

## Gender differences in chronic alcoholic and viral liver diseases

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**Summary.** Chronic liver disease progresses in men and women at different rates, regardless of the etiology of the disease itself. In general, the natural history of chronic liver disease is more favorable in women than in men.

The biological basis of these marked differences, in an organ that is not considered a classical hormone-dependent organ, is the presence in the liver of receptors both for estrogens and for androgens, which make the liver susceptible to changes in hormone levels during the various stages of reproductive life. In the literature, there are several studies that demonstrate, both in experimental animal models and in humans, that the presence of estrogens, at levels similar to those of the fertile period, is in principle protective against the development of a more severe disease, while on the contrary the effect of androgenic modulation has negative effects.

Estrogen protection disappears when a woman goes into menopause. As estrogen levels decrease, the tendency to develop a more pronounced fibrosis increases. Most importantly, there is a marked propensity to develop primary liver cancer, which in women over 65 has a similar incidence to that of men.

**Key words.** Gender, sex hormones, menopause, fibrosis, hepatocellular carcinoma.

*Differenze di genere nelle malattie epatiche croniche alcoliche e virali*

**Riassunto.** Le malattie epatiche croniche progrediscono nell'uomo e nella donna con velocità diverse, indipendentemente dall'eziologia della malattia stessa. Tendenzialmente, la loro storia naturale è più favorevole nel sesso femminile che in quello maschile.

La base biologica di queste marcate differenze, in un organo che non è ritenuto un organo classicamente ormono-dipendente, è la presenza nel fegato di recettori sia per gli estrogeni che per gli androgeni, che rendono quindi il fegato suscettibile alle modificazioni dei livelli ormonali durante le varie fasi della vita riproduttiva. In letteratura sono numerosi gli studi che dimostrano, sia nell'animale da esperimento che nell'organismo umano, come in effetti la presenza di estrogeni, a livelli analoghi a quelli del periodo fertile, sia in linea di massima protettiva nei confronti dello sviluppo di una malattia più severa mentre al contrario l'effetto della modulazione androgenica ha effetti negativi.

La protezione estrogenica viene a mancare quando la donna va in menopausa. Man mano che i livelli estrogenici di-

minuiscono, aumenta la tendenza a sviluppare una fibrosi più marcata in tempo più breve ed aumenta la propensione a sviluppare tumore primitivo del fegato, che nelle donne oltre i 65 anni ha un'incidenza simile a quella dell'uomo.

**Key words.** Genere, ormoni sessuali, menopausa, fibrosi, carcinoma epatocellulare.

### Introduction

Chronic liver disease (CLD) progresses differently in males and in females, with a generally faster course and with greater consequences in the long term (fibrosis, cirrhosis and hepatocellular carcinoma) in men than in women. This applies not only to virally associated conditions but also, with few exceptions, to CLD of different etiology. This favorable condition ceases when the hormonal status changes because of menopause. Its onset often coincides with a sharp acceleration of the course of disease and worsening of the therapeutic response. One likely explanation for this chain of events lies in the loss, in the body and liver, of the effect of estrogens because of menopause and the taking over of androgen action. We will review the physiologic basis of estrogen action on the liver, the changes occurring in men and women in the different reproductive periods, and the consequences of these changes. We will also examine the effects of gender in various hepatic conditions and the consequences on natural history and therapeutic outcome.

### Liver and sexual hormones

The molecular basis for the hepatic responsiveness to sexual hormones (estrogens and androgens) lies in the presence in the liver of both estrogen ( $\alpha^1$  and  $\beta^2$ ) and androgen<sup>3</sup> receptors. They are functional active receptors, with high affinity for the ligand. They mediate the action of sexual hormones on the liver, which responds with relevant modifications of its function. A typical example is the marked increase in the synthesis of several proteins (ceruloplasmin, corticosteroid binding globulin, thyroid-binding globulin and testosterone-estradiol

binding globulin) during pregnancy. On the other hand, the presence of beta estrogen receptors (mainly localized in the biliary epithelium) has been related with the modulation of cholangiocyte proliferation<sup>4</sup>.

