. 6)	15 suicides among bipolar, 7 suicides among patients with schizophrenia; results partly support neuroinflammation in bipolar disorder

Contd..

Table 1: Contd...						
Study	Origin	Source	Population	Measure(s)	Results	Observations
de Baumont et al. ^[36]	Brazil (São Paulo, SP and Porto Alegre, Rio Grande do Sul) and Portugal (Braga and Guimarães, Braga)	Stanley Neuropathology Consortium	29 bipolar, 29 schizophrenia, 30 nonpsychiatric controls	RNA extracted from frontal cortex to assess gene expression profile; cloned DNA for microarray analysis	Immune response and stress-related genes were differentially expressed between the control and the patient group; bipolar patients differed from those with schizophrenia in that the former showed an up-regulation of a set of 28 genes in bipolar disorder with respect to schizophrenia	Neuroinflammatory microglia activation is compatible with these data; the influence of medication has not been ruled out

*S100 β induces the expression of IL-1 β in cultured rat microglia;^[37,38] astrocyte synthesis of S100 β in AD may be triggered by microglial-derived IL-1.^[39] BA: brodmann's area; TNFR2: tumor necrosis factor receptor, type 2; IL-1 β : interleukin-1 beta; MIP 1 α /LD78: macrophage inflammatory protein-1 alpha/LD78; PrP^c: cellular prion protein; QRT-PCR: quantitative real-time polymerase chain reaction; MyD88: myeloid differentiation factor 88; NF- κ B: nuclear factor kappa B; GFAP: glial fibrillary acidic protein; iNOS: inducible nitric oxide synthase; NMDA: N-methyl-D-aspartic acid; HERV: human endogenous retrovirus; HERV-W: human endogenous retrovirus-W; AA: arachidonic acid; cPLA2: cytosolic phospholipase A2; sPLA2-IIA: secretory phospholipase A2-IIA; COX: cyclooxygenase; mPGES: membrane prostaglandin E synthase; cPGES: cytosolic prostaglandin E synthase; TNF- α : tumor necrosis factor α

It proved to be more tiresome to download all papers, but this allowed us to identify two papers that would otherwise have gone undetected. Surprisingly, the majority of suitable articles regarded postmortem studies. These studies ($n = 15$) [Table 1] favored the idea of the existence of neuroinflammation in bipolar disorder in their majority. Two provided indirect evidence for microglial activation, while four were not consistent with the presence of neuroinflammation in bipolar disorder. However, in one of these,^[23] it is possible that treatment could have set-off neuroinflammation. In fact, patients were receiving drugs such as lithium and valproate, which both interfere with the arachidonic acid cascade,^[45] one of the cross-roads of excitotoxicity and neuroinflammation.^[46] The evidence stemming from *in vivo* studies ($n = 5$) is consistent with the presence of neuroinflammation in bipolar disorder, also in consideration of the fact that most patients were sampled/tested when euthymic. The demonstration of peripheral benzodiazepine receptor alterations in bipolar I disorder patients may constitute a definitive demonstration,^[43] but it should also be considered that such alterations in the brain of people with bipolar disorder, which are consistent with microglial activation, may not be specifically related to the pathogenesis of bipolar disorder or any diagnosis, but rather to disease activity.^[47]

Causality effects, that is, whether it is bipolar disorder that once established, triggers neuroinflammation, either through the adoption of a reckless lifestyle that is likely to promote a metabolic syndrome that facilitates the onset of neuroinflammation, or rather it is neuroinflammation that has always existed in a given

individual that eventually ensued in bipolar behavior and disorder, cannot be probed. In fact, to demonstrate causality we need longitudinal study designs, and in the case of neuroinflammation and bipolar disorder all relevant articles were based on cross-sectional articles. While this was mandatory for postmortem studies, it was not for studies investigating living humans. Future studies should be able to tackle the causality question by studying the same patients across the various phases of their illness. However, the fact that the supposed neuroinflammation was present to some extent also when bipolar disorder was in its euthymic phases strongly argues against the lack of involvement of the brain immune system in bipolar disorder.

The studies included in our review were methodologically different, and their sample sizes were much variable. Investigations of neuroinflammatory markers in the CSF were all but one carried out in Sweden and conducted by the same Karolinska-Gothenburg group. One of these studies had to be excluded due to sample and data duplication,^[21] but all these studies were quite consistent in their conclusions, supporting the existence of neuroinflammation in bipolar disorder. This might have introduced a site bias. The markers tested each time in postmortem studies differed, even when the same research groups were involved. Hence, we had no population overlap, with the same group first reporting on some people and then on a bigger sample that comprised the formerly reported cases. We found no duplicates even when data referred to the same population in postmortem studies. There was a tendency in Bethesda-based groups to support neuroinflammation while the German groups were more skeptical about it [Table 1].

Table 2: Studies in living humans included in this review investigating microglia or neuroinflammation in bipolar disorder

Study	Origin	Population	Design	Results	Observations
Soderlund <i>et al.</i> ^[40]	Sweden (Stockholm-Linköping-Göteborg)	15 bipolar I, 15 bipolar II, all euthymic, 30 healthy volunteers	CSF cytokine concentrations assessed through immunoassay-based protein array multiplex system; cross-sectional	Higher IL-1 β and lower IL-6 levels bipolar than controls. Patients with recent manic/hypomanic episodes had significantly higher IL-1 β levels than those without	Evidence for neuroinflammation and for IL-1 β involvement; cross-sectional design is a methodological weakness
Stich <i>et al.</i> ^[41]	Germany (Freiburg, Greifswald)	40 bipolar versus 26 controls with pseudotumor cerebri	Paired CSF and serum samples analyzed through ELISA to detect the concentration of antibodies against <i>Toxoplasma gondii</i> , HSV types 1 and 2, CMV, and EBV. Specific AI > 1.4 = intrathecal specific antibody synthesis; oligoclonal bands = chronic neuroinflammation	Eight patients with bipolar disorder versus 1 control had AI > 1.4; 5 patients versus 0 controls had oligoclonal bands	Cross-sectional design; possible that some bipolar patients have autoimmune disorder; 1 of 5 patients with bipolar disorder show evidence of neuroinflammation
Isgren <i>et al.</i> ^[42]	Sweden (Göteborg-Stockholm); England [London]	21 euthymic bipolar disorder patients versus 71 age- and sex-matched healthy controls	Measurements of serum and CSF concentrations of 11 cytokines; IL-6 measured through singleplex assay; IL-1 β , IL-2, IL-4, IL-5, IL-8/CXCL8, IL-10, IL-12, IL-13, TNF- α , and IFN- γ through the MSD 96-well multi-array and multi-spot human cytokine assay; validation of IL-8 measurement re-analysis: 21 patients and 20 controls with Proseek Multiplex Inflammation I	CSF IL-8 only was higher in bipolar patients with respect to controls; validation reanalysis showed measurements to have been valid, but also showed no correlation between serum and CSF levels	Cross-sectional design is a limitation. The study favors the presence of neuroinflammation in bipolar disorder, but do not rule out that medication could account for the results; there was no correlation between central and peripheral data
Jakobsson <i>et al.</i> ^[18]	Sweden (Göteborg-Stockholm); England [London]	221 bipolar versus 112 healthy controls for serum sampling; 125 bipolar versus 87 controls for CSF sampling	MCP-1, YKL-40, sCD14, TIMP-1, and TIMP-2 measured through ELISAs	MCP-1, YKL-40, and TIMP-2 levels higher in patients with bipolar disorder than controls in CSF; serum and CSF MCP-1, YKL-40 levels correlated, but differences in CSF levels between bipolar patients and controls were independent from serum levels	Cross-sectional design is a limitation. The study favors the presence of peripheral chronic inflammation and neuroinflammation in bipolar disorder, but also stresses the fact that the two are independent
Haarman <i>et al.</i> ^[43]	The Netherlands (Groningen)	14 bipolar I versus 11 healthy controls	Dynamic 60-min PET scan after injecting [¹¹ C]-(R)-PK11195, a ligand of the peripheral benzodiazepine receptor that constitutes a microglial marker	Significantly increased of [¹¹ C]-(R)-PK11195 binding potential in bipolar patients versus controls in the right hippocampus; a trend toward the same finding was present for the left hippocampus	This study provides strong evidence for the presence of neuroinflammation in bipolar I disorder, but the sample was small. Furthermore, the cross-sectional nature of the design does not allow to establish causality

ELISA: enzyme-linked immunosorbent assay; CSF: cerebrospinal fluid; HSV: herpes simplex virus; CMV: cytomegalovirus; EBV: Epstein-Barr virus; IL-1 β : interleukin-1 beta; IFN- γ : interferon- γ ; MCP-1: monocyte chemoattractant protein-1; sCD 14: soluble cluster of differentiation 14; TIMP-1: tissue inhibitor of metalloproteinases-1; TIMP-2: tissue inhibitor of metalloproteinases-2; PET: positron emission tomography; AI: antibody index; TNF- α : tumor necrosis factor alpha; MSD: meso scale discovery; YKL-40: human cartilage glycoprotein-39

Despite our care to avoid studies of peripheral immunity in bipolar disorder, many of these studies appeared through our search. This was due to the fact that many of these papers speak about neuroinflammation without actually showing it. In many of these fascinating and fashionable papers, the question of how chronic inflammation and immune derangement would “enter” the brain and produce neuroinflammation is much

talked-about, but not proved. Another striking result of our search is the high number of reviews ($n = 96$), and especially of those focusing on this issue ($n = 40$). Most reviews mix-up peripheral and brain studies, reaching unwarranted conclusions. The activation of microglia must not be taken only as evidence of neuroinflammation, because it might also be consistent with a host of other functions, such as remodeling the

brain microstructure, contributing to plasticity and synaptic formation, and responses to environmental challenges.^[48] So, it is possible that neuroglial activation and precisely aberrant neuroglial function, may be responsible for some neuronal miswiring that is consistent with some psychotic symptoms that are frequently observed in bipolar disorder.

Summarizing, the evidence for the existence of neuroinflammation in bipolar disorder is more yes than no. However, the hypothesis of a preexisting peripheral inflammation passing in the brain and establishing a disease mode function, thus maintaining the disorder, is a mere supposition that is not based on evidence. Whereas neuroinflammation might lead to bipolar symptoms, it is not always true that neuroinflammation causes bipolar disorder, and it might be that only rarely is so. It is more likely that stress interacts with the personal constitution and epigenetic factors to cause both neuroimmune dysreactivity and bipolar disorder in susceptible individuals. As de Baumont *et al.*^[36] stated, schizophrenia and bipolar disorder may “arise from shared genetic factors, but that the resulting clinical phenotype is modulated by additional alterations mediated by microglia, possibly caused by interference of environmental factors at different times during neurodevelopment and early life, and/or epistatic interactions among groups of genes and environment.” All this should be borne in mind when projecting investigations to explore the relationship between neuroinflammation and bipolar disorder.

CONCLUSION

This review attempted to answer the question of whether neuroinflammation plays a role in the pathophysiology of bipolar disorder. Direct and indirect evidence points to some degree of possibility that this is the case, but studies heretofore are much heterogeneous in their methodology and conclusions, thus suggesting caution. It appears that the topic of neuroinflammation in bipolar disorder is a much under-investigated but over debated and highly reviewed issue. Finally, PubMed should be trusted, but alternative search engines should be used, lest precious articles are lost.

Acknowledgments

The authors wish to thank Ms. Mimma Ariano, Ms. Ales Casciaro, Ms. Teresa Pioreschi, and Ms. Susanna Rospo, Librarians of the Sant'Andrea Hospital, School of Medicine and Psychology, Sapienza University, Rome, for rendering precious bibliographic material accessible, as well as their Secretary Ms. Lucilla Martinelli for her assistance during the writing of the manuscript.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

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Neuroinflammatory modulators of oligodendrogenesis

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ABSTRACT

Oligodendrocytes are key neural cells that are responsible for producing myelin sheaths that wrap around neuronal axons in the central nervous system. Myelin is essential to insulate neurons and maintain a fast and saltatory propagation of action potentials along the axon. However, oligodendrocytes are very susceptible to damage, and thus demyelination may arise from a brain lesion or a neurodegenerative disorder. Consequently, demyelination produces a loss of axonal insulation leading to sensory or motor neuron failure. During adulthood, there are two main sources of oligodendrocytes: parenchymal oligodendrocyte precursor cells (OPCs) and subventricular zone derived OPCs. In this review, we will discuss oligodendrogenesis derived from these two sources, and also highlight their main extrinsic and intrinsic modulators. In addition, the neuroinflammatory mediators of oligodendrogenesis will also be assessed.

Key words: Demyelination, inflammation, neural stem cells, oligodendrocyte, remyelination

INTRODUCTION

Oligodendrocytes are the myelin-forming cells of the central nervous system (CNS). They are the last brain cells to be generated during development, making myelination a late event in brain maturation.^[1] Their cholesterol-rich membrane loops around neuronal axons creating a myelin sheath, which is a multilamellar spiral structure that protects neurons, ensures their survival^[1] and provides electrical insulation that enables faster transmission of action potentials along axons.^[2] Oligodendrocytes are essential for proper brain functioning and are easily affected by oxidative stress, so that demyelination is often a secondary event to brain lesions or pathologies.^[1] However, new oligodendrocytes are continuously generated during adulthood, which restore insulation of demyelinated axons and/or remodel existing myelin, an important role for functional plasticity, learning, and memory

formation. This process is called remyelination, one of the few spontaneous processes of regeneration that take place in the adult CNS.^[3] New oligodendrocyte production is therefore enhanced in response to a pathological insult such as demyelination. During the progression of a demyelinating disease, such as multiple sclerosis (MS), several inflammatory modulators are released by a variety of brain cells impacting the determination, proliferation, differentiation, migration, and maturation of oligodendrocyte precursor cells (OPCs), ultimately resulting in remyelination. New myelinating oligodendrocytes are derived from two main cell sources: early postnatal-derived OPCs that are present all over the brain parenchyma;^[4] and new OPCs that are continuously originated from a distinct group of transit-amplifying progenitors in the subventricular zone (SVZ) of the lateral walls of the lateral ventricles.^[4,5] In response to demyelination, both parenchymal OPCs and SVZ-derived OPCs produce new oligodendrocytes to recover from myelin loss.^[5]

Oligodendrogenesis is a process that is regulated by extrinsic and intrinsic factors. The main external stimuli are morphogens, growth factors, and extracellular

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10.4103/2347-8659.167311

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Cite this article as: Armada-Moreira A, Ribeiro FF, Sebastião AM, Xapelli S. Neuroinflammatory modulators of oligodendrogenesis. *Neuroimmunol Neuroinflammation* 2015;2:263-73.

