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Review

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Sex disparity in hepatocellular carcinoma owing to NAFLD and non-NAFLD etiology: epidemiological findings and pathobiological mechanisms

Amedeo Lonardo[1], Stefano Ballestri[2], Pierce K. H. Chow[3,4], Ayako Suzuki[5,6,7]

[1]Operating Unit of Metabolic Syndrome, Azienda Ospedaliero-Universitaria di Modena, Ospedale Civile di Baggiovara, Modena 41126, Italy.
[2]Operating Unit Internal Medicine, Ospedale di Pavullo, Azienda Unità Sanitaria Locale, Modena 41126, Italy.
[3]Division of Surgery and Surgical Oncology, National Cancer Center Singapore and Singapore General Hospital, Council 169610, Singapore.
[4]Surgery Academic Clinical Program and Office of Education, Duke-NUS Medical School, Singapore 169857, Singapore.
[5]Division of Gastroenterology, Duke University Health System, Durham, NC 27710, USA.
[6]Division of Gastroenterology, Durham VA Medical Center, Durham, NC 27705, USA
[7]Programme in Cardiovascular and Metabolic Disorders, Duke-NUS Medical School, Singapore 169857, Singapore.

Correspondence to: Prof. Amedeo Lonardo, Department of Internal Medicine, Ospedale Civile di Baggiovara, 1135 Via Giardini, Modena 41126, Italy. E-mail: a.lonardo@libero.it

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Abstract

Nonalcoholic fatty liver disease (NAFLD) exhibits sexual dimorphism, with men being more exposed than women to the risk of simple steatosis, nonalcoholic steatohepatitis fibrosis, and hepatocellular carcinoma (HCC), while the protection conferred to women seemingly disappears with aging and reproductive senescence (i.e., menopause). HCC, the most common primary liver cancer, which carries an ominous prognosis, may result from various genetic and non-genetic risk factors. NAFLD is now projected to become the most common cause of HCC. HCC also exhibits a definite sexual dimorphism in as much as it has a worldwide high male-to-female ratio. In this review article, we focus on sex differences in the epidemiological features of HCC. Moreover, we discuss sex differences in the clinical outcome and molecular pathobiology of NAFLD-HCC. By highlighting the research gaps to be filled, the aim of this review is to prompt future research of sex differences in HCC and facilitate developing personalized cancer prevention strategies, detection, and treatments to achieve better patient outcomes in NAFLD-HCC, considering sex differences in HCC pathobiology.

Keywords: Liver cancer, pathobiology, personalized medicine, sex differences

BACKGROUND

Hepatocellular carcinoma (HCC), the most common primary liver cancer (PLC), carries an ominous prognosis, and is the fourth most common cause of mortality owing to cancer[1,2]. The chief modifiers of HCC risk include geographic variability, demographics and severity of liver disease[2]. Cirrhosis, irrespective of aetiology, increases the risk of HCC[1]. On a global basis, the proportion of HCCs attributed to nonalcoholic fatty liver disease (NAFLD) is increasing owing to trajectories of declining HCV infection and escalating NAFLD[1,3,4]. Additional risk factors for the development of HCC are infection with HBV, alcoholic liver disease, aflatoxin, and genetic haemochromatosis[5].

Spanning a wide range of liver histology changes, NAFLD faithfully recapitulates the whole spectrum of alcoholic liver disease though it is observed in the nonalcoholic patient[6] and in the absence of other competing causes of (steatogenic) liver disease[7]. Similar to HCC, NAFLD accounts for a substantial clinical burden and exacts a heavy toll of healthcare-related expenses[8].

Sex disparities in various human diseases, from initial manifestations to disease outcome, are often encountered in clinical practice. In fact, sex and gender act as powerful modifiers of the top ten causes of mortality and morbidity, including heart disease, cancer, chronic lung disease, Alzheimer's disease, influenza and pneumonia, chronic kidney and chronic liver diseases[9]. Clear sex disparity exists in HCC, which is twice as common in men as in women[1]. NAFLD also exhibits multifaceted sexual dimorphism[10,11]. It occurs more often in men than in women of fertile age and is heavily affected by reproductive status[12]. Understanding these sex differences is the key to deciphering the pathophysiology of the disease as well as in guiding personalized care[12].

On this background of evidence, we aimed at illustrating our current knowledge of sex differences in HCC and clarifying gaps to be filled in future research, while placing special focus on NAFLD-related HCC.

