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Review

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Pathogenesis and biomarkers in ANCA-associated vasculitis

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Abstract

Anti-neutrophil cytoplasmic antibody (ANCA)-associated vasculitides (AAVs) are a group of systemic autoimmune diseases associated with serum ANCA positivity that affect small to medium vessels with inflammation and endothelial injury. This group includes several diseases: granulomatosis with polyangiitis (GPA), microscopic polyangiitis, eosinophilic GPA, and drug-induced AAV. A few AAVs are ANCA negative, but this form has decreased with the increase in detection methods. Different genetic, epigenetic, and environmental risk factors contribute to the pathogenesis of AAV. ANCA's role in the origin of vasculitis has led to a better grasp of the disease. Research has also improved the treatment, more precisely to tune its intensity, translating into better outcomes. However, there is still a gap to be filled with new potential and testable biomarkers for diagnosis, disease activity, and prognosis, which we discuss here.

Keywords: Pathogenesis, ANCA, ANCA-associated vasculitis, biomarkers

INTRODUCTION

Anti-neutrophil cytoplasmic antibody (ANCA)-associated vasculitides (AAVs) are a group of systemic autoimmune diseases that affect small to medium vessels with inflammatory and endothelial injury. In the early 1980s, the first case report mentioning ANCA presence in the serum of patients with segmental necrotizing glomerulonephritis triggered a new chapter in immune-mediated diseases[1]. The discovery of

the major antigens targets of ANCAs [myeloperoxidase (MPO) and proteinase 3 (PR3)] led to the creation of an inclusive group of AAVs. Classification criteria based on clinical findings and tissue biopsy, if possible or reasonable, have not always been widely accepted and new scores are being proposed[2,3].

The AAV group incorporates at least four particular diseases: granulomatosis with polyangiitis (GPA), microscopic polyangiitis (MPA), eosinophilic GPA (EGPA; formerly Churg-Strauss syndrome), and drug-induced AAV[4].

GPA is a necrotizing small-vessel vasculitis characterized by the involvement of the respiratory tract with granulomatous inflammation. These patients are mainly PR3-ANCA positive. In contrast, MPA preferably targets renal glomeruli small vessels and presents a histological pattern of necrotizing vasculitis normally lacking granulomatous inflammation. The serum of these patients commonly presents MPO-ANCA positivity.

The EGPA histological pattern is similar to GPA, except its main feature of eosinophil-rich reaction that involves small- to medium-sized vessels. Patients with this disease typically have associated adult-onset asthma and allergic sinusitis, eosinophilia, tissue infiltrating eosinophils, and around 50% of the cases have MPO-ANCA positivity.

Drugs such as propylthiouracil, hydralazine, or cocaine induce ANCA production and generate drug-induced AAV. The clinical features of this particular spectrum of AAV have a wide and global variation according to ethnic differences[5]. More commonly, MPA- and MPO-ANCA positivity is observed in East Asian countries, while GPA- and PR3-ANCA is more noticeable in Western countries[6].

Worldwide identification of these diseases prompted international guidelines and treatment management for remission and maintenance[7-9]. However, methods for the monitoring of AAVs activity are still lacking. In this review, we characterize the current knowledge regarding AAV's risk factors and pathogenesis and subsequently review newly discovered biomarkers for its diagnosis, monitoring, and prognosis.

RISK FACTORS OF AAV

A multiplicity of risk factors for AAV has been described in the literature, as summarized in Table 1.

Genetics

Even though familiar reports of AAV are rare[10], as for other autoimmune disorders, genome-wide association studies (GWAS) have been performed to further identify alleles related with patterns of susceptivity or resistance to AAV. Among these, the strongest associations were found for major histocompatibility complex class II (MHC II) and HLA gene locus on chromosome 6.

The differences in HLA are related to the ethnicity of the population and the appearance of certain circulating antibodies rather than the clinical appearance[11]. A GWAS conduced in the UK found strong associations between HLA-DPB1*04 and PR3-ANCA (OR = 7.03), MPO-ANCA and DQ loci (OR = 0.65), and HLA-DP allele and GPA (OR = 5.39)[11]. In Japan, population studies linked HLA-DRB1*09:01 with MPO-ANCA (OR = 1.57) and MPA (OR = 1.56). Curiously, HLA-DRB1*13:02 was suggested as a protective factor against AAV[12].