Received: 14-02-2015; **Accepted:** 15-06-2015

matrix elements while the internal stimuli important for oligodendrocyte formation are transcription factors and epigenetic regulators. Therefore, studying modulators capable of stimulating OPCs are of paramount importance and fundamental for future therapies concerning inflammatory and neurodegenerative disorders in which myelin sheaths are affected.

OLIGODENDROGENESIS FROM OPCs DURING DEVELOPMENT AND IN THE ADULT BRAIN PARENCHYMA

During CNS development, and also throughout adulthood, oligodendrogenesis is derived from OPCs. OPCs are a subtype of glial cell, characterized by the expression of the platelet-derived growth factor receptor α (PDGFR α), and the neuron-glia antigen 2 (NG2) proteoglycan [Figure 1].^[6] Other known markers for these cells are the O4 antigen and the transcription factors Olig1, Olig2, and Nkx 2.2.^[7] It should be noted that, these markers can be expressed in other cells, a combination of markers should be used to unambiguously identify OPCs.^[1] In the adult brain, OPCs comprise 3-8% of the total number of cells^[8] and are prevalent in the hippocampus and in all layers of the neocortex.^[9]

In the developing forebrain of mice, the entire oligodendrocyte population is generated from three phases of OPC proliferation and migration. The first phase occurs at embryonic day 12.5 (E12.5) and consists of a “wave” of OPC production, originated from ventral ganglionic eminences.^[10,11] At E15.5, the second phase takes place, emerging from the lateral and caudal ganglionic eminences.^[12] Finally, the third phase happens after birth, with origin in the cortex.^[12] These three phases are responsible for the generation of most adult oligodendrocytes in mice, which will migrate and populate most of the future brain.^[1]

In human CNS development, oligodendrocyte differentiation and maturation follow similar paths to rodents.^[13] This process has its beginning in the

second trimester of gestation and spans into birth and adulthood.^[14,15] Specifically, at 9 gestation weeks, early OPCs (NG2 and PDGFR α positive) arise from the ganglionic eminence and migrate to the cortex in the following weeks. Late OPCs, which show O4 immunoreactivity, are first detected in a small percentage at 15 gestation weeks, gaining more density in midgestation (c. 20-22 gestation weeks), especially in the subplate layer directly under the cortical plate. Finally, myelin basic protein-positive oligodendrocytes are rare at midgestation but show a steady population growth from that point on. Indeed, the first myelin sheaths can be found around 18 gestation weeks in the thalamus, spreading to the internal capsule at 21 gestation weeks.^[15]

Given the nature of oligodendrocyte production, one question arises: are the OPCs involved in these different phases functionally equivalent? There is evidence that each phase of OPC production can lead to the myelination of distinct brain regions,^[16] suggesting the existence of functionally different subpopulations of OPCs that serve separate functions. In fact, a study conducted in mice targeted differentially ventrally-derived OPCs (vOPCs) and dorsally-derived OPCs (dOPCs), as well as the oligodendrocytes generated by each class of OPCs (vOLs and dOLs respectively). This study shows that while vOPCs and dOPCs appear to have the same electrical properties, their migration and settling patterns are significantly different, to the point that vOLs and dOLs populate different forebrain and spinal cord regions at different timepoints during development (for example, while in adulthood the corticospinal and rubrospinal tracts are myelinated by dOLs; during early postnatal life, these regions are actually myelinated by vOLs).^[16] On the other hand, it has been shown that, if one of these subpopulations is eliminated, neighboring OPCs of different origins rapidly migrate and proliferate to generate the regular number of oligodendrocytes in the mature brain,^[12] which could imply that the subpopulations of OPCs are functionally equivalent.

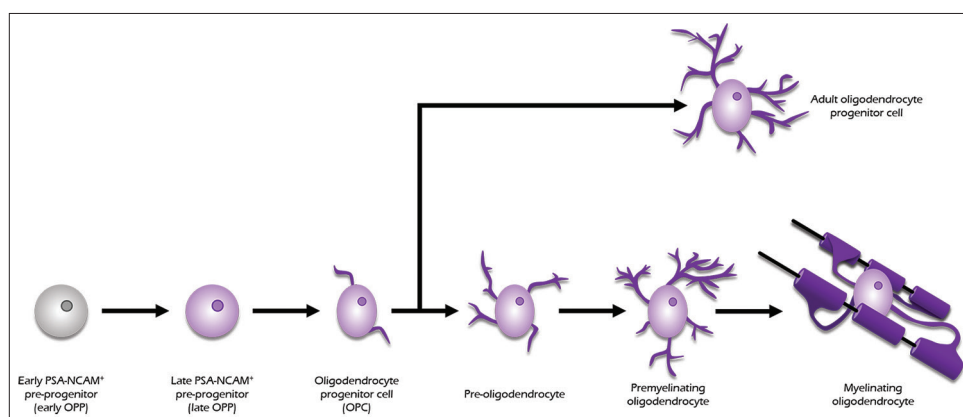


Figure 1: Diagram of the oligodendrocytic lineage progression: from early oligodendrocyte precursor cell to functioning mature myelinating oligodendrocyte

After CNS development, a small fraction of OPCs remains undifferentiated, in an immature slowly proliferative or quiescent state.^[17] These adult OPCs are morphologically equivalent and express the same markers as the OPCs present during development.^[18] However, they differ from the developing OPCs in growth factor responsiveness, migration capacity, and cell cycle length.^[19-21] Their cell density, although stable throughout adult life, is higher in white matter than in gray matter.^[22,23] Indeed, it has been shown that adult OPCs present a higher rate of proliferation in white matter, which is a possible explanation for the difference in cell density.^[22] It is possible to further divide white matter OPCs and gray matter OPCs by their characteristics. While white matter OPCs are proliferative and eventually lead to adult oligodendrogenesis, gray matter OPCs remain quiescent and immature.^[22]

Given these findings, could adult OPCs be a heterogeneous population, possibly with several distinct functions? Some studies show that the different characteristics observed in adult OPCs can be the result of environmental signals. Specifically, gray matter environment is described as an inhibitor of OPC proliferation and differentiation while white matter environment seems to favor OPC maturation.^[17,22,23] These differences may be linked to intrinsic cell mechanisms or to environmental cues. While there seem to be differences in the local microenvironment surrounding OPCs in white and gray matter, the different characteristics of white matter and gray matter OPCs can also be explained by intrinsic mechanisms, such as receptor desensitization. For instance, it is known that, in the developing spinal cord, PDGF-A mRNA has higher expression in the gray matter,^[24] which can lead to desensitization of the receptor (PDGFR α) and prolonged impairment of gray matter OPCs maturation.^[25] However, there are indeed molecular differences between white matter OPCs and gray matter OPCs, namely in the resting membrane potential and ion channels expression.^[26,27] Concerning the ion channels, two subpopulations of adult OPCs have been described: one completely devoid of voltage-gated Na⁺ channels, and another with functional channels, able to react to action potentials. Consequently, this second subtype can sense neuronal activity through axonal input and is more sensitive to ischemia.^[26] Another study corroborating the existence of functionally different subtypes shows that white matter OPCs can generate myelinating oligodendrocytes even if they are transplanted into other brain regions. Gray matter OPCs, in contrast, remain less efficient even if transplanted into white matter.^[28] Therefore, it seems that white matter OPCs are more prepared to generate new myelinating oligodendrocytes than gray

matter OPCs. What is the role of OPCs in gray matter remains a question to be explored.

In response to a demyelinating insult, the remyelination process is activated. New myelinating oligodendrocytes are mainly generated from early postnatal-derived OPCs that are present in the brain parenchyma.^[4] In a first phase, quiescent or slow-dividing OPCs are recruited to the damaged areas OPCs start to proliferate, migrate, and populate the demyelinated area. In a second phase, the recruited OPCs start to differentiate into mature oligodendrocytes as they form myelin sheaths around demyelinated axons.^[29] Oligodendrocytes derived from parenchymal OPCs are only able to migrate short distances, just populating damaged areas in the proximity of their progenitor cells.^[5]

OLIGODENDROGENESIS DERIVED FROM ADULT SUBVENTRICULAR ZONE NEURAL STEM CELLS

After birth, OPCs can be produced by adult neural stem cells (NSC), which are self-renewing, multipotent cells that generate most of the cells of the nervous system, such as neurons, astrocytes, and oligodendrocytes.^[30] These NSCs can divide in three different ways: symmetrically, originating in two new NSCs (expansion, symmetrical self-renewal); asymmetrically, originating one NSC and one differentiated cell (maintenance, asymmetrical self-renewal); or symmetrically, originating two differentiated cells (extinction, symmetrical commitment). Depending on the activation of specific signaling pathways and the presence of differentiation-inducing molecules, NSCs are capable of differentiating into cells of neuronal (neurogenesis) and glial (gliogenesis) lineages, particularly oligodendrocytes (oligodendrogenesis).^[31]

Neural stem cells exist in discrete regions of the adult mammalian brain where neurogenesis and oligodendrogenesis are highly regulated.^[32] The brain regions where these processes take place, that is where the NSC pools can be encountered, are called neurogenic niches. In adulthood, there are two main neurogenic niches in the brain: the SVZ of the lateral ventricles, and the subgranular zone of the dentate gyrus (DG) of the hippocampus.^[33]

In the SVZ, the NSC pool comprises type B cells, which are quiescent NSCs that originate type C cells, which are fast dividing transient amplifying cells.^[34] Most of these C cells will then differentiate into neuroblasts (type A cells), migrate along the rostral migratory stream^[35] to the olfactory bulb, and terminally differentiate into interneurons.^[36,37] SVZ-derived oligodendrogenesis originates from a minority of C cells that do not follow the previously explained cellular fate. Instead, they

produce OPCs, which migrate radially out of the SVZ into the surrounding cortex and white matter [Figure 2].^[5,38,39]

It should be noted that one NSC can generate either oligodendrocytes or neurons exclusively^[13] and that the number of oligodendrocytes produced by the SVZ NSC pool is significantly inferior to the number of olfactory interneurons.^[5] The relative quantity of oligodendrocytes and neurons is area dependent: while in the posterior zone of the SVZ, the ratio is one oligodendrocyte to three neurons; in the rostral zone, this ratio is 1:30.^[5] The ratio also changes dorsoventrally, due to environmental cues. The dorsal part of the SVZ is Wnt enriched, which favors OPC commitment.^[13] On the other hand, the ventral part is more exposed to bone morphogenic proteins (BMP), which inhibits OPC specification.^[40]

Contrary to the parenchymal OPCs, SVZ-derived OPCs, although a minority in the brain, can migrate long distances into the corpus callosum, striatum, and fimbria fornix, where they continue to divide or differentiate into mature myelinating and nonmyelinating oligodendrocytes.^[5] Adult SVZ progenitor cells mostly generate neuronal lineage cells and only a few oligodendrocytes; however, progenitor cells show some lineage plasticity in pathological conditions. In a demyelination context, OPC production in SVZ is favored to the detriment of neuronal precursor cells. These OPCs then migrate to the affected areas, where they differentiate into

oligodendrocytes, contributing to the remyelination process [Figure 3].^[4,5,41,42]

The generation of new oligodendrocytes from the SVZ is possible because adult NSCs, from which OPCs are derived, are embedded in the specialized and diverse microenvironments of the SVZ niche, which is responsible for regulating NSCs and their progenies' self-renewal and differentiation by receiving information from the brain and other tissues.^[43-45]

The first specialized microenvironment is the apical ependymal compartment where slow-dividing type B cells are in direct contact with the cerebrospinal fluid (CSF) present in the space of the lateral ventricles, through a specialized apical process surrounded by ependymal cells.^[43,44,46] The adult choroid plexus (CP) expresses and secretes to the CSF not only numerous trophic factors but also cytokines, which can influence the behavior of SVZ progenitor cells, modulating the self-renewal capacity, proliferation, and differentiation [Figure 2].^[43,44] Interleukin-1 β (IL-1 β) is one of the cytokines that are secreted and regulated by CP, which acts on type B cells through binding to IL-1 receptors to up-regulate vascular cell adhesion molecule 1 expression, modulating type B cells adherence to the SVZ niche. Insulin-like growth factor 2 (IGF-2) is also present in the adult CSF and regulates SVZ progenitor proliferation.^[44] Moreover, CP also secretes guidance molecules, such as chemorepulsive factors and chemoattractants, which regulate SVZ NSCs and their progenies' migration.^[42] CP changes its secretome under pathological conditions, leading to a different CSF cellular and molecular composition that will then influence the SVZ niche population. For example, during peripheral tissue inflammation, inflammatory information from the blood can have an impact in the CNS.^[47,48] Peripheral inflammation elicits the up-regulation of cytokines, adhesion factors, and signaling pathway genes, such as tumor necrosis factor- α (TNF- α), IL-1 β , and small inducible cytokine A2 transcripts that are under the regulation of the NF- κ B cascade, in the CP.^[47] Then the CP, through changes in CSF composition, will affect the SVZ niche population.