METHODS

The PubMed database was extensively searched for articles published as of the 31st of July 2020. The keywords used in our search include, but are not limited to: HCC, liver cancer, sex differences, gender differences, epidemiology, natural course, pathogenesis, risk factors, immune response, genetics, and sex hormones. Additional terms were used to search for articles reporting sex differences and/or the effect of sex hormones in specific mechanisms pertaining to carcinogenesis. Among the retrieved publications, only those that were deemed to be relevant based on consensus among the authors were retained.

EPIDEMIOLOGICAL MODIFIERS OF HCC RISK

Irrespective of its aetiology, HCC affects men more commonly than women owing to complex and multi-factorial reasons. This section reviews risk factors of HCC in general and discusses interactions between sex and risk factors.

Geographic area and ethnicity

Eastern Asia, Southeast Asia, and sub-Saharan Africa exhibit a high incidence and prevalence of HCC; Mongolia, China, Japan, Papua New Guinea, and Egypt are top-ranked countries[1]. By contrast, countries with a low incidence and prevalence include India, Russia, northern countries of South America, Argentina, European countries (except for southern countries), USA, and Australia with the rest of the world exhibiting intermediate rates of incidence and prevalence[1].

Multi-ethnic populations display a clear ethnic gradient. For example, in the United States, Asians/Pacific Islanders have been reported to have the highest incidence rate per 100,000 (11.7), followed by Hispanics (9.5), Blacks (7.5), while Whites had the lowest (4.2)[13].

Sex

With few exceptions, the male to female (M:F) ratio of the incidence of HCC ranges between 2 to 3 in the most of the countries, irrespective of whether they are high-rate areas or not, and are maximal in middle European countries (M:F ratio up to 5)[13,14]. In contrast, in Costa Rica, Colombia, Ecuador and Uganda, the M:F ratio of the incidence of HCC is smaller, ranging from 1.3 to 1.6[13,14].

The biological grounds underlying this sex disparity in the prevalence of HCC are incompletely defined and probably related to multiple behavioural, hormone-metabolic risk factors, and cancer biology. Sex differences in HCC pathogenesis are discussed below under sex disparity in HCC pathobiology.

The difference in the M:F ratio of the incidence of HCC among different countries is intriguing, suggesting potential race/ethnicity-sex interplay in HCC. At this point, sufficient data do not exist to delineate whether the difference is explained by a biological interplay and/or an interplay of gender attributes and culture/ethnicity.

Age

The overall incidence of HCC consistently peaks at 70 years in various countries worldwide, such as France, Italy, Japan, and USA (whites) and this is approximately 5-15 years before the peak occurrence of cholangiocarcinoma, the second most common PLC after HCC[15]. However, other authors report that the mean ages of diagnosis with HCC are 55-59 years in China and 63-65 years in Europe and North America[14]. In Qidong, China, where the HCC burden is among the world's highest, the age-specific incidence rates increase up to the age of 45 among men and then plateau; while increasing to the age of 60 and then plateauing among women[14]. A surveillance, epidemiology, and end results (SEER) Analysis (from 1988 to 2010) including 39,345 patients with HCC (Men 76%, women 34%) showed that men are diagnosed 4-7 years earlier than women across the race/ethnic groups[16]. These findings suggest that sex and age interact in the occurrence of HCC, implying that consideration of this interaction (as opposed to treating age and sex as independent variables) will be essential in future research.

Severity of liver histology

While cirrhosis is an almost essential pre-requisite for the development of HCC in those with HCV infection, infection with HBV exerts a more direct carcinogenic effect on the liver[1]. Similar to HBV infection and to alcoholic liver disease, NAFLD-HCC may occur in non-cirrhotic livers[14,15]. A Japanese descriptive study reported that men with nonalcoholic steatohepatitis (NASH) developed HCC at earlier liver fibrosis stages than women[17]. The study was too small to confirm the sex difference but provides an intriguing hypothesis pertaining to carcinogenesis. Further larger studies are warranted to investigate this.