Table 1. Anti-neutrophil cytoplasmic antibody-associated vasculitis risk factors

Risk factor		Evidence	
Genetics		Significant association HLA-DP locus and GPA; DQ loci and MPO-ANCA; HLA-DP allele and GPA SERPINA-1, α-1-antitrypsin allele deficiency, proteinase 3 gene (PR3) polymorphism and variant increasing PTPN22 activity	
Epigenetics		MPO and PRTN3 promoter methylation	
Exposome	Seasonality	Discrepant results in literature Onset observed in autumn and winter months	
	Latitude and UV radiation	Increased prevalence of MPA and GPA in hot and cold countries, respectively Higher latitudes correlate with higher GPA incidence	
	Infections	Exposure to toxic shock syndrome toxin-1 Nasal S. aureus colonization related to relapse rates	
	Pollution	Silica	2.5-fold increased risk, even higher for patients with renal involvement, GPA or MPA
		Farming	GPA associated with 12 months prior-visits and significant livestock exposure Gardening, namely digging, mowing and planting
		Other pollutants	Exposure to carbon monoxide, hydrocarbon and high organic solvent
	Drugs	e.g., propylthiouracil, methimazole, hydralazine, minocycline or cocaine	
	Smoking	Weak correlation	

GPA: Granulomatosis with polyangiitis; MPO: myeloperoxidase; ANCA: anti-neutrophil cytoplasmic antibody; MPA: microscopic polyangiitis.

Besides MHC genes, several single-nucleotide polymorphisms (SNP) associated with the AAVs have been found. The variant that increases the activity of PTPN22, which negatively regulates IL-10 (an immunosuppressive cytokine), increases the likelihood of PR3-ANCA (OR = 1.63). Oppositely, SNPs related to SERPINA1, PRTN3, and SEMA6A showed profiles protective against AAV[11].

Epigenetics

Gene expression regulation of MPO and PRTN3 genes were linked to DNA methylation and histone 3 lysine 27 (H3K27me3). MPO and PRTN3 promoter methylation are negatively correlated with patients with active AAV and increase during remission of the disease[13]. H3K27me3 trimethylation reduction was also associated with active disease by expressing aberrant MPO and PRTN3 genes[14].

Exposome

The initial report of AAV was coupled with an infectious disease[1]. More recently, AAVs have been shown to be triggered by infectious agents, namely after exposure to toxic shock syndrome toxin-1, a toxin secreted by *Staphylococcys aureus*, and relapses have also been associated with higher nasal *S. aureus* colonization[15,16].

Many other factors appear to be related with higher risk for AAVs. Seasonality shows discrepant results in the literature, but AAVs, namely GPA, seem to have a more prevalent onset in autumn and winter months. The prevalence of AAV subtypes also seems linked with temperature, latitude, and UV radiation[17,18].

As mentioned above, some drugs can precipitate AAV; thus, drug indications need to be weighed when studying the correlation between drugs and the risk for AAVs. Alopurinol seems to be the only drug that conferred a greater risk for developing GPA[19].

The role of airborne particles has also been studied, with multiple approaches suggesting a higher disease risk when being exposed to certain pollutants, namely silica, carbon monoxide, hydrocarbons, and high organic solvents and particles associated with farming and gardening activities. As for smoking, there is a weak correlation to higher risk for developing the disease[17,20-22].

ANCA PATHOGENICITY

ANCA patterns

Two major types of ANCA can be detected by indirect immunofluorescence of ethanol-fixated neutrophils. Perinuclear ANCAs (p-ANCAs) stain around the nucleus and are mainly composed of myeloperoxidase. Conversely, some ANCAs stain diffusely in the cytoplasm, namely cytoplasmatic ANCAs (c-ANCAs), of which the most important is PR3. Other "minor" ANCAs have been described: against α-enolase, azurocidin, bactericidal permeability-increasing protein (BPI), cathepsin G, elastase, defensin, lactoferrin, lysosome-associated membrane glycoprotein 2 (LAMP2), and moesin. These antibodies are mainly p-ANCAs and are rarely associated with vasculitis. Nowadays, enzyme-linked immunosorbent assay (ELISA) tests detect MPO and PR3 instead of p-ANCAs and c-ANCAs, which correspond to the staining pattern[15].