The second SVZ niche component is the basal vasculature, composed of blood vessels and a basal lamina rich in laminin.^[42] Here, type B cells have a long specialized basal process through which they interact with blood vessels, and fast-dividing/transit-amplifying type C cells are also in very close proximity to blood vessels.^[43-46] Endothelial cells secrete several diffusible signals, such as fibroblast growth factor-2 (FGF2),

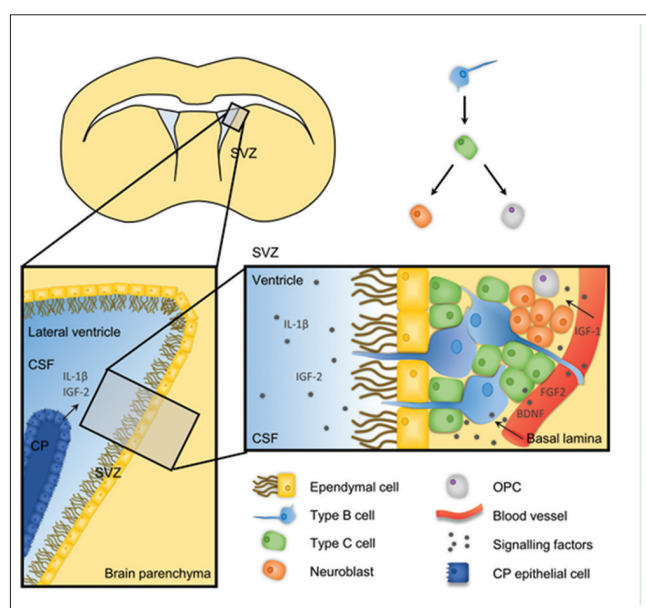


Figure 2: Schematic representation of the adult subventricular zone (SVZ) neurogenic niche. SVZ lines the lateral ventricles and is comprised of three main cell types: the multipotent type B cells that give rise to type C cells (fast dividing transient amplifying cells) that generate type A neuroblasts. Type B cells interact basally with blood vessels and apically with the cerebrospinal fluid (CSF). The composition of the CSF is modified by the choroid plexus, a thin vascularized membrane mainly composed by epithelial cells, which secretes several cytokines and trophic factors to the CSF

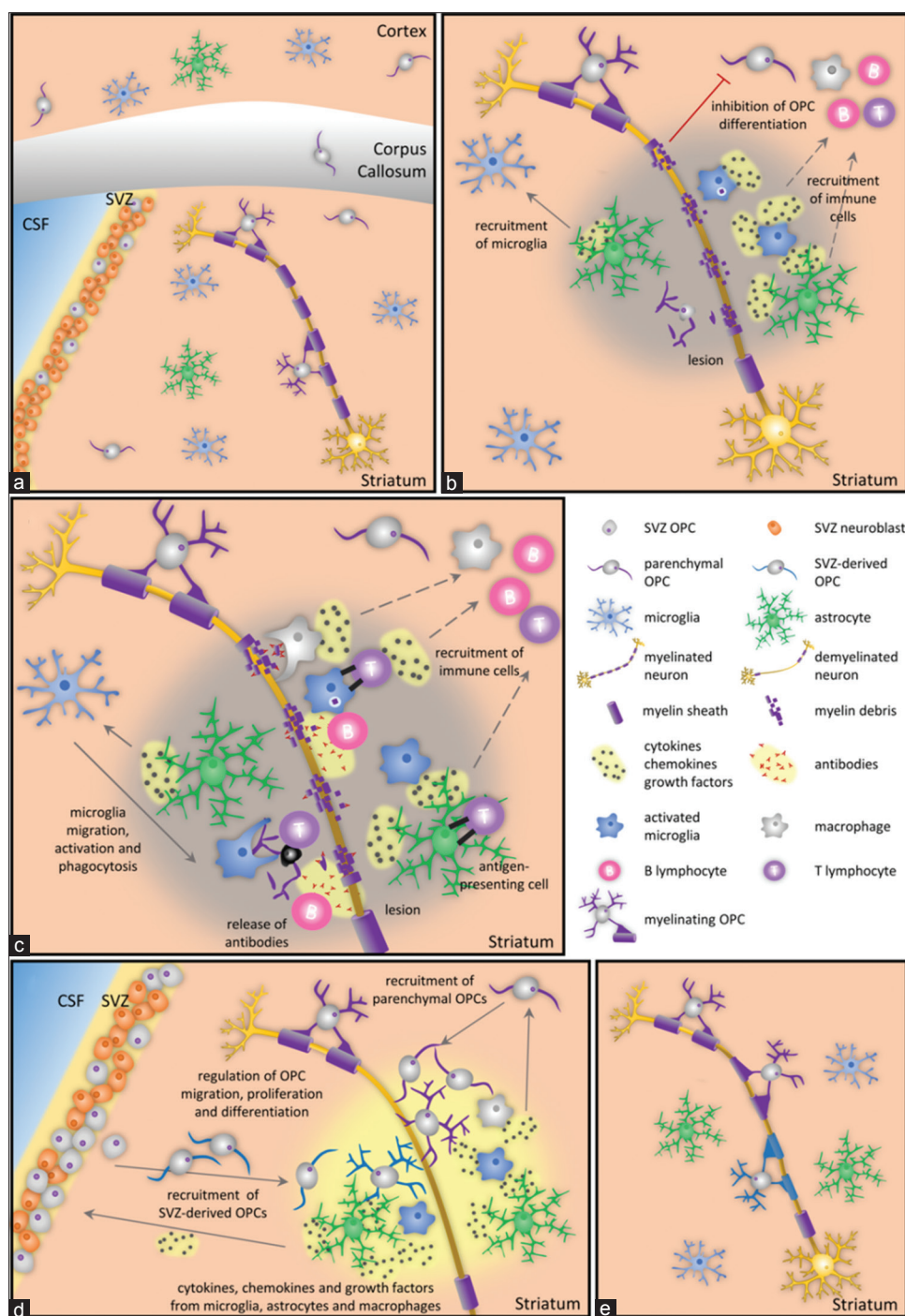


Figure 3: Schematic representation of the demyelination and remyelination processes. (a) in a basal condition, type A neuroblasts and oligodendrocyte precursor cells (OPCs) are continuously generated from neural stem cells, with neuroblasts being the great majority progeny; (b) after a demyelinating episode, nearby astrocytes and microglia are activated and release inflammatory mediators, increasing the permeability of the blood-brain barrier (BBB). By releasing chemokines, astrocytes recruit more microglial cells to the demyelinated area; (c) which phagocyte dead cells and myelin debris, as do macrophages that have crossed the BBB. Astrocytes and macrophages act as antigen-presenting cells to T lymphocytes that are then activated and attack the myelin sheath and dying cells. B lymphocytes produce autoantigens against myelin antigens functioning as opsonins; (d) microglia, macrophages, and astrocytes release mediators that mobilize parenchymal OPCs to proliferate, migrate, and differentiate into new myelinating oligodendrocytes in the demyelinated area. After a demyelinating episode, OPC production in the subventricular zone (SVZ) is favored in detriment of neuronal precursor cells. These SVZ-derived OPCs migrate to the demyelinated areas where they differentiate into mature myelinating oligodendrocytes; (e) new myelinating oligodendrocytes form a thinner myelin sheath around the demyelinated axon

IGF-1, brain-derived neurotrophic factor (BDNF), chemokines, among others, which also influence stem cell self-renewal, proliferation, and fate determination in the SVZ [Figure 2].^[44-46,49] In addition, cerebral endothelial cells promote differentiation of SVZ NSCs into oligodendrocytes, being important for the proliferation and migration of OPCs.^[42]

The extracellular matrix composition of the vascular basal lamina makes the basal lamina an important integration site for the exchange of signals between the SVZ progenitors and the main compartments of the SVZ niche, the vasculature and the CSF, because it provides, stores, and compartmentalizes growth factors and cytokines.^[42,45]

By changing the composition of CSF or the blood stream, it would be possible to modulate proliferation and or differentiation of SVZ progenitors with an impact on demyelinating diseases; however, these modifications should be tightly regulated to maintain brain homeostasis.

MODULATORS OF OLIGODENDROGENESIS

Extrinsic factors

The extrinsic factors include morphogens, growth factors, and signaling molecules delivered through blood vessels or associated with the extracellular matrix. For instance, during the development of both the brain and spinal cord, the relative levels of Sonic hedgehog (Shh), BMP, and Wnt/ β -catenin have been shown to play roles in oligodendrocyte determination.^[1] The motoneuron domain (pMN) is a restricted domain of the ventral ventricular zone of the embryonic spinal cord, and in mice, cells in this domain express the transcription factors Olig1 and Olig2.^[50] In this zone, Olig2 expression is crucial for the production of motoneurons and oligodendrocytes. In order for this transcription factor to induce oligodendrogenic cell fate instead of neuronal differentiation, a switch must occur.^[1] As seen in both mice and zebrafish, this switch is highly dependent on the level of Shh in the environment^[51] and on the Notch/delta pathway, which restricts the production of motoneurons, thus allowing oligodendrocyte determination.^[52] On the other hand, this switch is repressed if high level of BMPs and Wnt are locally present.^[53] It has also been shown that, during brain development, Shh promotes the generation of ventrally derived OPCs,^[11] while Wnt/ β -catenin and BMPs inhibit it. Curiously, in the adult brain, there is an apparent contradiction since Wnt3 (from the Wnt family) promotes oligodendrocyte specification in the SVZ.^[13]

Other extrinsic factors modulate oligodendrogenesis in the adult SVZ. Evidence shows that both factors secreted by blood vessels and factors connected to the extracellular matrix are capable of favoring OPC commitment.^[13,54,55] One of these factors is laminin, an element of the extracellular matrix. A study in mice shows that the elimination of laminin α 2-subunit leads to a reduction of the OPC population in the SVZ.^[56] Other trophic factors, such as PDGF^[57,58] and epidermal growth factor (EGF),^[59,60] contribute indirectly to oligodendrocyte lineage determination, through promoting OPC proliferation and maturation. Additionally, IGF-1 also contributes to oligodendrogenesis by blocking BMP signaling, both *in vivo* and *in vitro* [Table 1].^[61]

Intrinsic factors

The intrinsic factors that modulate oligodendrogenesis include transcription factors and epigenetic

Table 1: Main modulators of oligodendrogenesis in the adult brain

Signal	Effect
Extrinsic	
Morphogens	
Wnt ^[62]	Stimulates NSC proliferation/self-renewal
Notch ^[63,64]	Required for NSC proliferation, maintenance
Shh ^[65]	Required for NSC maintenance and OPC production
SIRT1 ^[58]	Inhibits oligodendrogenesis
BMP ^[66]	Inhibits oligodendrogenesis
Growth factors	
EGF ^[67]	Stimulates OPC proliferation and migration
FGF-2 ^[68]	Induces progenitor cell proliferation
IGF-1 ^[61]	Stimulates oligodendrocyte differentiation
PDGF ^[58]	Induces OPC proliferation and differentiation
Extracellular matrix elements	
Laminin ^[56]	Promotes OPC generation
Intrinsic	
Transcription factors	
ASCL1 ^[69]	Favors oligodendrocyte fate
Nkx6.1/6.2 ^[70,71]	Required for oligodendrocyte and motoneuron production in the pMN
Sox8/9 ^[72]	Promotes glial specification
Epigenetic markers	
miRNA-7a ^[73]	Promotes OPC commitment
Histone methylation ^[74]	Favors OPC production
Histone acetylation ^[75]	Inhibits OPC differentiation

NSC: neural stem cell; OPC: oligodendrocyte precursor cell; pMN: progenitors of motor neurons; Shh: sonic hedgehog; BMP: bone morphogenic protein; EGF: epidermal growth factor; FGF-2: fibroblast growth factor-2; IGF-1: insulin-like growth factor-1; PDGF: platelet-derived growth factor; ASCL1: achaete-scute homolog 1

regulators. The main transcription factor involved in oligodendrocyte determination is Olig2. This basic helix-loop-helix (bHLH) factor is induced by Shh^[76] and expressed in every stage of oligodendrocyte maturation, from OPC to myelinating oligodendrocyte.^[1] In the majority of the CNS, inactivation of Olig2 during development leads to a reduction in OPCs.^[76-78] In contrast, overexpression of Olig2 in neuroepithelium leads to enhanced OPC production in the CNS.^[79] Furthermore, the presence of Olig2 is sufficient to reprogram rat and mouse fibroblasts into induced OPCs.^[80,81] Although this factor is crucial to oligodendrocyte differentiation, Olig2 knockout mice are still able to produce some OPCs in the hindbrain, possibly through Olig1 compensation.^[76]

Another important transcription factor is Achaete-scute homolog 1 (Ascl1 or Mash1), which is also a bHLH factor.^[1] During development, absence of Ascl1 leads to a reduction of OPC production in the brain and spinal cord.^[82,83] However, this reduction can be compensated to normal values by Ascl2 and 3.^[83] After birth, Ascl1 is only expressed in C cells and OPCs in the SVZ^[69] and similarly to what happens during CNS development, elimination of this transcription factor leads to decreased OPC generation.^[69]

The Nkx and Sox families also play roles in oligodendrogenesis, even though they are not critical.^[84] Ablation of Nkx6.1/Nkx6.2 blocks the production of both oligodendrocytes and motoneurons in the pMN.^[70,71] Additionally, Sox9 knockout mice show deficits in glial specification, presenting a reduced number of oligodendrocytes and astrocytes.^[72] Furthermore, if there is a Sox8/Sox9 double inactivation, no oligodendrocytes are produced, which suggest that Sox8 and Sox9 serve redundant functions in relation to oligodendrogenesis.^[72]

In the last few years, epigenetic modulation of oligodendrogenesis has been gaining some importance, namely regarding the modulation by microRNA and histone modifications. One of the most described microRNA in oligodendrogenesis is miRNA-7a. This miRNA is highly enriched in OPCs and overexpressing it in neuronal progenitors during brain development promotes OPC commitment, both *in vivo* and *in vitro*.^[73] In contrast, blocking miRNA-7a function inhibits OPC generation and favors neuronal progenitors.^[73]

Histone modifications can also be important for oligodendrogenesis. For instance, it has been shown that oligodendrocyte production from NSCs, instead of other cellular fates, depends on histone deacetylases (Hdac) activity.^[85] Similarly, a study using Enhancer of zeste homolog 2 (Ezh2), a polycomb group protein involved in gene silencing through histone methylation, provided evidence that a higher rate of histone methylation (via Ezh2 overexpression) leads to an increase in oligodendrocyte production [Table 1].^[74]