Viral hepatitis

With the exceptions of Japan and Egypt (where HCV infection is the chief risk factor of HCC), in most high-risk countries, chronic HBV infection and aflatoxin B1 are the major risk factors for the development of HCC, whereas HCV infection, excessive alcohol consumption, and common metabolic disorders (diabetes, obesity and metabolic syndrome) prevail in low-rate areas[15]. Chronic drinking of alcohol > 80 g/day for over 10 years increases the risk of HCC by a factor of 5; and alcohol consumption enhances the risk of HCC in those with either chronic hepatitis C or NAFLD[10,18]. However, given that these data often combine both sexes, research needs to be conducted urgently to clarify the sex-specific thresholds of alcohol consumption that are associated with a raised HCC risk.

Family history

HCC commonly exhibits familial clustering, and family history of disease is a risk factor for the development of HCC. Interestingly, family history of HCC was identified as a favourable prognostic factor for improved survival particularly in those individuals whose tumours can be radically cured, even in the stage-stratified analysis[19]. In the study, female sex and younger age, non-diabetics, and lifetime non-drinkers were more common among individuals with first-degree family histories of HCC than among those without such histories[19]. The exact mechanisms underlying the above associations remain uncertain.

Genetic risk determinants of NAFLD and inherited metabolic liver diseases

Genetic variants associated with an increased risk of NAFLD, advanced NAFLD, and NAFLD-HCC appear to contribute to the risk of HCC in the general population. A recent study conducted using Danish and UK databases demonstrated that a genetic risk score using three genetic variants [i.e., patatin-like phospholipase domain-containing protein 3 (PNPLA3) p.I148M, transmembrane 6, superfamily member 2 (TM6SF2) p.E167K, and hydroxysteroid 17-beta dehydrogenase 13 (HSD17B13) rs72613567], is associated with an up to 12-fold higher risk of cirrhosis and up to a 29-fold higher risk of HCC in individuals from the general population from these countries[20].

Certain inheritable metabolic disorders such as hemochromatosis, α-1 antitrypsin deficiency, tyrosinemia, glycogen storage diseases and several porphyrias also increase HCC risk, although they account for a negligible HCC risk globally[21,22].

Other risk factors of HCC

Smoking and co-infection with HIV also contribute to the development of HCC[1]. Certain environmental factors or occupational factors, such as vinyl chloride, polycyclic aromatic hydrocarbons, aflatoxins, and aristolochic acid, a common ingredient of traditional herbal medicine, have been suggested to play a role in the development of HCC[23,24]. How these factors and underlying mechanisms intersect with sex and sex hormones in the development of HCC has not been fully elucidated.

Interaction between sex/gender and metabolic risk factors

Sexual dimorphisms in metabolism are well-known (recently reviewed elsewhere[9,10,12]) and likely account for sex differences in HCC risk. A few other risk factors have been suggested for sex/gender-interaction, which are also discussed in this section.

Obesity has been associated with a higher risk of HCC incidence in men than women, especially in non-Asians[25]. A recent study conducted in an Asian population found a different relationship between BMI and HCC risk according to sex, following a U-shaped and a linear curve in men and women, respectively[26]. Studies reported a stronger risk association between pre-diabetes/diabetes and HCC in men than women[26-28].

NAFLD has a definite sexual dimorphism; men are more prone than women to the risk of uncomplicated steatosis, NASH fibrosis, and HCC. However, aging and menopause are associated with the disappearance of protection in women[12,29,30].

Prospective studies indicate that regular alcohol intake, although within safe thresholds, is a risk factor for the progression to HCC among individuals with NAFLD[31]. Moreover, among those with HCC, alcohol use is more frequent in men than in women[26,32].

Additionally, men are more prone to acquire HBV and HCV infection, develop chronic hepatitis, cirrhosis and HCC than women[14].

CLINICAL SEX DIFFERENCES IN HCC

Recent large epidemiological studies conducted in the USA have shown that, compared to men, women with HCC presented with older age, higher frequency of NAFLD, non-cirrhotic HCC, less-advanced tumour stage (by size, local/vascular invasion, metastasis) and lower frequency of alcoholic liver disease[32-34].

