Review

Anabolic Androgenic Steroids and Hepatocellular Adenoma and Carcinoma: Molecular Mechanisms and Clinical Implications

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Abstract: Anabolic androgenic steroids (AAS) are a class of hormones that are used for hormonal replacement therapy in cases of male hypogonadism and for a few other medical conditions, mainly anemias, as well as for the female-to-male transition process. At the same time, AAS are widely abused for their muscle-building and strength-increasing properties. Among their side effects, androgens can exert a toxic effect on the liver, causing hepatotoxicity, but they can also induce hepatocyte proliferation and malignant transformation. Hepatocellular adenoma (HCA) and hepatocellular carcinoma (HCC) are two primary liver lesions that have been described as potentially related to AAS. This review provides an up-to-date analysis of how androgens can induce liver carcinogenesis and a comprehensive overview on the available data in the literature about AAS and primary liver tumors.

Keywords: Anabolic androgenic steroids; androgens; primary liver tumor; hepatocellular adenoma; hepatocellular carcinoma

1. Introduction

Hepatocellular adenoma (HCA) is a rare benign liver tumor that is significantly more frequent in women in of reproductive age or taking oral contraceptives. HCAs have been subclassified into six molecular subtypes that each yield a different risk of malignant transformation [1]. HCA in men is more rare, but it is associated with a higher risk of evolution to hepatocellular carcinoma [2].

Hepatocellular carcinoma (HCC) is the most frequent type of primary liver cancer. It represents a serious healthcare issue worldwide, accounting for more than 900,000 new cases/year and 830,000 deaths annually [3].

Up to 90% of HCC develops in the setting of a chronic liver disease, with liver cirrhosis defined as the main risk factor for the progression of hepatic carcinogenesis.

The prevalence of underlying liver disease etiologies varies among geographic areas, but the main causes are hepatitis B virus (HBV), hepatitis C virus (HCV), and alcohol abuse. Another significant cause of liver cirrhosis and HCC development in specific endemic areas is aflatoxin B1 chronic exposition [4].

Thanks to a systematic HBV vaccination program and the high rate of direct oral antivirals with a sustained virological response rate for HCV, we have observed a progressive and continuous increase in incidence of metabolic-associated liver disease, recently redefined as metabolic disfunction associated steatotic liver disease (MASLD), in the last decade [5]. So, the epidemiological scenario of hepatology is significantly changing, mainly in industrialized countries [6].

Regarding sex prevalence, HCC is more frequent in males (with a gender ratio of around 4:1). This significant gender disparity is partially justified by a higher incidence

of high-risk behaviors in men (e.g., alcohol abuse and injection drug use). Although there has been a significant increase in such behaviors in women, leading to an increase in the incidence of chronic liver disease and liver tumors, the different risk each gender has of developing HCC has not significantly changed [7,8].

These observations reinforce the hypothesis that intrinsic gender-specific variables are related to this difference in incidence rate.

It is now well known that the liver is a sexually dimorphic organ that is highly susceptible to the endogenous activity of sexual hormones, including estrogens, progesterone, and androgens. Sexual hormones’ receptors are constitutively expressed by liver cells, with a disparity in the gene expression patterns between men and women. Androgen, estrogen, and progesterone receptors are all present in hepatocytes; estrogen receptors are also present in Kupffer and stellate cells; and progesterone receptors are also present in cholangiocytes [9]. Several liver diseases (e.g., drug-induced liver injury, viral hepatitis, metabolic liver disease) are strongly involved with sexual-related gene expression pattern [10]. In particular, androgens and their nuclear receptors are involved in the pathogenesis of liver tumors.

Gender differences in HCC patients have been also observed in terms of clinical course and prognosis. HCC develops at an earlier age in males and has a more aggressive course and worse overall survival than in females [11,12]. Male patients with HCC tend to present more frequently with larger and multifocal tumors, with macrovascular invasion and extrahepatic spread at diagnosis, compared to female patients [13–15]. Consequently, men are less frequently eligible to receive curative treatments than women [16]. Lastly, male patients present a higher risk of early HCC recurrence after treatment [17].

Notably, estrogens have also a protective role, with the clinical implication that late postmenopausal women have the same overall survival than age-adjusted men [13], leading to a loss of the significance of all the previous cited gender differences in older people. Moreover, estrogen intake and oral contraceptive use have been shown to increase and reduce the prognosis in HCC females [18,19].

Anabolic androgenic steroids (AAS) are a class of drugs that are structurally related to testosterone, and which exert their function by binding to the androgen receptors (AR). Currently, AAS have very limited indications for the treatment of medical conditions. AAS are generally used in high, non-therapeutic dosages for their muscle-building and strength-increasing properties. This illicit consumption for recreational purposes is generally observed in young males in the context of gym practice or bodybuilding. The long-term use and/or abuse of AAS can cause hepatotoxicity and is related to a high risk of liver tumor development [20,21].

The aim of this review is to examine and summarize the role of androgens and AAS, for both therapeutical and recreational purposes, in liver carcinogenesis, and their clinical implications.

2. Anabolic Androgenic Steroids

AAS are a group of natural and synthetic hormones that share a chemical structure and biological effect. AAS have a steroid nucleus consisting of three cyclohexane rings and one cyclopentane ring. The term anabolic refers to their propriety of skeletal muscle building, whereas the term androgenic refers to their effect in the induction and maintenance of male secondary sex characteristics.