IMMUNE MEDIATORS OF OLIGODENDROGENESIS

When white matter is damaged as a result of an infection, a trauma or a neurodegenerative disease such as MS or vascular dementia, microglia, the brain's innate immune cells, are activated. Microglia removes infectious agents and apoptotic cells, through phagocytosis and by producing reactive oxygen species (ROS), TNF- α , nitric oxide (NO), IL-1 β , and prostaglandin E2.^[86] When microglia are chronically activated, sustained release of inflammatory factors, cytokines, and chemokines compromises the blood-brain barrier (BBB), resulting in vascular permeability to blood and circulating immune cells, such as T and B lymphocytes and macrophages, as well as recruitment of these peripheral immune cells to the lesion site.^[87] By recognizing their specific autoantigen presented by MHC class II molecules on the surface of antigen presenting cells, CD4⁺ T cells are activated and attack the myelin sheath.^[87] Antigens on MHC class I molecules also activate CD8⁺ T cells

to attack myelin, demyelinated axons, and dying motoneurons through the activation of the perforin pathway, the delivery of granzymes into the cells, or by Fas-Fas ligand interactions.^[86] Additionally, B lymphocytes produce autoantibodies against myelin antigens, degrading myelin sheath. Because infiltrating effector T cells, microglia and macrophages release cytokines and chemokines, inflammation will be exacerbated and consequently more T cells, B cells, and innate immune cells will be recruited to the lesion site, contributing to chronic neuroinflammation, and neurodegeneration [Figure 3].^[87]

Neuroinflammatory responses can be deleterious for cell survival, resulting in irreversible extensive damage to the brain, especially if they are prolonged in time.^[88] However, they have also been described as having beneficial effects and as being critical for the activation of the brain repair process, such as for the remyelination program. As a result of white matter damage, there is an accumulation of apoptotic cells and myelin debris in the lesion site, which have been demonstrated to be inhibitory to axonal regeneration, as well as affecting OPC differentiation into mature myelinating oligodendrocytes. However, through phagocytosis of cellular debris and apoptotic cells, microglia and brain infiltrating macrophages function toward repairing the damaged tissue, by promoting a pro-regenerative environment, promoting OPC recruitment and differentiation, thus favoring remyelination and axonal regeneration.^[29,89-91] For instance, ROS hydrogen peroxide (H₂O₂), released by macrophages and microglia, destroys damaged cells, affecting not only healthy surrounding cells, but also promoting proliferation and differentiation of NSCs into oligodendrocytes.^[92] Astrocytes have also been described to have an important, yet controversial, role in demyelinating diseases. Astrocytes have been shown to have an important role in both demyelination and remyelination.^[93-95] On the one hand, because astrocytes are antigen-presenting cells and release cytokines and chemokines, they contribute to myelin damage through an immune-mediated demyelination by recruiting inflammatory cells, such as T lymphocytes, microglia, and macrophages to the lesion site.^[95] On the other hand, astrocytes are described as being responsible for a successful remyelination through the regulation of the clearance of myelin debris^[94] and oligodendrogenesis in the lesion site [Table 2].^[93] Astrocytes promote OPC migration, proliferation, and differentiation after demyelination by secreting several factors, which have an impact on myelin repair.^[93] In fact, several cytokines and chemokines produced by microglia, macrophages, and astrocytes in response to brain injury have been described as having an essential

Table 2: Main immune mediators of oligodendrogenesis in the adult brain		
Signal	Cells	Function
CXCL10	Produced by astrocytes ^[94]	Promotes the migration of microglia and macrophages to the demyelinated areas to phagocytose the damaged myelin sheaths ^[94]
CXCL12	Produced by microglia and astrocytes ^[96] Binds to CXCR4 on the surface of OPCs ^[97]	Mobilizes and stimulates OPC differentiation into oligodendrocytes ^[96]
CXCL1	Produced by astrocytes ^[98] Binds to CXCR2 on the surface of proliferating OPCs and reactive astrocytes ^[98]	Prevents apoptosis of oligodendrocytes in demyelinated white matter areas ^[99]
CXCR2 blockade		Enhances OPC differentiation ^[100] Reduces the extent of microglia activation and the number of infiltrating inflammatory cells ^[100]
CNTF-IL-6 family	Produced by astrocytes ^[101]	Protects axons and OPCs ^[102,103] Controls SVZ-derived progenitor cells and OPC mobilization and migration toward demyelinated areas ^[104] Promotes oligodendrocyte maturation ^[93]
IL-1 β	Mainly produced by microglia, macrophages, astrocytes, and oligodendrocytes ^[105]	Promotes OPC protection and differentiation by stimulating microglia, macrophages, and astrocytes to produce growth-promoting factors, such as IGF-1, and also TNF- α and nitric oxide ^[106-108]
IL-4	Produced by T cell ^[109]	Promotes microglia activation which produces IGF-1 ^[109]
TNF- α	Produced by microglia and astrocytes ^[110] Binds to TNFR2 present on the surface of OPCs ^[111]	Promotes proliferation and accumulation of OPCs ^[111]
TNF- α depletion		Decreases proliferation of NG2+immature oligodendrocytes ^[111]

OPC: oligodendrocyte precursor cell; SVZ: subventricular zone; IGF-1: insulin-like growth factor-1; TNF- α : tumor necrosis factor- α ; NG2: neuron-glia antigen 2; IL-1 β : interleukin-1beta; IL-4: interleukin-4; TNFR2: tumor necrosis factor receptor 2; CNTF: ciliary neurotrophic factor; IL-6: interleukin-6

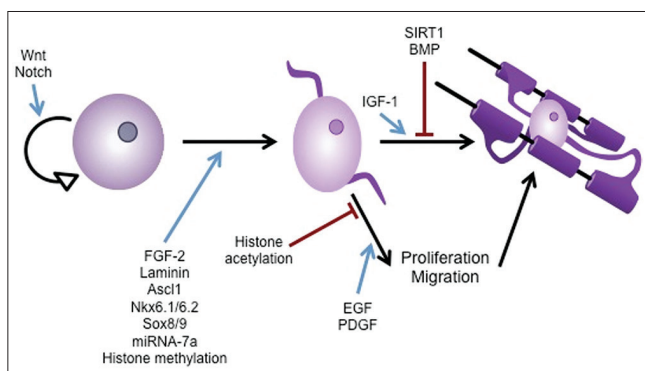


Figure 4: Schematic representation of regulators of oligodendrogenesis in the adult brain. Wnt and Notch positively regulate neural stem cells self-renewal. Fibroblast growth factor-2, laminin, Ascl1, Nkx6.1/6.2, Sox 8/9, miRNA-7a and histone methylation positively modulate oligodendrocyte fate. Histone acetylation inhibits while epidermal growth factor and platelet-derived growth factor receptor α promotes oligodendrocyte precursor cell differentiation. Sirt1 and bone morphogenic proteins block while Insulin-like growth factor-1 stimulates oligodendrocyte differentiation

role in the repair process, such as regulating OPC proliferation, migration, and differentiation into new myelinating oligodendrocytes [Table 2]. In other words, the inflammatory response seems not merely a cause for demyelination but rather a prerequisite for a successful remyelination.

With the progression of the demyelinating disease, or even with ageing, there is an impairment of the remyelination process due to a decrease of pro-oligodendrogenic signals and an increase of anti-oligodendrogenic signals from immune cells that compromises oligodendrocyte maturation and myelination, leading to high inflammation and cell death.^[42,106,112]

There are still contradictory actions of immune mediators that need to be clarified. The use of limited models of demyelination can be a cause for some of those differences. Thus, it is necessary to develop combined models that will help us to better understand the mechanisms of demyelination and remyelination. How cell immune mediators can be either beneficial or detrimental to the remyelination process and how these responses can change with aging are key questions to successfully develop remyelinating or neuroprotective therapeutic strategies.

CONCLUSION

In the CNS, myelin is produced and maintained by oligodendrocytes. Therefore, new treatments to overcome demyelinating disorders could be primed by targeting this type of cell. In fact, in a demyelinating disorder, parenchymal OPCs spontaneously remyelinate newly nude axons in damaged areas. Moreover, NSCs can be a source of new oligodendrocytes for use in regenerative medicine concerning myelin pathologies. In this review, we have highlighted some of the key players of oligodendrogenesis and that may be used in the future for therapies concerning demyelinating disorders [Figure 4].

Financial support and sponsorship

Sara Xapelli (SFRH/BPD/76642/2011) and Filipa F. Ribeiro (SFRH/BD/74662/2010) were funded by Fundação para a Ciência e a Tecnologia (FCT), Portugal.

Conflicts of interest

There are no conflicts of interest.

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Progress in mechanisms of acetylcholinesterase inhibitors and memantine for the treatment of Alzheimer's disease

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ABSTRACT

Alzheimer's disease (AD) is the most common causes of dementia in the elderly. Currently, only two classes of drugs, acetylcholinesterase inhibitors (AChEIs) and memantine are approved. AChEIs ameliorate cognitive and psychiatric symptoms in AD patients through activation of acetylcholine (ACh) receptors by increased synaptic ACh levels and also have protective effects against glutamate neurotoxicity and inflammation, whereas memantine appears to mainly protect against excitotoxicity and neurodegeneration. Herein, we review the pharmacologic properties of the available AChEIs and memantine, and focus on recent progress in the mechanisms of AD in relation to acetylcholinergic and glutamatergic involvement.

Key words: Alzheimer's disease, amyloid- β peptide, donepezil, memantine, tau

INTRODUCTION

As the world's population ages and life expectancy increases, many individuals are faced with an increased risk of developing dementia. The most common form of dementia is Alzheimer's disease (AD). About 35.6 million people worldwide are now suffering from AD, and the disease is expected to affect 115 million by 2050.^[1] Although this disease has been known about for over a century, there is no curative treatment available so far. At present, four drugs have been approved by the United States Food and Drug Administration for the symptomatic treatment of AD. The acetylcholinesterase (AChE) inhibitors donepezil, rivastigmine, and galantamine are suggested for managing mild-to-moderate AD, whereas donepezil and memantine, a

noncompetitive antagonist of N-methyl-D-aspartate receptors (NMDAR), is indicated for patients with moderate or severe AD.^[1-3]

Pathologically, AD is characterized by atrophy of the hippocampus and neocortex resulting from neuronal and synaptic loss, and the deposition of two proteinaceous lesions: senile plaques containing a core of amyloid-beta ($A\beta$) peptide and neurofibrillary tangles (NFT) composed of hyperphosphorylated microtubule-associated tau protein.^[3,4] It is well-accepted that the accumulation of $A\beta$ protein plays a central role in the pathogenesis of AD. The severity of dementia in AD correlates more strongly with cortical levels of soluble $A\beta$ species than with insoluble amyloid plaque burden.^[5,6] Experimentally, soluble $A\beta$ oligomers have been specifically shown to block hippocampal long-term potentiation (LTP), an electrophysiological correlate of learning and memory, *in vivo* and in brain slices.^[7-9] Understanding precisely how $A\beta$ impairs hippocampal synaptic function could enable the development of potential therapeutics for AD.

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10.4103/2347-8659.167305

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Cite this article as: Li SM, Mo MS, Xu PY. Progress in mechanisms of acetylcholinesterase inhibitors and memantine for the treatment of Alzheimer's disease. *Neuroimmunol Neuroinflammation* 2015;2:274-80.

Received: 14-03-2015; **Accepted:** 29-07-2015

Synaptic loss is one of the pathological hallmarks of AD and the best correlate of cognitive decline^[10,11] suggesting that it is a critical event in the pathophysiology of the disease. Several factors such as A β production, cholinergic dysfunction, NFT accumulation, inflammatory agents, oxidative stress, mitochondrial dysfunction, glutamate-mediated excitotoxicity, and genetic components are reported to be involved in the pathogenesis.^[3] Proposed explanations for the pathophysiology of AD include the cholinergic hypothesis,^[11] the soluble A β oligomers hypothesis,^[12] and the tau hypothesis.^[12,13]

CHOLINERGIC SYSTEM

Acetylcholine (ACh) is widely distributed in the nervous system and plays a critical role in cerebral cortical development, cortical activity, and learning and memory processes. Cholinergic neurons in the brainstem and basal forebrain project axons to many areas of the brain. All functions of the cholinergic system are controlled by the interaction of ACh with two families of receptors: muscarinic ACh receptors (mAChRs) and nicotinic ACh receptors (nAChRs).^[14]

Hippocampal cholinergic activity contributes to memory

Many studies have shown that hippocampal-dependent learning is associated with an increase in hippocampal ACh levels; thus, the elevation of extracellular ACh is thought to reflect hippocampal-dependent memory processes.^[15] Several behavioral studies have demonstrated that lesion-induced damage to cholinergic activity in the basal forebrain and its projections to the neocortex induced learning and memory deficits.^[16] Pharmacological experiments have further confirmed that cholinergic receptor agonists and acetylcholinesterase inhibitors (AChEIs) reduce the severity of cognitive dysfunction,^[17] whereas anticholinergic drugs cause learning and memory deficits in both animal and humans.^[18] Antagonists of mAChRs such as scopolamine, impair the encoding of new memories in animal models of learning and memory and produce cognitive impairment in humans.^[15]

It has been found that pharmacological activation of mAChRs or nAChRs produces an LTP-like increase in synaptic transmission in the hippocampal CA1 region.^[14] Blockade of the presynaptic inhibitory M2/M4 subtype of mAChRs by methoctramine increased ACh levels, and elicited a pharmacological LTP^[19] that shares a similar mechanism with tetanus-induced LTP.^[20] In accordance, both the endogenous release of ACh *in vivo* and the exogenous application of mAChR agonists *in vitro* facilitate the induction of LTP.^[14] Increasing endogenously released ACh specifically activates

the nAChR, facilitating LTP induction.^[21] Selective depletion of medial septum cholinergic neurons caused LTP impairment and glutamatergic synaptic current alteration in the hippocampus.^[22]

Glutamatergic effect

The facilitation of LTP by mAChR activation is thought to be mediated by enhancement of synaptic NMDAR activity either by direct alteration of NMDAR channels^[14] or by induction of Ca²⁺ release from endoplasmic reticulum stores.^[23] The mAChRs also inhibit a variety of potassium channels including small conductance calcium-activated KCa2 channels (SK channels).^[24] Therefore, mAChR activation might induce a parallel long-term enhancement of both α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) and NMDAR-mediated transmission.^[25]

It has been reported that chronic nicotine administration and *in vitro* acute nicotine treatment increases ACh release and enhances NMDAR responses in the hippocampus.^[26] One potential mechanism is that nicotine acts at presynaptic nAChRs to increase glutamate release onto postsynaptic NMDARs.^[27] The activation of nAChRs causes Ca²⁺ entry through receptor channels, which can trigger Ca²⁺ release from intracellular stores.^[28] Multiple lines of evidence also suggest that nicotine could act to ameliorate hippocampal-based learning deficits associated with changes in NMDAR function.^[29] Consistent with these studies, pretreatment with AChE inhibitors has been found to protect cortical neurons from glutamate neurotoxicity in a time- and dose-dependent manner through activation of nAChR.^[30]

Anti-inflammatory effect

The deposition of A β is the result of an imbalance between A β production and clearance. This imbalance leads to a situation of chronic inflammation in the brain. A β deposition contributes to the activation of astrocytes and microglia, and induces the production of a series of proinflammatory cytokines, chemokines, macrophage inflammatory proteins, leukotrienes, reactive oxygen species, and nitric oxide (NO).^[3,31,32] The neuroinflammatory cytokines may not only contribute to neuronal death, but they might also influence classical neurodegenerative pathways such as amyloid precursor protein (APP) processing and tau phosphorylation.