Studies regarding sex differences in survival rates have yielded conflicting results so far. Two recent large multi-centre US studies enrolling 5,327 patients with HCC (22.6% women) and 1,110 (23.5% women), respectively, reported higher overall survival rates among women after adjusting for confounding factors[33,34]. Consistently, one of these found that female sex was independently associated with early tumour detection [odds ratio (OR) 1.46] and response to first HCC treatment (OR 1.72)[33]. Conversely, other US studies found no sex-related difference in HCC prognosis[32,35], while Asian studies did[36-39]. Another US study showed age, sex, and ethnicity intersection in survival rates; women had higher survival rates from HCC than men before age 55, while after 65 years or among Hispanics, there was no such a survival difference between sexes[38]. Another US study also suggested a similar interplay of age and sex in HCC survival and further possible age-, sex-, and race/ethnicity-interaction in HCC survival[16]. Table 1 provides a synthetic overview of sex differences in risk factors, presentation, and outcome of HCC owing to NAFLD and non-NAFLD aetiologies. Future studies with proper consideration of these interactions are warranted to reconcile some inconsistency in the literature.

SEX DISPARITY IN HCC PATHOBIOLOGY

Chronic persistent injury induces wound-healing responses through the release of pro-inflammatory cytokines (e.g., IL-6, TNF-alpha) and increased oxidative stress in the liver[40]. The wound-healing process, together with persistent liver damage, promotes fibrogenesis and tissue DNA damages and facilitates hepatic carcinogenesis[41]. The liver tissue concentration of 8-OHdG, a marker of oxidative DNA damage, has been associated with epigenetic inactivation of tumour suppressor genes (i.e., methylated tumour suppressor genes)[42,43]. An increased number of methylated tumour suppressor genes was, in its turn, associated with a shorter time-to-HCC development in patients with chronic hepatitis C[44], demonstrating a mechanistic link of oxidative DNA damage, epigenetic alteration of tumour suppressor genes, and development of HCC. In NAFLD patients, chronic metabolic stress to hepatocytes aggravates oxidative stress, induces cellular protein/DNA damage, and promotes premature senescence of hepatocytes, contributing to an increased risk of HCC among obese individuals[12,45], even in the absence of cirrhosis.

Sex differences are well documented in cancer mechanisms[46]. Several well studied mechanisms accounting for sex differences are summarized in Table 2[47-56]. Compared to females, males are more susceptible to oxidative stress due to a higher NADPH oxidase activity, a lower NFR-2, and lower anti-oxidants[51,52,57], and have a higher induction of IL-6 by hepatic Kupffer cells under liver injury[54]. In contrast, higher physiological oestrogens protect females from HCC development via the anti-oxidative effects of estrogenic[51,57], anti-fibrotic effects[12], and inhibitory effects on IL-6 production by hepatic Kupffer cells[54]. The protective effects of oestrogen are lost after menopause, which may explain the fact that the male predominance observed in HCC incidence decreases with advancing age[58]. Gut microbiota also exhibits sex differences[56,59-61]. In an experimental mouse model, higher hepatic hydrophobic bile acids were observed in males, which was causally associated with a decreased expression of tumour-suppressive microRNA in the liver and increased incidence of HCC[61]. Importantly, similar sex differences in bile acid profile exist in humans[56], suggesting that sex-differences in gut microbiota and bile acid profile may contribute to male dominance in the development of HCC in man.

Despite the fact that evidence supports oestrogen exerting protective effects on the development of HCC, whether oestrogens have protective effects on the progression of HCC and patients' survival remains

Table 1. Sex differences in risk factors, presentation, and outcome of HCC owing to NAFLD and non-NAFLD aetiologies