ANCA production

Neutrophils participate in the innate immune defense. These cells form networks of extracellular fibers, primarily composed of DNA from neutrophils, which bind pathogens[23], the so-called neutrophil extracellular traps (NETs). An imbalance in NETs' production is known to participate in ANCA production. These traps are degraded by serum DNAse I. Nevertheless, in some circumstances, as in the case of propylthiouracil administration, NETs become resistant to DNAse I. The overexposure to NETs promotes the production of autoantibodies against neutrophils. Meanwhile, the traps are formed, and the content of neutrophilic granules, including PR3 and MPO, becomes mixed with chromatin fibers, which bind to DNA. By this physiological step, the antigenicity of PR3 and MPO might suffer modifications[24]. However, only a second step that prevents a complete degradation of NETs seems to be specific to some autoimmune diseases, such as AAVs and systemic lupus erythematosus[24,25]. This impaired NET degradation coincides with the lower serum DNAse I activity observed in patients with AAV when compared to healthy individuals[26]. Moreover, a later step triggers the autoimmune reaction by presenting misfolded proteins/antigens, in which cryptic epitopes are then recognized in class II MHC molecules by the immune system[27].

PATHOGENESIS OF AAV

Facing a bacterial or viral infection, dendritic cells produce tumor necrosis factor (TNF)-β and interleukin-6 (IL-6). These inflammatory markers stimulate the differentiation of T cells into T helper 17 (Th17). In turn, Th17 cells produce IL-17, an important cytokine that can induce the production of TNF-α or IL-1β by macrophages. These proinflammatory cytokines then prime the innate immune system, namely neutrophils and the formation of ANCAs. Additionally, the autoreactive response is sustained by imbalances of T regulatory cells (Treg) that regulate the differentiation of Th17 and are associated with increased risk of AAV[28,29].

The priming of the neutrophil also occurs by the complement system, namely C5a by the altern complement pathway, as described in animal models[30], which is related to hypercoagulability states in patients with AAV due to tissue factor release[31].

Upon priming, neutrophils express surface ANCA antigens. These ANCAs, besides binding to the corresponding antigens, also bind to the Fcγ receptor in neutrophils. This positive feedback induces an excessive activation of these neutrophils, promoting cytokine production and reactive oxygen species and lytic enzymes release[32], further incrementing the inflammatory status. ANCA-stimulated neutrophils release NETs that contribute to the local inflammatory response and epithelial damage [Figure 1]. At the same time, more neutrophils are activated either directly by NETs or indirectly by circulating ANCA.