A growing body of studies using donepezil has shown that donepezil does not function solely at the level of ACh, but also has potent anti-inflammatory effects in AD patients, a tauopathy mouse model and lipopolysaccharide (LPS)-treated animals.^[33] Donepezil inhibits proinflammatory gene expression directly

resulting in reduced secretion of tumor necrosis factor- α , NO, and interleukin-1 β in LPS-treated BV2 cells, a murine microglia cell line.^[34] Furthermore, donepezil may inhibit neuronal death and cognitive decline by repressing oligomeric A β -triggered inflammatory pathways in microglia.^[35] Thus, donepezil-mediated attenuation of the release of inflammatory mediators may result from inhibition of protein expression of proinflammatory molecules.

The cholinergic pathway has been shown to exert anti-inflammatory effects on several diseases such as rheumatoid arthritis,^[36] inflammatory bowel disease,^[37] sepsis,^[38] and cardiovascular diseases.^[39] On the other hand, nAChR has been shown to possess anti-inflammatory properties in macrophages,^[40] and the activation of $\alpha 7$ -nAChR significantly inhibits the production of proinflammatory cytokines.^[41] It has been demonstrated that AChEI treatment may favor a Th2-mediated immune response by activating B-lymphocytes and increasing immunoglobulin production.^[42] Galantamine-enhanced microglial A β phagocytosis to promote A β clearance requires the combined action of an ACh competitive agonist and the allosterically potentiating ligand for nAChRs.^[43] Furthermore, plasma anti-A β_{1-42} antibody levels in AD patients were found to be significantly increased after AChEI treatment,^[44] thus suggesting that increasing the endogenous response against A β might provide new insights for AD therapy. Recently, several promising studies have been conducted in phase II and phase III trials using active and passive immunotherapies, respectively.^[45]

GLUTAMATERGIC SYSTEM

Glutamate is one of the most prominent neurotransmitters in the body. It is present in over 50% of the nervous tissue.^[46] It plays a prominent role in a variety of brain functions including synaptic transmission, neuronal growth and differentiation, synaptic plasticity, learning and memory, and other cognitive functions.

The role of the glutamatergic system is to convert nerve impulses into a chemical stimulus by controlling the concentration of glutamate at the synapse. It is well-accepted that LTP induction triggers the NMDAR, and therefore, activates the AMPA receptor in the CA1 region.^[47,48] NMDAR activation allows Ca²⁺ to enter the postsynaptic cell, which subsequently triggers a number of kinase pathways and increases protein transcription. This process strengthens synapses and increases synaptic density, thus allowing fast adaptations of network activity which are critical for information processing.^[49]

Neuroexcitotoxicity

Glutamate excitotoxicity has been hypothesized to have a role in AD pathogenesis. Dysfunction of glutamate transporters has been implicated in this pathway.^[50] It has been reported that hippocampal excitatory amino acid transporter 1 (EAAT1) and EAAT2 expression is significantly reduced in AD,^[49] further reinforcing the notion of a deficit in glutamate clearance in AD brain. In addition to uptake defects, the abnormal release of glutamate from vesicle stores has been implicated as a source of excess extracellular glutamate in AD.^[51] Excessive activation of glutamate receptors leads to a number of deleterious consequences including impairment of calcium buffering, generation of free radicals, and activation of the mitochondrial permeability transition that results in release of apoptogenic proteins into the cytosol, where they trigger caspase-dependent apoptosis or promote autophagy.^[52]

We and others have demonstrated that A β inhibits glutamate uptake in rat cortical synaptosomes, cultured cells, and acute brain slices.^[9] These findings are also consistent with an intracerebroventricular injection of A β into rat brain, which causes a rapid increase in interstitial fluid glutamate levels without altering gamma-aminobutyric acid or aspartate.^[53] The hydrophobic A β oligomers may bind principally to membrane lipids, and thereby, secondarily interrupt the structure and function of synaptic transmembrane transporters (glutamate transporters), leading to increases in extracellular glutamate concentration.

Activation of extrasynaptic receptors

Electron microscopic studies have shown that most plasmalemma receptors are extrasynaptically located, whereas only 1-2% of cell membrane receptors are located at synaptic sites in the hippocampus.^[54] Thus, the chemicals distribute in the extracellular fluid and bind preferentially to these vastly extrasynaptic receptors. Extrasynaptic NMDARs, that is, receptors that are not activated during low-frequency synaptic events, can be found at various locations, such as the cell body, the dendritic shaft, the neck of the dendritic spine, and adjacent to the postsynaptic density. It has been found that synaptic NMDAR activity is extremely important for neuronal survival, whereas the extrasynaptic NMDARs are coupled to cell death pathways.^[55] Using both whole-cell recording and Fluo-4 calcium measurements, we confirmed that A β rapidly and significantly increases extrasynaptic NMDA responses. Soluble A β oligomers activate extrasynaptic NR2B-containing NMDARs, thus increasing downstream calpain signaling and p38 mitogen-activated protein kinase activity.^[9] Several studies have demonstrated that selective

NR2R antagonists prevent A β -induced synaptic dysfunction.^[9] Consistent with these findings, low concentrations of memantine have been shown to target extrasynaptic NMDAR.^[56] Both studies and related reports suggest that A β oligomers disrupt glutamate uptake or trigger glutamate release from glial cells, thus increasing glutamate levels to induce synaptic dysfunction.

BEHAVIORAL AND PSYCHOLOGICAL SYMPTOMS IN DEMENTIA

AD is a neurodegenerative disorder associated not only with a decline in cognitive abilities, but also with frequent manifestation of noncognitive symptoms (such as anxiety, depression, apathy, and psychosis) and other conduct disorders that impair daily living.^[57] It has been proposed that the behavioral and psychological symptoms of dementia in AD patients are due to an imbalance of different neurotransmitters (ACh, dopamine, noradrenaline, and serotonin) in specific brain regions responsible for emotional activities (parahippocampal gyrus, dorsal raphe, and locus coeruleus) and cortical hypometabolism.^[58]

There is increasing awareness that the cholinergic system plays a role in emotion and noncognitive behavior and may be involved in neuropsychiatric symptoms of AD.^[59,60] Other evidence indicates that monoamines, in addition to ACh, are also involved in the pathogenesis of AD and other dementia disorders. The increased activity and altered serotonergic modulation as a result of dopaminergic neurotransmission are associated with agitated and aggressive behavior, respectively.^[61] Chronic administration of donepezil has been reported to reduce the incidence of neuropsychiatric symptoms in patients with mild to moderately severe AD.^[62] Thus, the stimulation of monoaminergic activity in conjunction with AChE activity may provide an effective treatment option for AD and accompanying psychiatric disorders.

COMPARISON OF DONEPEZIL AND MEMANTINE

It is well-established that AChEIs inhibit the action of the ACh-hydrolyzing enzyme AChE to boost ACh levels, and thus, alleviate disease symptoms associated with the progressive loss of cholinergic function in AD. In contrast, memantine acts at the NMDAR to lower the pathologically increased tonic level of excitation of the glutamatergic synapse at rest. Although AChEIs significantly improve learning and memory, memantine behaves like other NMDAR antagonists

and has been reported to inhibit hippocampal LTP,^[63] disrupt cognitive flexibility, and impair memory and locomotor behaviors.^[64,65] Interestingly, a comparison between the effects of donepezil and memantine on spatial memory in the APP23 mouse model using a complex dry-land maze test showed that donepezil treatment significantly improved moving time, whereas memantine improved resting time, thus suggesting that donepezil may influence memory acquisition and memantine influences memory retrieval.^[66]

Donepezil administration increases dopamine and norepinephrine levels in the dorsal hippocampus and decreases extracellular norepinephrine and serotonin levels in the ventral hippocampus.^[67] In contrast, memantine decreases dopamine and serotonin in the dorsal hippocampus and increases 3-methoxy-4-hydrophenylglycol in the ventral hippocampus. Although memantine is recognized as a moderate affinity, noncompetitive, reversible NMDAR antagonist, it has been demonstrated that memantine enhances synaptic transmission in an mAChR-dependent manner in the mouse hippocampus,^[68] and may interact more potent with cholinergic receptors than with NMDAR.^[69] Acute systemic or local administration of either memantine or donepezil significantly increases ACh levels in the neocortex and hippocampus of rats.^[70]

EFFICACY OF DONEPEZIL AND MEMANTINE ON THE TREATMENT OF AD

AChEIs are considered the standard treatment of the mild-to-moderate stage of AD,^[71] whereas memantine is suggested for moderate-to-severe AD patients.^[72] Clinically, donepezil at 10 mg/day significantly improves cognitive, neuropsychiatric, and global function, thus reducing caregiver burden.^[62,72] Increasing the daily dose to 23 mg/day was found to be safe and tolerated in patients with moderate-to-severe AD.^[73,74] Memantine has been found to improve global cognition, functional communication, and some behavioral symptoms (agitation and aggression).^[75,76] Interestingly, donepezil and memantine also have differential behavioral effects: donepezil affects depression, anxiety, and apathy whereas memantine mainly affects agitation, aggression, and delusions.^[77,78] A recent clinical review suggests that combination therapy with donepezil and memantine for AD could be safe and well-tolerated for moderate-to-severe AD.^[79] However, there are no significant benefits of the combination of donepezil and memantine over donepezil alone on cognitive function.^[80] Thus, combination therapy may be more effective in improving neuropsychiatric behaviors than cognition because of their complementary activity.

CONCLUSION

AChE inhibitors ameliorate the cognitive and psychiatric symptoms in AD patients through increased synaptic ACh levels to activate AChRs and protect against glutamate neurotoxicity and inflammation, whereas memantine appears to mainly protect against excitotoxicity and consequent neurodegeneration. AChE inhibitors exert neuroprotective effects by improving cholinergic mediated memory function, enhancing glutamatergic responses and acting as anti-inflammatory agent. Memantine is efficient at preventing the deleterious actions of A β oligomers mainly due to its selectivity for the extrasynaptic NMDARs. Therefore, AChE inhibitors could be used for the earlier to later stages of AD, but memantine should preferentially be used only in the later phase of AD.

Acknowledgments

This work was supported by research grants from the State Key Development Program for Basic Research of China (2011CB510000), the National Natural Science Foundation of China (81271428, 81471292, and 81430021), and a grant supported by assisting research project of science and technology for Xinjiang (201591160).

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

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The endonuclease VIII-like proteins: new targets in the treatment of ischemic stroke?

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ABSTRACT

Oxidative deoxyribonucleic acid (DNA) damage is one of the major causes of neuronal injury in ischemia. The endonuclease VIII-like (NEIL) DNA glycosylases have a specific role in recognition and removal of oxidative DNA damage. The NEIL family includes NEIL1, NEIL2, and NEIL3, that differ in substrate specificity, catalytic efficiency, and subcellular/tissue distribution. This opens for a situation-dependent phenotype in their absence. In this review, we will discuss the current knowledge on the involvement of the NEILs in ischemic stroke and discuss the potential of these enzymes to serve as new targets in the treatment of ischemic stroke.

Key words: Endonuclease VIII-like proteins, ischemic stroke, targets

INTRODUCTION

According to the World Health Organization health report, stroke is the leading cause of disability in adults and accounts for 5.5 million deaths worldwide, equivalent to 9.6% of all deaths.^[1] Ischemic stroke is the most common type of stroke, accounting for about 87% of all stroke events.^[2] Oxidative stress, which is a disturbance in the oxidant-antioxidant balance leading to the potential cellular damage, is widely recognized as one of the major factors contributing to the pathophysiologic progressing of the brain in an ischemic stroke.^[3] Oxidative stress is also known to play a role as a vital factor of ischemia reperfusion, which is one of the pathological events after ischemic brain.^[4] Oxidative stress not only could lead to structural changes of the proteins which can cause the loss of biological activity, but also create oxidative deoxyribonucleic acid (DNA) lesions in addition to damage to other cellular constituents.^[5] Oxidative

DNA damage can trigger cell death after the trauma,^[6] especially in neurons which are more susceptible to damage than astrocytes.^[7] Accumulated cell loss will gradually contribute to disease progression and eventually lead to poor prognosis in ischemic stroke.^[8] Maintaining genome integrity is essential for cell survival and the main DNA repair system for repairing oxidative DNA damage is base excision repair (BER).^[9] However, the exact impact of BER is still not fully understood, as most studies have been performed in models where BER is either present or absent. As it is well known that unbalanced BER is detrimental to the cell, one might speculate that suppressing full activation after ischemic insult could be beneficial to restore cell. It should be remembered that the killing period occurs after the stroke, during reperfusion when repair process is expected to be maximal. The DNA repairing capacity of BER may affect the survival rate of neurons and change the pathological outcome of ischemic stroke.