Authors	Study characteristics	Sex differences		
		Risk factors	Tumour features	Outcome
Yang *et al.*[16]	Retrospective cohort from a national registry, US, 39345 HCC patients, 9557 (24%) F, diagnosed between 1988-2010	F older age (67 years *vs*. 61 years)	F more liver-limited (32% *vs*. 26%) and less metastatic (14% *vs*. 16%) disease	F better overall survival (11 months *vs*. 10 months; HR 0.93) independent of age, race, disease stage, or treatment. The protective effect of sex was greatest in patients aged 18-44 years (14 months *vs*. 10 months; HR 0.75) and it was lost after 65 years. No survival sex difference among Hispanics
Ladenheim *et al.*[35]	Retrospective cohort, US, 1,886 HCC patients, 437 (23.2%) F, diagnosed between 1998-2015	F older age (64 years *vs*. 60 years); M more HCV+ (43% *vs*. 37%), alcohol use (63% *vs*. 35%) and smoking (58% *vs*. 31%)	F less likely to present with tumours > 5 cm (30% *vs*. 40%) and more likely to be diagnosed by routine screening (66% *vs*. 58%)	No significant difference in median survival (30.7 months *vs*. 33.1 months)
Wu *et al.*[32]	Retrospective cohort single centre, Hawaii (US), 1,206 HCC patients, 307 (25%) F, diagnosed between 1993-2017	F older age (66.0 years *vs*. 62 years), more NAFLD/NASH (22% *vs*. 7%) M more HCV+ (43% *vs*. 37%), alcohol (53% *vs*. 12%) and smoking (68% *vs*. 38%)	F smaller mean size at diagnosis (5 cm *vs*. 6 cm), less vascular invasion (7.5% *vs*. 12%). F more likely to undergo HCC surveillance but less to undergo liver transplant	Similar overall survival. Mortality predictors at MVA: NAFLD/NASH for both M and F, age and smoking for M. Transplant predictive of survival for M
Lai *et al.*[39]	Retrospective cohort single centre, Asia (Taiwan), 516 consecutive HCC patients, 118 (22.9%) F, who received surgical resection between 2000-2007, F-up > 10 years	F more HCV + (37% *vs*. 23%); lower HBV + (59% *vs*. 73%)	F less micro-vascular invasion (25% *vs*. 36%)	Similar overall survival. F better recurrence-free survival and distant metastasis-free survival in patients with alpha-fetoprotein M 35 ng/mL, independent of other clinical variables
Rich *et al.*[33]	Retrospective cohort single centre, US, 1,110 HCC patients, 258 (23.5%) F, diagnosed between 2008-2017	F older age (63 years *vs*. 59 years), more NAFLD (27% *vs*. 8%) M more alcohol alone (17% *vs*. 5%) or with HCV+ (33% *vs*. 15%)	F *vs*. M earlier- BCLC stage tumours (53% *vs*. 44%) but similar liver function	F < 65 years had better overall survival than M (18.3 months *vs*. 11.2 months). However, older F and M had similar overall survival (15.5 months *vs*. 15.7 months) at UVA F sex associated with lower mortality (HR 0.82), early tumour detection (OR 1.46) and response to first HCC treatment (OR 1.72) at MVA
Phipps *et al.*[34]	Retrospective cohort multi-centric, US, 5,327 HCC patients, 1,203 (22.6%) F, diagnosed between 2000-2014	F more NAFLD (23% *vs*. 12%) and less alcoholic liver disease (5% *vs*. 15%)	Non-cirrhotic HCC higher among F (17% *vs*. 10%). F less-advanced HCC by tumour, node, metastasis staging and a higher proportion within Milan criteria (39% *vs*. 35%)	F greater overall survival (2.5 ± 2.9 years *vs*. 2.2 ± 2.7 years)

AFP: alpha-fetoprotein; BCLC: barcelona clinic liver cancer; F: female; F-up: follow-up; HR: hazard ratio; M: male; MVA: multivariate analysis; NAFLD: non-alcoholic fatty liver disease; NASH: non-alcoholic steatohepatitis; OR: odds ratio; US: United States; UVA: univariate analysis

uncertain. A few epidemiological studies implicated a potentially favourable effect of oestrogen on HCC survival, by demonstrating a beneficial association of exogenous oestrogen use with overall survival among women with HCC[62] and the better overall survival rates of women compared to men, which, however, disappears in advanced age[16]. However, possible beneficial effects of oestrogen on HCC survival have not been tested in experiments, and the mechanisms, if any, through which oestrogens affect HCC survival, remain uncertain.

HCC tumour tissue expresses oestrogen receptors, although the clinical and pathological significance of these remain controversial[63,64]. The positive expression rates of oestrogen receptors among HCC cases also