It is worth noting that although estrogens are associated with female sex and androgens with males, both types of receptors are present in the liver and the effect of sexual hormones on it is the end result of the fine balance between them. This balance varies throughout life as estrogens undergo remarkable changes depending on the reproductive status while androgen levels are much more stable. This will be associated with much higher consequences in women, as is the case in hepatitis C-positive CLD<sup>5</sup>.

The favorable action of estrogens in the liver is linked to their powerful antioxidant, antifibrogenic and anti-inflammatory action. The antioxidant action has been experimentally demonstrated in the model of dimethylnitrosamine-induced fibrosis in rats<sup>6</sup>. In a diet-induced model of liver fibrosis in zebrafish<sup>7</sup>, exposure to estrogens was associated with protection against the development of fibrosis, as menopausal female fish were as susceptible as male fish to fibrosis while fertile female fish were totally protected. Additionally, estrogens are also able to prevent hepatic oxidative damage, inflammatory cell injury and cell death by suppression of AP-1 and NF-kappa B activation and induction of Bcl-2 expression<sup>8,9</sup>.

## Virus-associated conditions

### *Hepatitis B virus*

A marked gender imbalance has always been reported for hepatitis B virus infection, starting from very old reports<sup>10</sup>. One of the hypothesized mechanisms lies in the presence of an androgen-responsive element in the enhancer I of hepatitis B virus<sup>11</sup>. An even greater disproportion has been reported during the progression of liver disease, with the highest rates in patients with hepatocellular carcinoma (HCC)<sup>12</sup>. Furthermore, HBV DNA levels (known to correlate with the course of the disease and the risk of developing HCC) are significantly higher in males than in females<sup>13</sup>. Another extremely relevant difference between males and females is the higher rate of spontaneous seroclearance of HBeAg in females: in a cohort of 1289 HBV carriers, 439 of whom HBeAg positive, the factors independently influencing HBeAg/anti-HBe seroconversion were female sex ( $p = 0.002$ ), genotype B ( $p < 0.001$ ), pre-core 1896 ( $p = 0.043$ ), baseline ALT and HBV-DNA. No difference was found between males and females for HBV-DNA clearance<sup>14</sup>.

A higher prevalence of more advanced CLD has been reported in males vs younger females. This difference

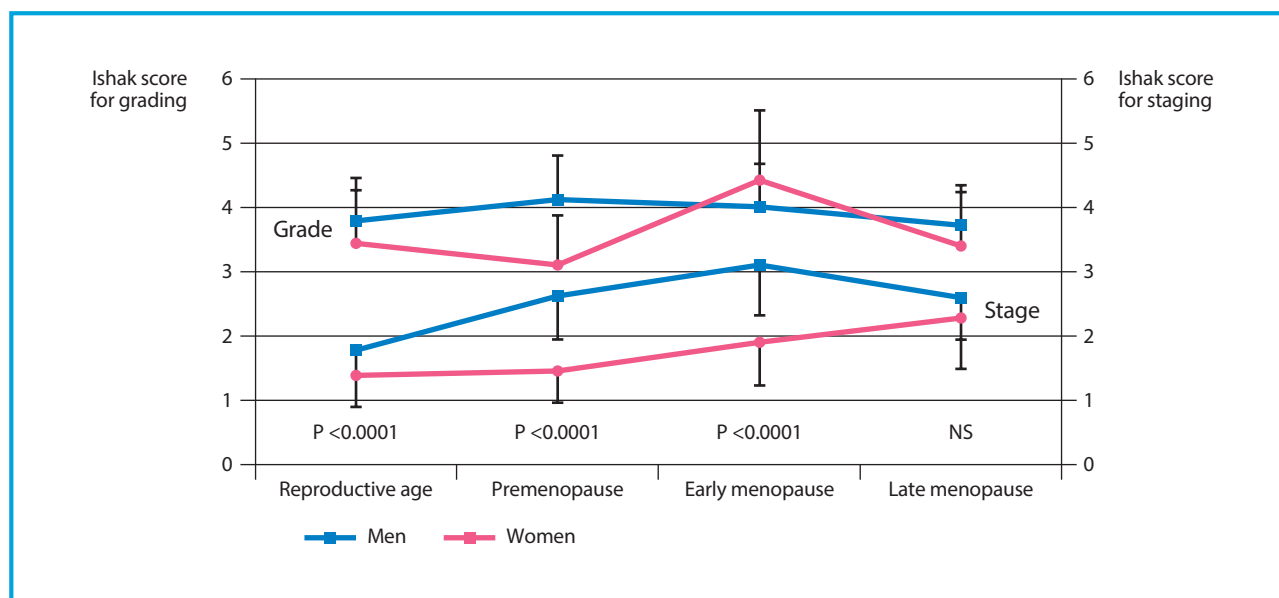
attenuates when older women are considered (MR Brunetto, personal communication). This becomes even more evident when HCC prevalence is studied. HCC prevalence in females vs males younger than 50 years is significantly lower than that in subjects older than 50 years<sup>15</sup>, further underlining that for women, aging and onset of menopause concur in leveling risk compared with males.