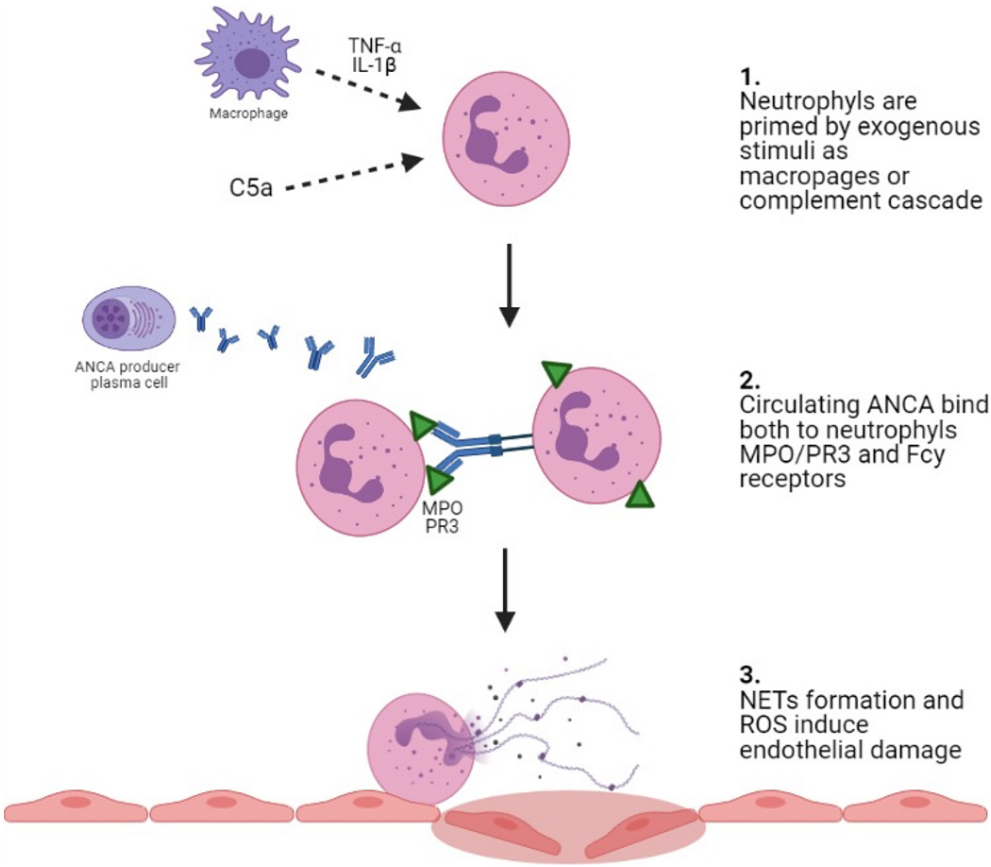

Figure 1. The inflammation cascade of anti-neutrophil cytoplasmic antibody (ANCA)-antibody associated vasculitis. ANCAs produced by plasma cells bind to myeloperoxidase and proteinase 3 produced by activated neutrophils (mediated by factors such as TNF-α or IL-1β). The neutrophil activation stimulates more neutrophils, leading to the production of extracellular traps (NET) and reactive oxygen species, leading to tissue damage, granulomatosis, and necrosis (image created in biorender.com). MPO: Myeloperoxidase; PR3: proteinase 3; NETs: neutrophil extracellular traps; ROS: reactive oxygen species; TNF-α: tumor necrosis factor α; IL-1β: interleukin-1β.

B cells are also activated by $CD4^+$ T presenting cells, through the presentation of antigens resulting from NETs[33]. Another important mechanism may be by the release of B-cell-activating factor (BAFF) or B-lymphocyte stimulator (BLyS) from activated neutrophils[33].

In GPA, chronic exposure to *Staphylococcus aureus* by nasal mucosa colonization might be the pathogenic trigger[34], by priming a local innate immune response that, under the right circumstances, may end up in AAV development. In the case of EGPA, which features eosinophilia, a vascular endothelial cell release of eotaxin 3 has been implicated in the tissue infiltration of eosinophils. These eosinophils secrete major basic proteins, eosinophilic cationic proteins, and neurotoxins that destroy the tissue[35]. Despite this particularity, the mechanism of MPO-ANCA production in these patients is still undisclosed.

BIOMARKERS IN AAV

Finding non-invasive markers capable of diagnosis and predicting disease activity, prognosis, and therapeutic options is a well desired goal for numerous studies related to AAV. As such, many studies have focused on the search for practical non-invasive markers to track AAV, as discussed below and summarized in Table 2.

Table 2. Anti-neutrophil cytoplasmic antibody-associated vasculitis biomarkers and their usefulness