Table 2. Sex differences relevant to the pathomechanisms of HCC in NAFLD

Authors	Study characteristics	Sex differences		
		Risk factors	Outcome	Impact on HCC
Pre-hepatic factors increasing hepatic metabolic stress				
Lemieux *et al.*[47]	Total body fat and abdominal adipose tissue were evaluated in 89 men and 75 women using CT	Visceral adiposity	After adjusting for total body fat mass, men had significantly higher values of visceral adipose tissue volume and areas, measured by CT, than women. An increase in total fat mass was associated with a significantly greater increase in visceral adipose tissue volume in men than in women	Higher FFA release in males induces inflammation, insulin resistance, and lipotoxicity and fosters a tumour-promoting environment in the liver and may contribute to an increased recurrence of HCC
Laughlin *et al.*[48]	A cross-sectional study to measure serum leptin, adiponectin and sex hormone levels in 1510 community dwelling men and postmenopausal women aged 50-92 years	Visceral adipokines	Serum adiponectin and leptin levels were higher in women than in men. In both sexes, adiponectin concentrations were lower, and leptin levels higher, with increasing BMI and waist girth	A higher adiponectine level may protect women from developing HCC via the activation of AMPK and p38α[49]
Lönnqvist *et al.*[50]	BMI and age matched obese subjects (22 male and 23 female) undergoing elective surgery were evaluated for visceral fat lypolysis	Visceral fat lipolysis	Catecholamine-induced rate of FFA mobilization from visceral fat to the portal venous system is higher in obese men than in obese women, probably due to a larger fat-cell volume but also to a decrease in the function of α_2-adrenoceptors, an increase in the function of β_3-adrenoceptors, and an increased ability of cyclic AMP to activate hormone-sensitive lipase	Higher FFA release in males induces inflammation, insulin resistance, and lipotoxicity and fosters a tumour-promoting environment in the liver and may contribute to an increase recurrence of HCC
Oxidative stress/Senescence				
Augustine *et al.*[51]	Compared Nqo1 mRNA and protein expression and activity in males and females before and after applying known inducers using SD and August Copenhagen x Irish (ACI) rat strains	NAD(P)H: quinone oxidoreducatase 1 (Nqo1)	ACI rats showed minimal differences in Nqo1. In SD rats, Nqo1 mRNA, protein, and activity levels were significantly higher in females than in males. Female SD rats showed greater induction than male	Higher Nqo1 may lead to greater protection against oxidative stress and thus decreased susceptibility to carcinogens
Kratschmar *et al.*[52]	The interaction among corticosteroid, 11b-HSD1, and NFR-2 was evaluated using transfected HEK-293 cells and hepatic H4IIE cells. The hepatic expression levels of 11b-HSD1 and NFR-2 target genes were also compared between male and female Han Wistar rats	NFR-2	The study using the cell lines demonstrated that glucocorticoids, activated by 11b-HSD1 and acting through GR, suppress the Nrf2-dependent antioxidant response. This research also demonstrated that the hepatic expression of 11b-HSD1 was higher in male rats vs. female rats while the Nrf-2 target genes (HMOX1, NQO1 and ABCC3) were lower in male vs. female rats, confirming the above-demonstrated pathway	Higher activity of 11b-HSD1 and/or corticosteroid may lead to suppressed antioxidant response, which may lead to higher oxidative DNA damage
DNA damage/repair				
Hofer *et al.*[53]	DNA SSB and ALS were measured in blood samples from 99 subjects (age: 19-31 years) living in Stockholm, Sweden. Oxidative DNA damage was also analyzed using the DNA repair glycosylase FPG as well as HPLC-ECD for specific analysis of 8-oxodG	Oxidative DNA damage	Males had higher levels of SSB + ALS than females, although no difference was seen for oxidative lesions. There was no correlation between FPG sites and 8-oxodG. In females, there was a positive correlation between FPG levels and BMI and a negative correlation between SSB + ALS and fruit intake	Men are associated with a higher risk of oxidative DNA damage
Immune response				

Naugler *et al.*[54]	In mice administered with DEN, HCC incidence, and its relationship with hepatic IL-6 induction, Toll-like receptor adaptor protein MyD88, and oestrogen were evaluated in male and female mice	IL-6	A higher HCC incidence was observed in male vs. female DEN-induced hepatocarcinogenesis model. The higher incidence of HCC in males was associated with higher MyD88-dependent induction of IL-6 in male hepatic Kupffer cells under liver injury. Estradiol inhibited IL-6 production by hepatic Kupffer cells	Higher induction of IL-6 in Kupffer cells under liver injury partly explains higher incidence of HCC in males while estrogens protect females from HCC, in part, via reducing IL-6
Fibrosis				
Yasuda *et al.*[55]	Using the DEN model, hepatic fibrosis was compared between male and female rats	Stellate cell activation/ fibrogenesis	In male rats the induction of fibrotic response was significantly stronger than in female rats. Estradiol reduced hepatic fibrogenesis in male rats while concomitant administration of a neutralizing antibody against rat estradiol enhanced fibrogenesis. Oophorectomy in the female rats had a fibrogenic effect	Higher oestrogen protects premenopausal women from advanced hepatic fibrosis, a major risk factor of HCC
Tumor suppressor genes				
Xie *et al.*[56]	The mechanistic link between microbiota and hepatocellular carcinogenesis using a STZ-HFD induced NASH-HCC murine model and compared results for both sexes	Bile acid/ microbiota	STZ-HFD feeding induced a higher incidence of HCC in male mice, which was associated with increased intrahepatic retention of hydrophobic BAs and decreased hepatic expression of tumor-suppressive microRNAs. Metagenomic analysis showed differences in gut microbiota involved in BA metabolism between male and female mice. Treating STZ-HFD male mice with 2% cholestyramine led to significant improvement of hepatic BA retention, tumor-suppressive microRNA expressions, microbial gut communities, and prevention of HCC	Sex differences in microbiota lead to higher intrahepatic retention of hydrophobic BAs, decreased tumor suppressor microRNA in the liver, and an increased incidence of HCC in male mice