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BER is initiated by DNA glycosylases that recognize and excise the damaged bases of DNA.^[10] Depending on the type of lesion the BER pathway is initiated

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Cite this article as: Yang LX, Wang W, Zhang X, Zhu Q, Zhao Q, Zhao G. The endonuclease VIII-like proteins: new targets in the treatment of ischemic stroke?. *Neuroimmunol Neuroinflammation* 2015;2:281-6.

Received: 24-04-2015; **Accepted:** 21-08-2015

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DOI:
10.4103/2347-8659.167308

by one of 11 different DNA glycosylases including UNG1, UNG2, SUMUG1, thymine-DNA glycosylase, methyl-binding domain 4, methylpurine-DNA glycosylase, OGG1, muty glycosylase homologue, NTHL1, endonuclease VIII-like (NEIL) 1, NEIL2, and NEIL3.^[11] The subsequent pathway can divide into two sub-pathways: short-patch BER (SP-BER) and long-patch BER (LP-BER).^[12] The repair processes consist of five steps: excision of the base, incision, end processing, and repair synthesis, including gap filling and ligation.^[11] In SP-BER, a pol β -mediated single nucleotide incorporation is followed by strand ligation, catalyzed by the XRCC1/DNA ligase III complex. If BER is initiated by NEILs glycosylases, after N-glycosidic hydrolysis the termini is catalyzed by β , δ -elimination. Then the 3'-phosphate is cleaved by polynucleotide kinase, producing a 1NT gap with 3'-OH terminus.^[13,14] LP-BER is characterized by alternating flap endonuclease 1 cleavage and pols β synthesis or the strand displacement DNA synthesis concerted by pols β and δ/ϵ . At last, the gap is sealed by DNA ligase I.^[14] OGG1, NTHL1, NEIL1, NEIL2, and NEIL3 are recognized to be the five major DNA glycosylases to remove oxidative base lesions.^[10] NEILs play a critical role in the repair of oxidative DNA damage. Accumulating evidence suggests that NEILs may relate to diseases in central nervous system, for example, ischemic stroke, neurodegeneration disease, and neurological autoimmune disease with consistent results.^[15] However, our understanding of the functions and potential uses of NEILs in ischemic stroke is still limited.

Thus, the purpose of this review is to summarize the current knowledge on the involvement of the NEILs in ischemic stroke and aim to search for a new target in the treatment of ischemic stroke.

CHARACTERISTICS OF NEIL

In 2002, several groups working independently worldwide discovered three genes in the mouse and human genomes encoding DNA glycosylases belonging to the Fpg/Nei superfamily and having a primary structure more similar to that of the NEIL protein.^[16-19] These proteins were named NEIL1, NEIL2, and NEIL3.^[16-19] The characteristics of human NEIL genes and proteins are shown in Table 1.

The discovery of these three new DNA glycosylases generated strong interests by scientists to investigate their biochemical functions and the corresponding mechanisms. We summarize the substrate specificities of NEILs in Table 2.

Interestingly, NEILs substrates largely overlap with the OGG1 and NTHL1 substrate spectra indicates that NEILs may have a role in DNA repair that is unique to other DNA glycosylases.

NEIL1, NEIL2, and NEIL3 can also be distinguished from each other by the preferred lesions in different DNA structures, which demonstrate their unique biological roles in the regulation of cell cycle. NEIL1 interacts with proliferating cell nuclear antigen (PCNA) and PCNA can stimulates NEIL1 activity suggesting its special role in replication.^[20] NEIL2 is involved in repairing oxidized bases in the transcribed genes of mammalian cells, in particular, lesions in the mutagenic cytosine oxidation product 5-hydroxyuracil of the transcribed strand. In this function, NEIL2 associates with ribonucleic acid (RNA) polymerase II and the transcriptional regulator heterogeneous nuclear ribonucleoprotein-U.^[21] The NEIL3 repairs lesions in DNA with single-stranded regions^[22,23] and this phenomenon may demonstrate its potential role in cell proliferating, embryonic development, and neurogenesis.

NEIL1 expression patterns

Many studies have aimed at clarifying the expression patterns of NEIL1. The NEIL1 shows ubiquitous expression in all tissues examined in a human, including the brain.^[17-19] More specifically, NEIL1 was identified in all brain regions analyzed in human, and especially in the cerebellum, neocortex, and hippocampus.^[24] Rolseth *et al.*^[24] showed that expression of NEIL1 increases with age by *in situ* hybridization studies in mouse brain. Englander and Ma^[25] found out that the expression of NEIL1 in the mature brain increases 1.5-2.5-fold compared to the embryonic brain.

The NEIL1 activity has also been detected in mitochondria in the brain.^[26] In accordance with its age-dependent expression, the activity in mitochondria also correlates with age, but with important differences: mitochondrial NEIL1 in the

Table 1: The characteristics of human NEIL genes and proteins

Gene	Gene length nucleotides	Number of exons	Coding sequence, nucleotides	CL	Amino acids	Protein size (kDa)	SL	CCD	C/I
NEIL1	8258	9	1173	15q24.2	390	43.6	N and M	Yes	I
NEIL2	17683	4	999	8p23.1	332	36.7	N and M	Yes	I
NEIL3	53102	10	1818	4q34.3	605	67.7	N	Yes	I

CL: chromosomal location; SL: subcellular location; N: nucleus; M: mitochondria; CCD: cell cycle dependent; C/I: constitutive or inducible protein

Table 2: The substrates specificities of NEILs

Name	Mono-/bifurcate	Base	Substrates specificity	DNA
NEIL1	B	Pyrimidines/ purines	Sp, Gh, DHT, DHU, Tg, 5-OHC, 5-OHU, 8-oxoG, FapyG, and FapyA	ssDNA/ dsDNA
NEIL2	B	Pyrimidines/ purines	Sp, Gh, DHT, DHU, Tg, 5-OHC, 5-OHU, and 8-oxoG	ssDNA/ dsDNA
NEIL3	M/B	Pyrimidines/ purines	Sp, Gh, FapyG, and FapyA	ssDNA

Sp: spiroiminodihydantoin; Gh: guanidinohydantoin; DHT: 5,6-dihydrothymine; DHU: 5,6-dihydrouacil; FapyG: 2,6-diamino-4-hydroxy-5-formamidopyrimidine; FapyA: 4,6-diamino-5-formamidopyrimidine; 5-OHC: 5-hydroxycytosine; 5-OHU: 5-hydroxyuracil; Tg: thygly; 8-oxoG: 8-oxoguanine; ssDNA: single-stranded DNA; dsDNA: double-stranded DNA

cortex region is age-dependent and maximal in the middle-age. However, in the hippocampus, one of the neurogenic regions in the brain, mitochondrial NEIL1 is stable throughout a lifetime. By using a 5-OHdU containing bubble substrate, Gredilla *et al.*^[26] found that mitochondrial NEIL1 activity showed an age-related change in the cortex with a significant peak at middle-age in the cortical region, but not in the hippocampus where no significant change occurred during the lifespan. The distinct age-dependent, subcellular- and tissue-distribution suggests that the role of NEIL1 is strongly connected to site-specific conditions.

In conclusion, the widespread expression of NEIL1 in mammals demonstrated its unique role in the maintenance of gene integrity.

NEIL2 expression patterns

By using Northern analysis, NEIL2 was found to have the highest expression in the skeletal muscle and testis, moderate expression levels in the brain and heart and a very low level expression in placenta, lung, liver, kidney, and pancreas.^[17] The same study also showed that NEIL2 mRNA level did not significantly change through the cell cycle and that NEIL2 was predominantly mitochondrially localized.^[17] NEIL2 expression had also been detected in embryonic, neonatal, and adult rat brain and the expression level increased 1.5-2.5-fold in the mature rat brain compared to the embryonic brain, which is the same as NEIL1.^[25] In the human brain, NEIL2 showed a widespread expression pattern in accordance with OGG1, NTH1, and NEIL1.^[24] Rolseth *et al.*^[24] also found that NEIL2 had a similar expression pattern as NEIL1 by detecting expression of NEIL2 in mouse brain during postnatal development, but with a slightly increased expression with age. In summary, NEIL2 has a widespread expression pattern in human and rodent and the subcellular localization of NEIL2 is in mitochondria and in the nucleus. This distribution

and tissue specificity of NEIL2 suggest that it may serve as a critical factor to maintain the integrity of the genome lifelong.

NEIL3 expression patterns

Compared to NEIL1 and NEIL2, The NEIL3 has a very distinct expression pattern.^[18] Among all the human adult tissues, NEIL3 is only expressed at detectable levels in the thymus and testis, which indicates that NEIL3 might have a specialized function associated with proliferative capacity. Torisu *et al.*,^[27] studied the expression level of NEIL3 in human but only found NEIL3 expression in thymus. In mouse tissue, NEIL3 mRNA was expressed in thymus, spleen, and bone marrow.^[27] By developing NEIL3-null mice, Torisu found that NEIL3-null mice looked healthy for at least 24 weeks after birth. Furthermore, NEIL3-null male mice were viable and fertile. According to these findings, Torisu *et al.*^[27] concluded that NEIL3 was not required for maintenance of testis function but had a potential function in the development of the hemopoietic system. In the central nervous system, NEIL3 mRNA expression has been investigated in different brain areas of human adults by Northern blot hybridization.^[24] NEIL3 could not be detected in any brain region of adult humans. In contrast, by studying the expression of NEIL3 in mouse brain during postnatal development, NEIL3 transcripts can be observed in the subventricular zone (SVZ), hilus of the hippocampal formation, the rostral migratory stream, and the Purkinje cell of the cerebellum in P3 mice brain. In 1-month-old mouse brain, the NEIL3 was detected in layer V of the neocortex and only in a few cells in the SVZ and in the 1-year-old brain only in layer V of the neocortex. These results indicate that the expression of NEIL3 declines with age, and it is selectively expressed in brain regions associated with neurogenesis. NEIL3 expression has also been detected in regions rich in neurogenesis in the embryonic brain^[28] and in two recent studies NEIL3-null models showed a decreased differentiation potential of neural stem cells.^[29,30] NEIL3-null mice showed learning and memory deficits and reduced anxiety-like behavior, and synaptic irregularities in hippocampal neurons.^[31] All together, these results suggest that NEIL3 may have a specific role in neurogenesis in the central nervous system.

The NEIL3 has been shown to be highly expressed in many tumor tissues, which supports the notion that NEIL3 might be associated with proliferation capacity.^[32-34]

Regarding the subcellular localization of NEIL3, two studies have consistently found that NEIL3 is expressed in the cell nucleus.^[18,27] Two additional

studies showed that NEIL3 was cell cycle-regulated with the highest expression in the G2 phase.^[32,33] Thus far, NEIL3 proteins have not been found in the mitochondria.^[23]

In conclusion, distribution of NEIL3 is very different from other DNA glycosylases which suggests a special function of NEIL3 in a mammal. In the central nervous system, NEIL3 expression in both human and mouse brain has been localized to regions where neurogenesis takes place. NEIL3 seems to be upregulated in tumor tissues compared to normal tissues. In summary, the expression patterns of NEIL3 suggest that mammalian NEIL3 seems to be highly expressed in cells that have high proliferative potential.

THE NEIL AND ISCHEMIC STROKE

NEIL1

The interest in the relationship between NEILs and stroke arises from the work done by Rolseth *et al.*^[35] By observing BER activities in organotypic hippocampal slice culture exposed to oxygen and glucose deprivation, the authors found that CA1 has a lower capacity than CA3/FD in base lesions removal under basal conditions, which may be correlated with the low expression levels of both NEIL1 and NEIL2.^[35] This study has not only demonstrated the reasons why CA1 was vulnerable to ischemic stroke but also revealed the potential unique role that DNA glycosylases might play in ischemic stroke.^[35] By addressing the effect of hyperoxic reoxygenation and therapeutic hypothermia on the development of brain damage after asphyxia in newborn pigs, Dalen *et al.*^[36] found out that NEIL1 is significantly downregulated in the hippocampus, cortex, striatum, and liver upon hypothermia without any detectable effect on the accumulation of oxidative DNA damage in genomic DNA. In addition, like OGG1, NEIL1 expression in the brain is unaffected by hyperoxia. A recent study by Canugovi *et al.*^[37] demonstrated that NEIL1 can be linked to changes in ischemic stroke. Due to the increasing brain damage caused by reducing the incision capacity on a 5-hydroxyuracil-containing bubble substrate, NEIL1 gene knockout mice exhibited impaired memory retention in a water maze test, but no abnormalities in motor performance, anxiety, or fear conditioning. These results indicate that NEIL1 plays an important role in learning and memory and in the protection of neurons against ischemic injury. Interestingly, NEIL1 gene knockout mice display a specific metabolic phenotype, which is attributed to the increased mitochondrial DNA damage.^[38]

In conclusion, there is evidence that NEIL1 might play an important role in ischemia stroke, but the exact functions of NEIL1 during the pathophysiologic development of cerebral ischemia are still unknown.

NEIL2

As previously mentioned, Rolseth *et al.*^[35] found that similarly to NEIL1, the expression of NEIL2 is not changed in response to OGD treatment in CA1. To date, we still know very little about the relationship between NEIL2 and ischemic stroke. Future studies are needed to completely understand if and how NEIL2 could be associated with ischemic stroke.

NEIL3

Similarly to NEIL1, the transcription of NEIL3 is significantly reduced in the hippocampus and cerebellum by hypothermia as observed in a study on newborn pigs exposed to hypoxia, however, no significant effect on the accumulation of oxidative DNA damage in genomic DNA was found.^[36] In order to understand the relationship between NEIL3 and hypoxia-ischemia, Sejersted *et al.*^[29] carried out an experiment on NEIL3 gene knockout (NEIL3-null) mice *in vitro* and *in vivo*. Interestingly, after hypoxia-ischemia, there is no increase of cellular damage or death *in vivo* in NEIL3-null mice at an early stage, but a significant deficit in reconstituted neuronal tissue after 42 d. NEIL3-null neurospheres exhibited poor growth and skewed differentiation that could explain the poor outcome of NEIL3-null mice after hypoxic ischemia. In agreement with the aforementioned expression pattern, this study has demonstrated that NEIL3 seems to predominantly play a role in neurogenesis. NEIL3-null mice also showed learning and memory deficits and reduced anxiety-like behavior, and synaptic irregularities in hippocampal neurons.^[31] Future studies addressing the role of NEIL3 in neuronal tissue in ischemic stroke will shed more light on this issue.