### *Hepatitis C virus*

The first indication of a strict relationship between gender and hepatitis C virus (HCV) infection comes from modeling studies by Poynard et al.<sup>16</sup> that showed that male sex has a stronger association with fibrosis progression than virological factors. Fibrosis progression in females was much slower. This was attributed by the authors to a positive effect of younger age at infection. Di Martino et al.<sup>17</sup> were among the first to identify a relationship with menopause, showing an accelerated progression of fibrosis in HCV-positive women after menopause. These Authors did not report a beneficial effect of hormone-replacement therapy (HRT), while a later study from another French group reported a potential benefit from HRT<sup>18</sup>. A retrospective study from our group<sup>6</sup> in more than 700 consecutive male and female patients, pair-matched by age in order to divide them in reproductive subgroups (fertile, premenopausal, early menopausal, late menopausal), with biopsy-proven chronic hepatitis C demonstrated that severity of liver fibrosis in women worsens in parallel with increasing estrogen deprivation. At multivariate analysis, male sex was independently associated with more severe fibrosis in the groups corresponding to pre-menopausal ( $p = 0.048$ ) and early menopausal ( $p = 0.004$ ) but not late menopausal pairs, indicating that estrogen protection is still active soon after the onset of menopause but is totally lost afterwards. We also showed the importance of the estradiol/testosterone ratio: it is the balance between the two that determines the ultimate effect on fibrosis progression (Figure 1)<sup>5</sup>.

Apart from fibrosis progression, menopause plays a negative role also in the outcome of antiviral IFN-based therapies. While women in fertile age had significantly better sustained virological response (SVR) than males of comparable age, menopausal women had a similar or even worse response than males<sup>19</sup>. Presence of menopause was shown to be the only independent factor for failure of antiviral therapy in genotype 1 patients<sup>19</sup>. The resistance of menopausal women to antiviral therapy has been partially overcome by first-generation protease inhibitors<sup>20</sup> and not completely solved by the new directly acting antivirals.

Pathogenetically, the critical event is the shift from a low inflammatory to a pro-inflammatory state. In



**Figure 1.** Different course of necro-inflammation activity (grade) and fibrosis (stage) scores in the four subgroups (divided by reproductive phase) of females (pink lines) and age-matched male patients (pale blue lines) with HCV-positive chronic hepatitis. Levels of significance are indicated for each female/male subgroup (modified from ref 5).

menopausal women, the level of circulating pro-inflammatory cytokines like IL-6, serum and TNF-alpha (important factors in the creation of hepatic damage) is significantly increased compared to women of childbearing age<sup>19</sup>.

This has a series of consequences in many different organs and tissues, including the bones, heart, brain, adipose tissue, and, among others, the liver. The consequences are consistent in HCV-positive women: in these women, HCV infection and menopause concur in inducing higher necro-inflammatory activity, increased hepatic steatosis, and eventually faster progression toward cirrhosis. Estrogen deprivation induces a rapid onset or a rapid worsening of the metabolic syndrome (increased intra-abdominal fat, atherogenic lipid profile, steatosis, insulin resistance) suggesting that the greater severity of liver disease after menopause may be at least partly explained by the occurrence of metabolically-mediated damage in association with that induced by the virus<sup>18</sup>.

Another recently described condition, which is likely to be related both with the ability of the HCV to replicate in extra-hepatic sites and with the induction of low-grade inflammation, is premature ovarian senescence, which is in turn associated with fewer live births, higher rates of miscarriage and gestational diabetes<sup>21</sup>. From this newly recognized condition comes the proof of concept of a pathogenic link with both infection and inflammation, as obtaining SVR with antiviral therapy reverses the condition and prevents miscarriages<sup>21</sup>.