Approach	Biomarker	Function	Description
Diagnosis	PR3-ANCA and MPO-ANCA	Distinguish from healthy patients and between AAV subtypes	Present in patients with GPA and MPA subtypes, respectively
	cfDNA	Distinguish between AAV subtypes	Increased levels of cfDNA in PR3-ANCA GPA patients compared to
	sCD163	Distinguish from healthy patients	Higher urinary levels of sCD163 in patients with AAV
	Eotaxin-3, IgG4 and CCL17/TARC	Distinguish between AAV subtypes	Increased levels of Eotaxin-3, IgG4 and CCL17/TARC in EGPA compared to other AAV subtypes
	12-HETE	Distinguish between AAV subtypes	Higher exhaled breath concentrations of 12-HETE in EGPA patients than healthy patients
Prognosis	Recognizable N-terminus of MPO heavy chain	Evaluate disease prognosis	Recognizable N-terminus of MPO heavy chain are more prone to develop severe disease
	C3	Evaluate disease prognosis	Low serum C3 levels is associated with poorer outcomes
Disease activity	PTX3+ and HMGB+	Distinguish disease activity status	Concentrations of PTX3+ and HMGB+ were higher in active AAV compared to remission
	cfDNA	Distinguish disease activity status	Concentration levels are higher in disease activity phases namely in PR3-ANCA positive GPA
	MCP-1, AGP, KIM-1, NGAL	Detect renal flares	Urinary levels of MCP-1, AGP, KIM-1, NGAL are increased in renal flares of AAV
	sCD163	Distinguish disease activity status	Higher urinary levels of sCD163 in disease activity phases
	ESR, CRP and Calprotectin	Distinguish disease activity status	Higher levels of ESR, CRP and Calprotectin in disease activity phases
	Eotaxin-3, IgG4 and CCL17/TARC	Distinguish disease activity status	Increased levels of Eotaxin-3, IgG4 and CCL17/TARC in disease activity phases
	sRAGE	Distinguish disease activity status	Assessing mild/limited disease activity
	EMPs and CECs	Distinguish disease activity status	EMPs and CECs levels positively correlate with disease activity
	$CD25^+$ T-cells Th17 cells	Distinguish disease activity status	$CD25^+$ T-cells inversely correlate with disease activity Th17 cells positively correlate with disease activity
	T follicular helper (Tfh) cells Tfh2/Tf1 ratio	Distinguish disease activity status	Tfh cells positively correlate with disease activity Tfh2/Tfh1 ratio shift
	Bb, C3a, C5a and soluble C5b-9	Distinguish disease activity status	Bb, C3a, C5a and soluble C5b-9 urinary levels positively relate with disease activity.
Relapse risk	PR3-ANCA	Evaluate relapse risk	Increased PR3-ANCA levels positively relate with higher relapse risk
	Calprotectin	Evaluate relapse risk	Elevated calprotectin levels in patients who relapsed
	EPCs	Evaluate relapse risk	Reduced levels of EPCs positively relate with higher relapse risk
	B-cells	Evaluate relapse risk	Incomplete B-cell depletion and B-cell repopulation after rituximab treatment relate with higher relapse rate
	CD8+ T-cells	Evaluate relapse risk	CD8+ T-cells levels positively relate with higher relapse risk
Response to treatment	PR3-ANCA	Evaluate response to treatment	PR3-ANCA positive respond better to treatment with Rituximab

GPA: Granulomatosis with polyangiitis; MPO: myeloperoxidase; ANCA: anti-neutrophil cytoplasmic antibody; MPA: microscopic polyangiitis; AAV: associated vasculitides; ESR: erythrocyte sedimentation rate; CRP: C-reactive protein; EGPA: eosinophilic GPA; EMPs: endothelial microparticles; EPCs: endothelial progenitor cells.

ANCA

The value of ANCA as a diagnostic marker is mainly irrefutable. Occasional cases of "ANCA-negative" AAV are acknowledged, decreasing by the day with ANCA testing methodology improvement[36,37]. False-negative results can still be found because in some cases ANCA binds to the circulating ceruloplasmin[38].

The value of ANCA goes beyond its simple diagnostic value. The ANCA-positive patient subtypes, namely PR3-ANCA and MPO-ANCA, differ from one another regarding genetic basis, epidemiology, clinical manifestations, histological findings, response to therapy, and pathogenesis. These facts sustain the reason to distinguish between PR3-ANCA and MPO-ANCA patients, meaning GPA (commonly associated with PR3-ANCA) and MPA (commonly associated with MPO-ANCA)[36,39].

Whereas the ANCA diagnostic value is no longer under doubt, its role as a disease activity marker has been the subject of frequent studies with varying outcomes[40]. Discrepant results regarding disease activity have been a matter of discussion in the literature[41-43]. This misalignment in the results can be interpreted by differences in the epitopes and affinities of ANCAs. Patients with a recognizable N-terminus of MPO heavy chain are more prone to develop a severe disease[44], while patients with low-affinity despite high titers of MPO-ANCA have a lower vasculitic disease activity[45]. Some clinical studies show a direct link between an increment in PR3-ANCA levels during complete remission and an increased risk of relapse[46,47], while others find a direct link between PR3-ANCA-positive patients and relapse probability compared to MPO-ANCA patients[48,49].