CONCLUSIONS AND FUTURE PERSPECTIVES

As a consequence of high oxygen metabolism, an efficient BER pathway is activated to ensure genomic stability and brain homeostasis. NEILs have been discovered in 2002 and since then studies have reported that these proteins are mainly expressed in the brain. However, all these data have not clearly elucidated the expression pattern and the relationship between the changes in NEILs and ischemic stroke. Although, there is evidence that DNA glycosylases deficiency impacts on brain function in animal models, the molecular mechanism is still unknown. In the future, there is hope that by characterizing the effect of NEILs on subclasses

of neurons, astrocytes, and microglia as well as other pathways of BER, we might be able to understand how NEILs contribute to ischemic stroke and find new targets for the treatment of ischemic stroke.

Financial support and sponsorship
Nil.

Conflicts of interest

There are no conflicts of interest.

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Isolated neurosarcoidosis presenting with recurrent hydrocephalus

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ABSTRACT

Sarcoidosis is an inflammatory process that is characterized by the formation of noncaseating granulomas. This protean disease may afflict nearly any organ system, including the central nervous system. Here, we present a case of isolated neurosarcoidosis that initially presented with hydrocephalus requiring ventriculoperitoneal shunt placement. The patient's hydrocephalus recurred multiple times and required two additional shunt placements over the 3-year course of her illness. Due to the lack of systemic involvement, sarcoidosis was only diagnosed after a tissue biopsy of a *Cauda equina* lesion. This case highlights the importance of tissue diagnosis and the diagnostic workup for sarcoidosis in cases of cryptogenic hydrocephalus.

Key words: Biopsy, hydrocephalus, inflammatory, neurosarcoidosis

INTRODUCTION

Sarcoidosis is an inflammatory disease that may present in a multitude of forms due to its ability to involve virtually any organ in the body. Involvement of the central nervous system (CNS) occurs in 5-25% of sarcoidosis patients.^[1-3] The most common symptoms associated with neurosarcoidosis are cranial nerve palsies, paresthesia, headache, weakness, ataxia, aseptic meningitis, peripheral neuropathy, and cognitive impairment [Table 1]. Hydrocephalus is a much rarer complication of neurosarcoidosis that was not found in one series of 91 cases and only reported in a few case reports.^[4,7-10] Neurosarcoidosis patients commonly have evidence of the involvement of the thoracopulmonary system. Isolated neurosarcoidosis (sarcoidosis limited solely to the nervous system) is relatively rare. The prevalence

of isolated neurosarcoidosis among neurosarcoid patients varies from series to series with older studies reporting 1-3% and more recent studies reporting 11-17%.^[4,11] Here, we report a case of a patient with isolated neurosarcoidosis that presented with hydrocephalus ultimately requiring placement of three ventriculoperitoneal shunts and eventually diagnosed by biopsy of *Cauda equina* lesions.

CASE REPORT

A 38-year-old African-American woman presented to an outside hospital with a 2-year history of headaches, a 5-month history of difficulty walking, dizziness refractory to meclizine, and a 3-day history of blurry vision. A neurological exam demonstrated nystagmus on upgaze, left gaze, and right gaze. She also had an ataxic gait and papilledema. Head computed tomography (CT), and magnetic resonance imaging (MRI) revealed communicating hydrocephalus. Analysis of cerebrospinal fluid (CSF) obtained by lumbar puncture (LP) demonstrated cell count, protein,

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DOI:
10.4103/2347-8659.167307

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Cite this article as: Hitti FL, Kennedy BC, Odia Y, Riley CS, Sheth SA. Isolated neurosarcoidosis presenting with recurrent hydrocephalus. *Neuroimmunology and Neuroinflammation* 2015;2:287-90.

Received: 15-04-2015; **Accepted:** 06-08-2015

and glucose levels within normal limits. A presumptive diagnosis of idiopathic hydrocephalus was made. A right frontal external ventricular drain was placed followed by right frontal ventriculoperitoneal shunt placement. A postoperative head CT revealed a collapsed right lateral ventricle and unchanged left lateral, third, and fourth ventricles. Due to symptomatic improvement, the unresolved hydrocephalus was followed without further intervention.

Five months later, the patient presented to the outside hospital with a headache, nausea, vomiting, ataxia, dizziness, and blurred vision. Other than an opening pressure of 25 cm of H₂O and glucose of 97 mg/dL, CSF analysis was within normal limits. Angiotensin-converting enzyme (ACE) levels were not tested. Head CT revealed left unilateral

obstructive hydrocephalus secondary to a trapped ventricle with a left-to-right midline shift, so a left frontal ventriculoperitoneal shunt was placed. Although postoperative MRI revealed decreased hydrocephalus, the third and fourth ventricles remained prominent. No postcontrast enhancement was seen [Figure 1a and b].

Other than one shunt revision, she remained relatively stable for 3 years after which she presented to the outside hospital with unsteadiness, double vision, and left leg weakness. Head CT revealed collapsed lateral and third ventricles and a persistent dilatation of the fourth ventricle, which prompted placement of a ventriculoperitoneal shunt in the fourth ventricle. She was discharged, but her condition continued to deteriorate, possibly due to over-shunting or disease progression and she was readmitted after 2 weeks. At this point, she transferred her care to our institution.

Upon arrival, her neurological exam demonstrated direction-changing nystagmus, asymmetric proximal lower extremity weakness (iliopsoas right 3/5, left 2/5), and ataxia on finger-nose-finger and heel-shin testing. There were no sensory deficits, and the patient did not complain of fecal or urinary incontinence. Head CT demonstrated bilateral frontal and right suboccipital approach ventricular catheters and a decompressed ventricular system. Hypo-density, suggestive of edema, in the cerebellum prompted treatment with Decadron. MRI revealed sulcal enhancement bilaterally along the frontal lobes and internal auditory canals that suggested leptomeningeal disease [Figure 1c and d]. MRI of the spine revealed diffuse nodular leptomeningeal enhancement throughout the spinal

Table 1: Signs and symptoms of neurosarcoidosis

Sign or symptom	Percent affected (%)	References
Cranial neuropathy	52-73	[3,4-6]
Aseptic meningitis	7-24	[3,4-6]
Peripheral neuropathy	6-24	[3-6]
Cognitive impairment	2-27	[4,5]
Seizures	2-20	[4-6]
Myopathy	9-12	[3,6]
CNS space occupying lesion	2-11	[4-6]
HPA axis dysfunction	2-11	[4-6]
Hydrocephalus	4-9	[3,6]
Paresthesia	43	[4]
Headache	37	[4]
Weakness	33	[4]
Ataxia	24	[6]
Myelopathy	21	[4]
Encephalopathy	11	[6]
Hemiparesis	7	[4]
Guillain-Barré syndrome	5	[5]
Radiculopathy	3	[4]

CNS: central nervous system; HPA: hypothalamic pituitary adrenal

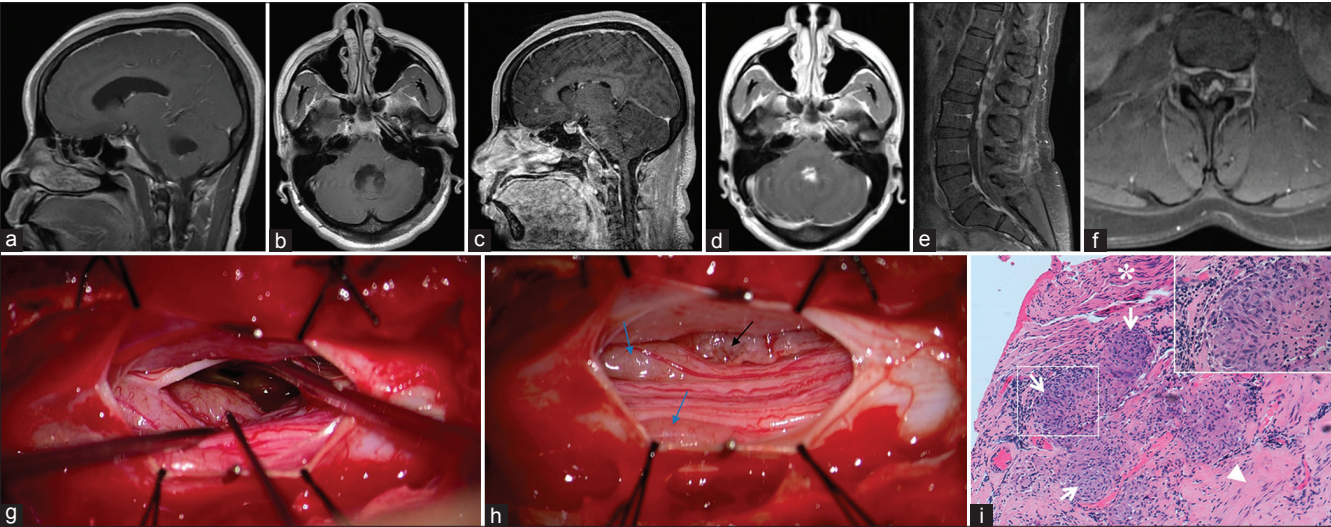


Figure 1: (a and b) Outside institution T1 postcontrast magnetic resonance imaging (MRI) (5 months after initial presentation); (c-f) T1 postcontrast MRI obtained upon admission to our institution (3.5 years after initial presentation) that demonstrates leptomeningeal enhancement throughout the central nervous system; (g and h) Intraoperative photographs of the exposed nerve roots at the L3 level of the *Cauda equina*; (g) Micrograph before the biopsy. The yellow-gray, nodular, hyper-vascular lesion intimately related to the leptomeninges that was biopsied is marked by a black stimulation probe; (h) Micrograph demonstrating the lesion after biopsy (black arrow) and two lesions (blue arrows) that produced motor potentials upon stimulation; (i) Photomicrograph depicting confluent nonnecrotizing granulomas (arrows) involving fibrocollagenous tissue of leptomeninges (arrowhead), and nerve fibers (asterisk); H and E, x100. Inset shows one highlighted granuloma at x400

cord and *Cauda equina* [Figure 1e and f]. Opening pressure was normal following LP and CSF analysis revealed lymphocytic pleocytosis (88 WBCs/uL, 94% lymphocytes), markedly elevated protein (868 mg/dL), and an ACE level of 15 U/L (normal, 0-2.5 U/L). Cytology demonstrated lymphocytosis without malignant cells, and flow cytometry was negative for lymphoma. The workup for infection was negative. Chest X-ray and CT of the abdomen and pelvis were negative for mass lesions or signs of systemic sarcoidosis. She experienced only transient improvement on steroids, so they were tapered off.

Discussion at a multidisciplinary case conference yielded a broad differential diagnosis that included inflammatory processes (neurosarcoidosis or other connective tissue diseases), primary or secondary CNS neoplasm (ependymoma and lymphoma), or a chronic infectious leptomeningitis. Biopsy from the L3 *Cauda equina* region was recommended as the lowest risk and highest yield target. A region of a root that did not produce a motor potential when stimulated was biopsied [Figure 1g and h]. Hematoxylin and eosin stained sections demonstrated numerous noncaseating granulomas [Figure 1i]. No organisms were identified with acid-fast or Grocott's methenamine silver stains. These biopsy findings support a diagnosis of neurosarcoidosis.

DISCUSSION

Here, we describe a case of isolated neurosarcoidosis presenting with hydrocephalus that over three years appeared to require three separate shunt catheters, likely due to progressive occlusion of the foramina of Monro and Sylvian aqueduct or possibly due to elevated CSF protein levels. Hydrocephalus is a rare symptom of neurosarcoidosis with few case reports.^[7-10] This is, to our knowledge, the first report of isolated neurosarcoidosis presenting with hydrocephalus. Furthermore, noteworthy is the development of a trapped lateral ventricle and necessity of multiple shunt placements. While there has been one report of neurosarcoidosis requiring multiple shunt placements, the patient described had systemic sarcoidosis making diagnosis more straightforward.^[8] The protracted course of our patient's undiagnosed illness was likely due to the absence of systemic disease.

Upon initial presentation, the diagnostic workup, including CSF analysis, was unrevealing, and she was diagnosed with idiopathic hydrocephalus at the outside institution. The leptomeningeal enhancement, later found on MRI, generated a broad differential diagnosis that included infectious, inflammatory, and neoplastic

diseases. Due to the protracted course of her illness and the presence of multiple lesions throughout her brain and spinal cord, ependymoma, and neurosarcoidosis were placed highest on the differential diagnosis. Several years into her disease course, her CSF profile became remarkable for the nonspecific findings of lymphocytosis, elevated protein and elevated ACE.^[5,12,13] While her CSF analysis was normal early in her disease course, this has been previously reported in other cases of neurosarcoidosis.^[6,7]

Because the appropriate clinical and radiographic findings are nonspecific, sarcoidosis diagnosis generally requires biopsy.^[1-3] For isolated neurosarcoidosis, diagnosis is made even more challenging by the absence of thoracopulmonary involvement, a hallmark present in 90% of patients.^[1,2]

Biopsy for sarcoidosis is also critical because patients may not show substantial improvement with an empiric trial of steroids, as was true for this patient. Many have advocated the use of immunosuppressants such as azathioprine, methotrexate, infliximab, or mycophenolate mofetil alone, or in addition to steroids in patients who do not respond to corticosteroid monotherapy.^[1,2,14-16] The risks of long-term immunosuppressive therapy warranted a definitive diagnosis of isolated neurosarcoidosis, given its rarity. Since neurosarcoidosis was indeed the diagnosis, the patient was initiated on 80 mg prednisone daily and 500 mg mycophenolate mofetil twice a day.