### Alcoholic liver disease

It is well known that alcohol abuse determines more severe damage in females than in males. This is both due to lower levels of alcohol dehydrogenase in females, as well as to the different body distribution between males and females linked to the higher fat/water ratio in females. After the consumption of the same amount of alcohol, females reach higher blood alcohol concentrations. It has been shown that men and women who share the same drinking pattern have a significantly different risk of death (60% in females and 40% in males vs light drinkers)<sup>22</sup>.

Alcohol is an important co-factor in determining severity of liver disease in patients with virally associated liver disease. The relationship with the hepatitis B virus (HBV) has been well known for a long time<sup>23</sup>. As most HBV carriers are males, the relationship was mostly evident in males.

Most recently also the cooperation with HCV has become evident. Several reports have underlined the impact of alcohol abuse (an avoidable cause of liver damage) on the occurrence and progression of CLD<sup>24</sup>. A nation-wide study on nearly 100,000 French patients clearly showed the extremely relevant contribution of alcohol abuse on HCV burden. All aspects of the natural history related to HCV were affected. This study confirmed that males are 4 times more likely to have a combined etiology than females<sup>25</sup>.

In the transplantation setting, combined etiology (most frequently alcohol and HCV, occurring twice as

often in males vs females) worsens the outcome of liver transplantation, not as survival but mostly as degree of severity of fibrosis<sup>26</sup>.

Apart from these biological differences, males and females also exhibit social and biological differences in terms of alcohol-related problems. Men drink more, have more alcohol-related social problems than women, and have a higher prevalence of heavy drinking and drinking-related problems. Alcohol abuse has been identified as a substantial problem in Europe. A recent report by the WHO Regional Office for Europe indicated that 1 in every 7 deaths in men and 1 in every 13 deaths in women in the group aged 15-64 years is related to alcohol consumption<sup>27</sup>. This difference can be attributed to several factors but perhaps the most relevant is the difference in consumption patterns between genders and their different drinking habits: women drink more often but in lower amounts than men, who drink less frequently but consume much larger quantities of alcohol. There are substantial differences in drinking patterns also after liver transplantation and this, together with psychosocial issues, influences LT outcome, with the male sex again displaying the worst outcome (5-year survival rate 58% in males and 78% in females)<sup>28</sup>.

### Hepatocellular carcinoma

Hepatocellular carcinoma (HCC) is the expected complication of CLD, regardless of its etiology. The increase in incidence has recently slowed while mortality is still rising. Nevertheless, there are subgroups of subjects (e.g., men in the 55-to-64 age group) in whom incidence is steadily increasing<sup>29</sup>. On the other hand, the prevalence of nonalcoholic steatohepatitis is sharply rising, and this contributes to the lack of more positive modifications of its epidemiology<sup>29</sup>. In this study as well as in the majority of the studies reported in the literature, men are significantly more affected by HCC (2 to 4 times) than females. The male-to-female ratio is greater in areas with a high incidence of HCC (e.g., China and sub-Saharan Africa) and is very likely a consequence of the unbalanced proportion of M/F ratio in hepatitis B<sup>12</sup>. Investigating the risk factors for HCC in Italy, we observed that along with HBV, HCV and alcohol, male sex was one of the independent risk factors (7.4% of patients with HCC were males vs 1% females)<sup>12</sup>. These data were confirmed by the Italic database, which has collected the data of more than 5000 patients with HCC from 21 liver units all over Italy. Male prevalence, regardless of the observation period, was almost three times higher compared to women<sup>30</sup>. Along the same line, Shimizu et al.<sup>15</sup> showed that chronic hepatitis B progressed more rapidly in males than in females, and non-alcoholic fatty liver disease, cirrhosis and HCC occurred predominantly in men and

postmenopausal women, suggesting that the more rapid progression may be due, at least in part, to lower production of estradiol and a reduced response to the action of estradiol.