The role of ANCA subtypes in predicting response to treatment is also a subject under scrutiny. Some studies suggest that adult PR3-ANCA-positive patients respond better to rituximab than to conventional induction/remission maintenance treatment with cyclophosphamide and azathioprine, leading to the belief that ANCA serotype may guide the type of treatment in AAV[50].

Biomarkers derived from neutrophil activation - NMPs and NETs

Neutrophil microparticles (NMPs) are membrane vesicles that induce endothelial damage in AAV, whereas NETs, composed of DNA, histones, and neutrophil proteins and released by ANCA-stimulated neutrophils, contain antigenic components including PR3 and MPO[51].

Removal of NMPs by filtration abolished a pathological trigger connected to endothelial activation, suggesting a target for therapeutic plasma exchange in AAV. Despite the suggestion, clinical utility remains unvalidated. A recent study concluded that MPO patients' NMPs express higher levels of pentraxin-3 (PTX3), high mobility group box 1 protein (HMGB1), and tumor necrosis factor-like weak inducer of apoptosis (TWEAK) when compared to healthy controls[52]. Concentrations of $PTX3^+$ and $HMGB1^+$ were significantly higher in active AAV patients compared to those in remission[52]. PTX3 serum levels were strongly correlated with the Birmingham vasculitis activity score[52].

Moreover, Lange *et al.*[53] detected a significant increase in serum circulating free DNA (cfDNA) levels in PR3-ANCA GPA patients compared to EGPA and concluded that there is an association between concentration of cfDNA and disease activity. These findings suggest that abnormal formation and/or insufficient clearance of NETs may contribute to increase the levels of cfDNA in GPA. The detection of cfDNA levels or NETs may serve as a marker of disease activity in AAV, namely PR3-ANCA-positive GPA[53,54].

Urinary biomarkers

Urinary biomarkers could be ideal for the non-invasive monitoring of disease activity and renal involvement.

Lieberthal *et al.*[55] found that urinary levels of monocyte chemotactic protein 1 (MCP-1), α-1-acid glycoprotein (AGP), kidney injury molecule 1 (KIM-1), and neutrophil gelatinase-associated lipocalin

(NGAL) presented statistically significant increases during renal flares among patients with AAV. Of those, MCP-1 chemokine granted the best discrimination between active renal disease and remission[55]. MCP-1 is in fact the most promising urinary biomarker for AAV still under research, with multiple studies suggesting usefulness in the assessment of vasculitis and monitoring treatment[55-57].

Urinary soluble CD163 was presented as an even more promising biomarker of renal vasculitis than urinary MCP-1[58]. CD163 is a soluble form of a high affinity scavenger receptor for the hemoglobin-haptoglobin receptor complex and functions as an innate sensor for bacteria. Patients with small vessel vasculitis (which includes GPA, MPA, and EGPA) had markedly higher urinary sCD163 levels than patients in remission, disease controls, or healthy controls[58]. However, serum sCD163 levels failed to distinguish infections from active disease, which may limit its use[59].

Inflammatory markers

Traditional inflammatory markers such as ESR (erythrocyte sedimentation rate) or CRP (C-reactive protein) are non-specific markers for AAV.

Monach *et al.*[60] evaluated whether these markers were able to distinguish severe AAV [BVAS for Wegener's granulomatosis (BVAS/WG) ≥ 3 at screening] from remission. All subjects with severe active vasculitis at screening were followed during remission at Month 6, of whom 24 out of 28 showed significant decline[60]. Although they are not specific, traditional inflammatory markers correlate with the activity of the disease.

Eosinophilia is the hallmark of EGPA; however, the number of circulating eosinophils may not be an adequate biomarker for active EGPA since the eosinophil count usually drops dramatically and rapidly after treatment with glucocorticoids. Indeed, the treatment effect, mainly glucocorticoids, may directly cause the mispresenting comparison between active and inactive EGPA. Eotaxin-3, immunoglobulin G4 (IgG4), and CC chemokine ligand 17 (CCL17/TARC) levels were higher in active EGPA compared to healthy controls, inactive EGPA, and/or other diseases featuring vasculitis or hypereosinophilia[35,61,62]. Interestingly, eotaxin-3 levels were lower in hypereosinophilic syndromes than EGPA, underpinning a potential diagnosis use[35,61].