CONCLUSION

This report highlights the importance of considering neurosarcoidosis in the differential diagnosis in patients with unexplained recalcitrant hydrocephalus.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

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Angioplasty and stenting for a young stroke patient diagnosed as cerebrovascular fibromuscular dysplasia

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ABSTRACT

Fibromuscular dysplasia (FMD) is a noninflammatory, nonatherosclerotic, and multifocal vascular disease, commonly involving the cerebral and renal arteries. Cerebrovascular stenosis and spontaneous dissection resulting from cerebrovascular FMD (cFMD) is one of the important causes of young stroke. Here, we reported the case of cFMD in a 28-year-old male patient with stroke. Digital subtraction angiogram demonstrated a dissecting aneurysm in the carotid artery and multiple stenoses in both vertebral arteries. Endovascular angioplasty with balloon predilation and stenting was successfully performed for the patient when the medical treatment was not adequate. The follow-up showed a remarkable improvement and no recurrence of stroke.

Key words: Angioplasty, fibromuscular dysplasia, stent, therapy, young stroke

INTRODUCTION

Fibromuscular dysplasia (FMD) is an uncommon vascular disease that occurs more often in young women. FMD mainly involves the renal and cerebral arteries.^[1] Cerebrovascular FMD (cFMD) can be complicated by stroke and associated with headaches, and it can also be associated with intracranial aneurysms with a high risk of subarachnoid hemorrhage. The cerebrovascular stenosis and dissection, as a result of FMD, is one of the important causes of stroke in the young (15-45-year-old). A case report of cFMD treated with angioplasty and stenting in a young male patient is presented, along with an overview of the available literature.

CASE REPORT

A 28-year-old male patient was admitted to our stroke unit ward of Department of Neurology in the Second

Affiliated Hospital of Soochow University because of ischemic stroke with inarticulate speech and paralysis of right limbs. The patient had no medical history of traditional cerebrovascular risk factors, like hypertension. The patient also had no personal or family history of stroke. The dysphasia and slight right-sided weakness occurred without the definite causes for 1-day before the admission without any prior warning. Magnetic resonance imaging revealed a new infarction lesion on the left temporal lobe and occlusion of the left internal carotid artery (ICA). The ultrasound sonography also demonstrated the occlusion in the left ICA. Antiplatelet (clopidogrel 75 mg and aspirin 100 mg q.d. p.o.), and statin therapy (atorvastatin 40 mg q.n. p.o.) were given to this patient, diagnosed as acute cerebral infarction without a clear etiology. During this hospitalization for 7 days, logaphasia, and more severe paralysis of the right limbs occurred. Then, he was transferred to our stroke unit ward for further vascular evaluation and interventional therapy. The positive neurological signs were as follows: incomplete logaphasia, central facial paralysis, right-sided hemiparesis (4/5), and positive Babinski sign. Other

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10.4103/2347-8659.167310

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Cite this article as: Xu JP, Cao YJ, Xiao GD, Zhang CY, Shi JJ. Angioplasty and stenting for a young stroke patient diagnosed as cerebrovascular fibromuscular dysplasia. *Neuroimmunol Neuroinflammation* 2015;2:291-4.

Received: 08-06-2015; **Accepted:** 06-08-2015

laboratory tests about thyroid function, autoimmune antibodies, and vasculitis indicators were all normal.

Digital subtraction angiography (DSA) showed a dissecting aneurysm of the C1 segment of right ICA and subtotal occlusion of the left ICA [Figure 1a and b]. Anterior communicating artery was open, and the left middle cerebral artery (MCA) territory got collateral blood flow from the right MCA [Figure 1c]. Severe bilateral stenosis ($> 70\%$) was also revealed on the V2 segment of both vertebral arteries (VA) [Figure 1d-f]. Then angioplasty and stenting was performed for this young patient.

First, the vertebral angioplasty and stenting was performed and then the left ICA was stented because of the carotid sinus reaction. A dissecting stenosis on the initial segment of the left VA stenosis was found during the angiography before stenting. Firstly, one expanding stent was placed on the dissecting site, and then another two stents ($4\text{ mm} \times 60\text{ mm}$ and $4\text{ mm} \times 40\text{ mm}$) were delivered and deployed to cover the long stenotic lesion. A final angiography demonstrated an excellent stent placement across the stenotic lesion of the left VA and left vertebral angiogram revealed a good flow in vertebral and basilar arteries [Figure 1g].

After advancing the 8F guiding catheter within the C1 segment of the left ICA, a microwire (0.014 inches) was delivered through the subtotal

occlusive site and predilation was performed with a balloon ($2.5\text{ mm} \times 15\text{ mm}$) at 6 atm. Then, the stent ($4\text{ mm} \times 40\text{ mm}$ Xpert Stent System) was deployed over the stenosis and postdilation was performed with a balloon at 6 atm. Angiography after stenting showed the revascularization of the subtotal stenosis and good flow across the stent with complete disappearance of this stenosis [Figure 1h]. After the procedure, antiplatelet therapy (clopidogrel 75 mg and aspirin 100 mg daily) was sequentially administered for 3 months, and then aspirin is taken prophylactically. During the follow-up period of 3 years, this patient was normal at 3 months after discharged from our hospital, and no recurrent stroke occurred.

DISCUSSION

FMD is a noninflammatory, nonatherosclerotic vascular disease that commonly involves the renal and internal carotid arteries. The young patient has multiple vascular stenoses, but no cerebrovascular risk factors; therefore, cFMD can be diagnosed. The prevalence of symptomatic renal artery FMD is about 4 in 1000, and the prevalence of cFMD is probably half that.^[1-4] FMD usually affects the females from 15 to 50 years of age and accounts for around 10% of cases of renal artery stenosis.^[1-4]

Although the etiology of FMD is not well understood, several mechanisms have been proposed. For example,

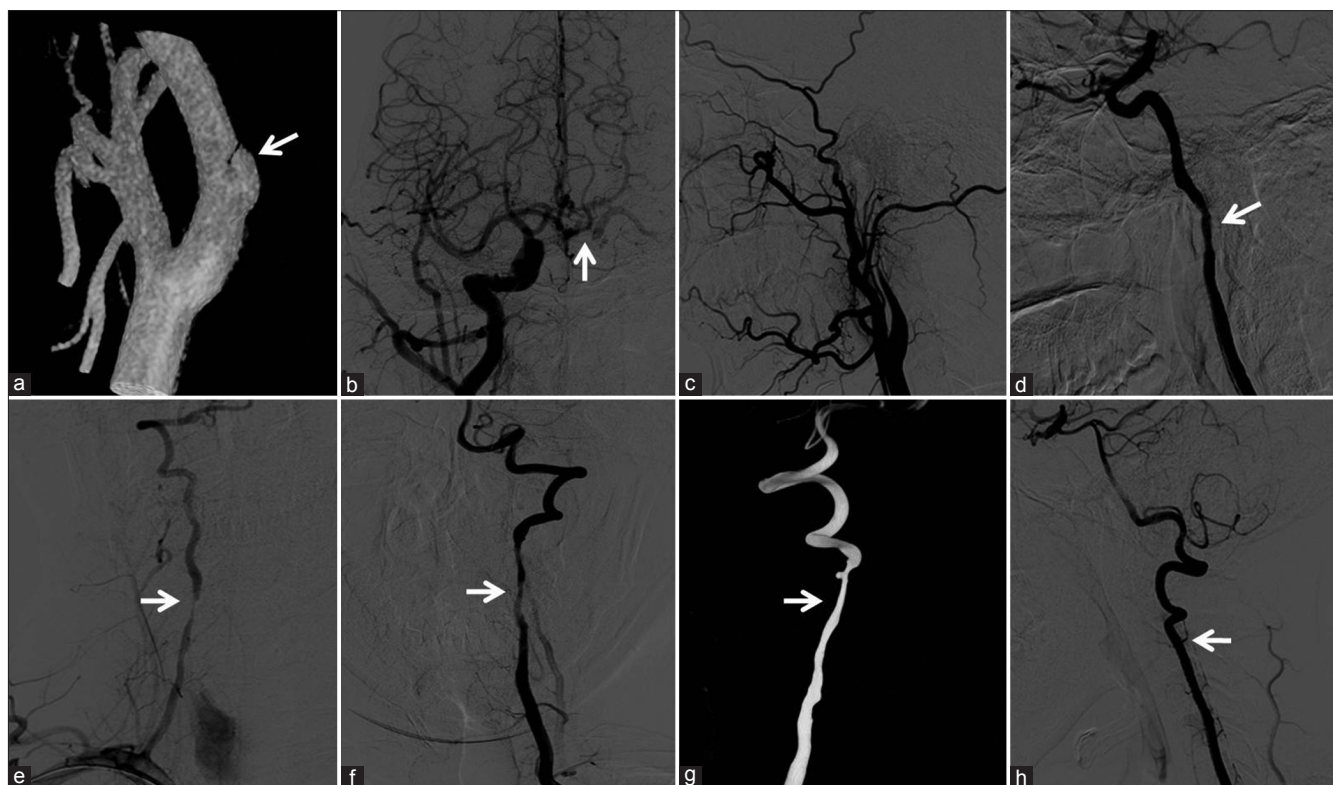


Figure 1: (a) The dissecting aneurysm on the C1 segment of right internal carotid artery; (b) "rat tail sign" of the left internal carotid artery before predilation; (c) the anterior communicating artery was open and the left middle cerebral artery territory got collateral blood flow from the right middle cerebral artery; (d-f) severe bilateral stenosis on the V2 segment of both VAs; (g) left vertebral arteries after stenting; (h) left internal carotid artery after balloon predilation

genetic predisposition, hormonal factors, and arterial wall ischemia.^[1,4] The pathogenesis of FMD remains unclear. A number of theories have been proposed, including the environmental factors such as smoking and estrogen, as well as genetic factors; however, about 10% of patients with FMD have an affected family member.^[5]

cFMD may be asymptomatic or associated with a variety of nonspecific symptoms, including headache, tinnitus, vertigo, lightheadedness, and syncope.^[1] The clinical manifestations of cFMD are variable and depend on a number of factors, including the distribution of vascular bed involvement and the type and severity of the vascular lesions.^[6,7] The more specific neurologic syndromes of TIA, amaurosis fugax, stroke, Horner's syndrome, and cranial-nerve palsies may be the first presentation of FMD involving the carotid or VA.^[8] And the most feared and serious sequela of cFMD include TIA, stroke, subarachnoid hemorrhage, and cervical artery dissection. It shows that FMD is present in about 15-20% of patients with a spontaneous dissection of carotid or VA.^[9] And multiple cervical artery dissections are more common in patients with an underlying arteriopathy, such as FMD.^[10]

Noninvasive imaging modalities for diagnosing FMD include Doppler ultrasound, computed tomographic angiography, and magnetic resonance angiography. The accepted gold standard for the diagnosis of cFMD is DSA. "String of beads pattern" in the pathological carotid or renal arteries is an important and most common angiographic finding and is present in over 90% of cases. However, it was not observed in this present case. This invasive test should be considered for those symptomatic patients in whom intervention is contemplated or for cases in which there is uncertainty about the patient's diagnosis or severity of the disease.^[11]

Medical therapy and revascularization are the two major treatment options for cFMD patients. As previously discussed, antiplatelet therapy is the mainstay of the medical therapy. For symptomatic patients with carotid or vertebral artery FMD, who have suffered a dissection, angioplasty with stenting may be performed. The indications for intervening in cFMD are for those in whom antiplatelet or anticoagulant therapy is contraindicated or less effective and for those cFMD patients with pseudoaneurysm formation, usually the result of a prior dissection.^[12]

The patient was successfully treated with angioplasty with stenting. During the follow-up period, no

adverse events and complications were observed suggesting that angioplasty with stenting may be a safe and effective treatment method for this condition.

Financial support and sponsorship

This study was supported by the National Natural Science Foundation of China (Grant No. 81471195) and the second affiliated hospital of Soochow university preponderant clinic discipline group project funding (Grant No. XKQ2015002).

Conflicts of interest

There are no conflicts of interest.

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Journal articles not in English	Zhang X, Xiong H, Ji TY, Zhang YH, Wang Y. Case report of anti-N-methyl-D-aspartate receptor encephalitis in child. <i>J Appl Clin Pediatr</i> 2012;27:1903-7. (in Chinese)
Journal articles ahead of print	Odibo AO. Falling stillbirth and neonatal mortality rates in twin gestation: not a reason for complacency. <i>BJOG</i> 2018; Epub ahead of print [PMID: 30461178 DOI: 10.1111/1471-0528.15541]
Books	Sherlock S, Dooley J. Diseases of the liver and billiary system. 9th ed. Oxford: Blackwell Sci Pub; 1993. pp. 258-96.
Book chapters	Meltzer PS, Kallioniemi A, Trent JM. Chromosome alterations in human solid tumors. In: Vogelstein B, Kinzler KW, editors. The genetic basis of human cancer. New York: McGraw-Hill; 2002. pp. 93-113.
Online resource	FDA News Release. FDA approval brings first gene therapy to the United States. Available from: https://www.fda.gov/NewsEvents/Newsroom/PressAnnouncements/ucm574058.htm . [Last accessed on 30 Oct 2017]
Conference proceedings	Harnden P, Joffe JK, Jones WG, editors. Germ cell tumours V. Proceedings of the 5th Germ Cell Tumour Conference; 2001 Sep 13-15; Leeds, UK. New York: Springer; 2002.
Conference paper	Christensen S, Oppacher F. An analysis of Koza's computational effort statistic for genetic programming. In: Foster JA, Lutton E, Miller J, Ryan C, Tettamanzi AG, editors. Genetic programming. EuroGP 2002: Proceedings of the 5th European Conference on Genetic Programming; 2002 Apr 3-5; Kinsdale, Ireland. Berlin: Springer; 2002. pp. 182-91.
Unpublished material	Tian D, Araki H, Stahl E, Bergelson J, Kreitman M. Signature of balancing selection in Arabidopsis. <i>Proc Natl Acad Sci U S A</i> . Forthcoming 2002.

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