ALS: alkali labile sites; AMPK: AMP-activated protein kinase; BA: bile acids; CT: computed tomography; DEN: diethylnitrosamine; FFA: free fatty acids; FPG: DNA repair glycosylase; IL-6: Interleukin-6; NASH-HCC: nonalcoholic steatohepatitis-hepatocellular carcinoma; SSB: single strand breaks; SD: sprague dawley; STZ-HFD: streptozotocin-high fat diet

significantly varies in the literature, probably due to the differences in the methodologies and populations of the studies (e.g., ethnicity, sex), as well as stage and aetiologies of disease[64]. HCC tumour tissues express both oestrogen receptor alpha (ERα) and beta (ERβ), and also a variant form of ERα (vERα), which lacks exon 5 in the hormone-binding domain[65]. Compared to patients with ER-negative HCC, patients with ER-positive HCC have a shorter survival rate after curative resection[66]. Several randomized controlled trials were conducted to ascertain whether blockage of oestrogen signalling in HCC by the anti-oestrogen tamoxifen would improve the survival of patients with HCC. However, the results were consistently negative[67,68]. The presence of the liver vER receptor in the tumour is a strong negative predictor of survival in inoperable HCC patients and is a marker of clinical aggressiveness compared to wild-type ERαn[69,70]. HCC positive for the vER receptor is unresponsive to tamoxifen but responds to megestrol[71].

Sex differences in HCC biology have extensively been explored in recent years in functional signatures of differentially expressed genes[71,72], expression quantitative trait loci (eQTL)[73], and cancer-driver genes[73]. These studies strongly suggest that HCC in males and females are biologically distinct and may respond differently to treatments. This is in agreement with epidemiological data summarized in Table 1. However, no randomized controlled trials have demonstrated sex differences in HCC treatment response or clinical outcomes. The consideration of sex and women's reproductive history in clinical studies designs and

analyses is warranted in future trials to better understand whether and how these factors may modify treatment response to specific therapeutic targets and influence clinical outcomes.

CONCLUSIONS AND RESEARCH AGENDA

A robust line of research has shown multifaceted sexual dimorphisms in the NAFLD domain[10-12]. It is important to note that the observed sex differences in NAFLD are not linear throughout the course of the disease but rather mechanism-specific. Further studies will eventually contribute to more effectively reducing the NAFLD-HCC incidence by delineating sex differences in individual pathways and therefore allowing the development of a personalized approach in preventing NAFLD progression. Similarly, sex differences in HCC epidemiology have not been fully characterized with proper consideration of women's reproductive status/history. Therefore, we recommend that sex/gender and reproductive history should be considered in future clinical and epidemiological HCC studies. Further mechanistic understanding, together with the epidemiological characterization of sex differences and the impact of reproductive history will predictably help clinicians by allowing more accurate risk stratification and personalized therapeutic approaches in the future. A scoring system combining genetic and non-genetic HCC risk factors while considering biological disparities by sex and reproductive status may improve our future care, although sufficient data to develop such a scoring system is pending future research.

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Authors' contributions

Made substantial contributions to conception and design of the study and performed data analysis and interpretation: Lonardo A, Suzuki A
Wrote the section on the epidemiology: Ballestri S, Lonardo A
Contributed to the section on pathobiology: Chow PKH, Suzuki A
Edited the final draft: Lonardo A, Ballestri S, Chow PKH, Suzuki A
Prepared the revised version of the manuscript based on Reviewers' suggestions and comments: Lonardo A, Suzuki A

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