Pepper *et al.*[63] suggested calprotectin as a potential disease biomarker in patients with AAV by showing that patients with AAV had higher monocyte and neutrophil cell surface calprotectin expression than healthy controls and that its levels increased following treatment withdrawal and were significantly elevated in patients who relapsed.

12-Hydroxyeicosatetraenoic acid (12-HETE) measurement in exhaled breath concentrates (EBC) is a very different approach to distinguishing EGPA from asthma or hypereosinophilic syndromes. Szczeklik *et al.*[64] identified markedly higher concentrations of 12-HETE in EBC of EGPA than of other compared diseases, even though 19 out of 23 patients were receiving glucocorticoids and one third were clinically in remission.

Clinical trials also provide promising markers, such as MMP-3, TIMP-1, and CXCL13 (BCA-1)[60], which need further investigation with proper endpoints. sRAGE (serum advanced glycation end products) is another particular biomarker that may reflect the burden of granulomatous inflammation in GPA[65] and therefore be useful for assessing mild/limited disease activity.

Endothelial damage biomarkers

Two important biomarkers of endothelial injury were studied in AAV: endothelial microparticles (EMPs) and circulating detached mature endothelial cells (CECs).

EMPs are complex vesicular structures released from activated or apoptotic endothelial cells that play a singular role in inflammation, coagulation, endothelial function, and angiogenesis. Their dysregulation disturbs the vascular homeostasis, contributing to the progression of vascular diseases, such as vasculitis[66,67]. For this reason, research on EMPs identified a positive correlation with disease activity when compared with patients in remission[68]. These same EMPs may generate excess thrombin and thrombotic complications, namely in children with systemic vasculitis[69] and cerebral vasculitis of the young[70].

CECs have also been described as a biomarker of disease activity in vasculitis[71]. These are necrotic, highly activated endothelial cells that detach from the vessel wall and positively correlate with vasculitis in adults and children[67,71]. Upon endothelial lesion, bone marrow-derived endothelial progenitor cells (EPCs) increase in order to perform endothelial repair. These events have been described to be predictive of early relapse in adults with AAV, in whom a reduced number of circulating EPCs has been observed[72].

Von Willebrand factor (VWF) is yet another biomarker of disease to be confirmed in adult AAV patients. This plasma protein, synthesized primarily by megakaryocytes and endothelial cells, mediates platelet aggregation and adhesion. VWF levels increase in response to endothelial injury or activation, and it has been described as a biomarker of disease activity in childhood CNS vasculitis that was not confirmed in adults[70,73].

B cell, T cell, and cytokines

B-cell subset populations have drawn study interest, with data from observational studies suggesting that incomplete B-cell depletion and B-cell repopulation after rituximab treatment is associated with a significantly higher relapse rate[74,75].

B-cell subset populations of interest include regulatory B cells (Bregs), such as $CD5^+$ cells[40]. Measurement of Bregs showed initial promise with a lower $CD5^+$ B-cell count correlating with active disease, but a subsequent analysis from the RAVE study found that this count was not predictive of disease relapse, severity, or treatment failure[76,77].

In some studies, elevated levels of B-cell activating factor (BAFF) have been found in patients with AAV active disease, with a corresponding fall after treatment. However, BAFF levels are affected by corticosteroid treatment, rendering it an inadequate biomarker in AAV[78].

T-cell involvement in AAV was previously discussed. CD25 and CD28 T-cell markers were already correlated with disease activity. $CD25^+$ marker on T cells was inversely related to disease activity, however it is not clear if its population reflects a cause or effect of the disease[39]. $CD28^+$ T cells are associated with a high risk of relapse, displaying markers associated with T-cell survival and memory T-cell levels; thus, the $CD8^+$ T-cell profile might be able to identify a group of AAV patients more likely to relapse[39]. Another subset of T cells, Th17 cells, has been identified as a potential driver of the disease. Th17 cells seem to be autoantigen-specific and are also spotted in renal lesions of AAV patients[79]. von Borstel *et al.*[80] analyzed $CD19^+CD24^{hi}CD38^{hi}$ B cells from the peripheral blood of AAV patients in remission and healthy controls. They found a negative correlation between Th17 cells and Breg and concluded that Th17 cells in AAV patients are at least partially controlled by Breg, highlighting another population that may be responsible for the disease and a target for its control[80]. Indeed, targeting B cells by the use of rituximab has shown a sustained remission at Month 28 when compared with other maintenance options[81,82]. However, as discussed above, B-cell activity has not been predictive of disease relapse. Other research groups found that an imbalance of Th17 and regulatory T (Treg) cells may also be of potential use to monitor AVV patients

with renal involvement[83]. An increase of Th17 cells and a decrease of Treg cells, along with a downward trend for IL-2 and IL-4 and an upward one for IL-6, IL-10, TNF-α, IFN-γ, and IL-17A, were observed in AVV patients[83].