Gender seems to have a role also in HCC survival. Using the 'Surveillance, Epidemiology, and End Results (SEER)' database, Yang et al.<sup>14</sup> evaluated the impact of age, sex, race, and ethnicity on the survival of subjects with HCC. They showed that not only men are 4 to 8 times more likely to develop hepatocellular carcinoma (HCC) than women, but even more interestingly that the positive effect of female sex on overall survival (OS) was present only in patients younger than 64 years, whereas the survival advantage disappeared in patients older than 65 years. This underlines once more the dual role of female sex, with lower morbidity and better natural history during fertile age and rapid loss of these advantages after occurrence of menopause.

Apart from the role played by hormonal senescence, there are several relevant topics, which are more broadly associated with estrogen and androgen receptors in the liver. In several studies, high blood testosterone levels and low androgen receptor (AR) exon-I CAG repeats (20 repeats) have been associated with a higher HCC occurrence rate in men with chronic HBV infection<sup>31,32</sup>. The enzyme steroid 5-reductase type II (SRD5A2), which converts testosterone into dihydro-testosterone, has also been associated with the occurrence of HCC<sup>33</sup>. The genetic polymorphism SRD5A2 V89L has been related to different enzyme activity, with the V variant (both VV and VL) resulting in a higher enzyme activity than the L variant<sup>34</sup>. Studies on HBV transgenic mice carrying the entire HBV genome demonstrated that male HBV mice produced a higher level of HBV than female HBV mice and that the castration of male mice led to a reduction in HBV levels in mouse serum<sup>35</sup>. Another study on liver-specific knockout of the AR significantly reduced HBV DNA levels in mouse liver<sup>36</sup>. These data suggest that androgens and their receptors might be crucial in stimulating HBV replication *in vivo*. Interestingly, in a recent study Tian et al.<sup>37</sup>, using a HBV transgenic mouse model in which HBV genomic DNA and androgen-receptor (AR) short hairpin RNA (shRNA) were introduced into the liver of naïve mice by hydrodynamic injection showed that the effect of androgens on HBV was dependent on the AR, whereas the effect of the AR on HBV was only partially dependent on androgens.

As regards estrogens, there are data that oral contraceptives or HRT are associated with a lower risk of HCC, indicating that a longer exposure to estrogen in female HBV carriers might be beneficial in reducing HCC risk<sup>38</sup>. On the contrary, more than 70% of HCC female patients carry reduced levels of estrogen receptors (ER)<sup>39</sup>. In a complex study, performed in a mouse model of dieth-

yl-nitrosamine-induced HCC, Naugler et al.<sup>40</sup> demonstrated that the ER-mediated inhibition of interleukin-6 secretion from Kupffer cells was able to drastically cut the risk of developing HCC in male mice. Estrogens seem to suppress HBV replication and a high dose of estrogen has been shown to reduce serum HBV e antigen levels in a HepG2-transplanted mouse model<sup>41</sup>. Wang et al. investigated whether the estrogen pathway negatively regulates the HBV life cycle and the mechanisms involved in a study on HBV transgenic mice and showed that estrogens can suppress viral load in vivo. They also observed that estrogen can repress the transcription of HBV genes by up-regulating ER, which interacts with and alters binding of HNF-4 to the HBV enhancer I. These findings might account for the lower viral load and reduced incidence of liver cancer in HBV-infected women than men<sup>42</sup>. All these studies strongly imply in female subjects that estrogen has a protective role against the development of HCC, in contrast to the promoting role of androgens in male subjects.

## Conclusions

All these findings suggest that gender plays an important role in chronic liver disease. As shown in Table 1, the difference between males and females in this setting is higher in the young population and seems to decrease after menopause due to the loss of the protective role of estrogens.

Different strategies and therapies should therefore be evaluated taking into account the etiology and the severity of the underlying liver disease, as well as age and gender.

### Key messages

- Gender disparities in chronic liver disease are striking.
- These differences may be partially explained by the presence of sex hormone receptors in the liver.
- Women show less severe symptoms, a better natural history and better response to therapies.
- Onset of menopause markedly changes this favorable picture.
- Post-menopausal chronic liver disease is similar in severity and course to that of males.

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#### Financial support

The experimental studies cited in this review were supported by Regione Emilia-Romagna [Grants "PRU (Programma di ricerca Regione-Università) 2010-2013"] (to E.V.)

*Conflict of interest statement:* the Authors declare no conflicts of interest.

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