T follicular helper (Tfh) cells and T follicular regulatory (Tfr) cells balance is critical for humoral immune responses and relevant in autoimmune diseases[84]. AVV patients present increased circulating Tfh cells ($CD4^+CXCR5^+CD25^-CD127^{interm-hi}$), decreased Tfr cells ($CD4^+CXCR5^+CD25^+CD127^{lo-interm}$), and elevated Tfh/Tfr ratios compared with healthy control[84]. Moreover, this study observed a Tfh2/Tfh1 shift and increased plasma IL-21 level associated with AAV and disease activity[84]. This suggests Tfh phenotype as a promising biomarker for AVV disease activity.

Complement

Alternative complement pathway activation is a fundamental way of developing disease. Gou *et al.*[85] found that urinary levels of Bb, C3a, C5a, and soluble C5b-9 were significantly higher in active disease.

Recently, the role of C5a in ANCA-induced neutrophil activation was shown, relating the generation of C5a by supernatants from ANCA-stimulated neutrophils with the role of C5a mediated effects via its specific receptor, allowing neutrophils to generate ROS in response to ANCA[31]. It was demonstrated that the lack of C5a receptor induces resistance to ANCA-induced disease, namely experimental anti-MPO vasculitis with the significant attenuation of the neutrophil glomerular influx and lower albuminuria, showing a potential therapeutic target in AAV[86].

In the first AAV human study of C5a, Yuan *et al.*[87] demonstrated higher levels of C5a in patients with active AAV compared with AAV in remission. Similarly, plasma levels of fragment Bb were significantly higher in active AAV than in patients in remission or healthy controls. Those levels positively correlated with the number of crescents on renal histology, vasculitis activity score, and serum inflammatory markers[88]. More recently, the ADVOCATE study group investigated an C5 receptor inhibitor in patients with ANCA-associated vasculitis-avacopan, which proved noninferior but not superior to prednisone taper with respect to remission at Week 26 and was superior to prednisone taper with respect to sustained remission at Week 52[89]. This result represents a role for C5 as a possible biomarker, as described above in the pathogenesis of the disease.

Finally, Manenti *et al.*[90] reported that low serum C3 levels at diagnosis is associated with poorer patient and renal outcomes in AAV patients; however, there were no significant association between serological and pathohistological phenotypes and serum C3 levels.

CONCLUSION

In this review, we discuss an updated understanding of the pathogenesis and novel biomarkers of AAV. The knowledge gap in the pathophysiology and follow-up of AAV diseases has raised research interest. Innovations in methodology, such as modern "omics", and new discoveries from other fields have incremented the AAV pathogenesis perception. Genetic, epigenetic, and environmental factors identification, along with the role of neutrophils, other immune cells, and humoral factor contributions, have now contributed to another milestone in AAV research. However, disease activity monitoring, treatment, and relapse prediction are not yet effective. Our most recent knowledge on the pathogenesis is identifying pivotal players in this disease and engendering new biomarkers soon to be studied in clinical settings. However, the demonstration of either the very high sensitivity or the very high specificity of new biomarkers has not yet reached clinically relevant outcomes.

Future collaborative research and interactions between basic and clinical research would be a good strategy for the comprehensive understanding of the etiology and pathogenesis of AAV and ultimately for improving the diagnosis, treatment, and prognosis of these patients.

DECLARATIONS

Authors' contributions

Wrote and reviewed the biomarkers: Almeida S
Wrote and reviewed the pathogenesis: Neves MP
Wrote and reviewed the introduction: Da Silva Domingues V
All authors contributed for the final version of the paper.

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Conflicts of interest

All authors declare that there are no conflicts of interest.

Ethical approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

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