AAS could be administered by intramuscular injection or by oral ingestion. Intramuscular formulations are based on vegetable oils in which AAS are dissolved, with the addiction of aromatic compounds to increase AAS solubility. These molecules could be chemically modified by esterification of the 17β-hydroxyl group in order to prolong their half-life (e.g., unmodified testosterone and enanthate have a half-life of approximately 10 min and 4.2 days, respectively) [22,23]. AAS reach systemic circulation by direct diffusion or by lymphatic drainage.
Orally ingested AAS are rapidly absorbed by the gastrointestinal tract and reach the liver via the portal system. A large amount of absorbed AAS is metabolized in the liver before entering in the systemic circulation, leading to a significant decrease of oral bioavailability. Esterification also helps to directly enter the lymphatic system and bypass liver first-pass metabolism. Despite this chemical modification, the bioavailability of oral AAS remains drastically lower than that of the parenteral form [24].

These drugs might be prescribed for the treatment of medical conditions, but they are generally used (or abused) for their secondary effects of muscle building and increased strength.

The main conventional indication of testosterone and AAS is hormonal replacement therapy in male hypogonadism. AAS could also be prescribed for aplastic anemia, Fanconi’s anemia, paroxysmal nocturnal hemoglobinuria, hereditary angioedema, and specific forms of osteoporosis. Additionally, they represent the cornerstone of the female-to-male transition process.

Concerning their “non-medical” use, AAS are usually bought on the internet or through local dealers by bodybuilders and professional athletes for the increase of muscle mass and to improve sports performances. Both their trade and their use are illegal in many countries.

AAS side effects are very common, especially if assumed at supraphysiological dosage. Apart from the desired muscular effect, patients may experience sex-related side effects (e.g., male-pattern hair loss, hirsutism, dysphonia, clitoral hypertrophy, menstrual cycle disturbances), endogenous testosterone suppression-related side effects (e.g., testicular atrophy, oligo/azoospermia, erectile disfunction), and systemic adverse events (e.g., erythrocytosis, hypertension, left ventricle hypertrophy, dyslipidemia, acne, decreased renal function, proteinuria). Their higher association with prostate cancer has been postulated, but the available data are still inconclusive [21].

Among systemic adverse events, hepatotoxicity has also been described, demonstrated by a mild elevation of blood aspartate aminotransferase (AST), alanine aminotransferase (ALT), lactate dehydrogenase (LDH), and gamma-glutamyl transpeptidase (GGT) [25]. Fortunately, severe manifestations, such as jaundice, pruritus, and acute hepatic failure, are extremely rare [26,27]. It should be considered that AAS users generally engage in intense muscular exercise and weightlifting at least three times per week. AST, ALT, and LDH are also present in skeletal muscle tissue, so this training regimen could affect these markers measurement on blood. Conversely, bilirubin and GGT are not affected by exercise; therefore, the increase of these markers might be more specific to liver damage in this cohort of patients. In order to reduce the risk of overdiagnosis, it is strongly suggested to perform blood tests after at least one week of abstinence of exercise.

AAS have also been associated with the development of peliosis hepatitis [28], hepatocellular adenomas, and carcinomas [29]. Even though their actual incidence is not well established, it is supposed to be very low.

3. Androgens and Liver Carcinogenesis

Androgens are steroidal sex hormones that are produced by the ovaries, testes, and adrenal glands. They are essential for both males and females, but their serum concentrations are significantly higher in men [30].

Testosterone is the predominant androgen hormone in adult men, and it plays a pivotal role in sex characteristics development and reproduction mechanisms. At physiological concentrations, about 96–98% of serum testosterone is transported by binding proteins (mainly albumin, sex hormone-binding globulin, and corticosteroid-binding globulin) [31]. As a lipophilic molecule, it freely diffuses through cellular membranes, and it is bioactivated by the cytosolic 5α-reductase into dihydrotestosterone (DHT). DHT is considered the most potent endogenous androgen, with a 5- to 10-fold higher AR affinity than testosterone [32].

The binding between androgens and AR allows a conformation change of the AR and its translocation into the nucleus. Here, the AR–androgen complex interacts with specific
DNA sequences, triggering the androgen response elements (AREs) and regulating the transcription of genes involved in cell growth and survival [33,34]. (Figure 1).

Among the different prooncogenic signaling pathways enhanced by androgens, the Wnt/β-catenin is one of the most studied and has the highest amount of evidence. AR activation led to a higher transcription of the cell cycle-related kinase (CCRK) regulator that phosphorylates the inhibitor of cytosolic β-catenin, de-activating it [35]. The translocation of β-catenin to the nucleus drives the transcription of its target genes with cell proliferation and anti-apoptotic activity (e.g., c-myc and cyclin D1).

AR seem to promote the Enhancer of Zeste Homolog 2 (EZH2) gene, another oncogene that reduces the expression of the inhibiting signaling of Wnt, allowing the binding to a higher amount of its receptor, preventing β-catenin from degradation [36].

AR expression in HCC tissue is increased compared with that in normal liver and its overexpression seems to be related to disease progression and survival [37]. In vivo studies have shown that transgenic AR knockout mice develop HCC later and less frequently than wild-type mice [38]. Similarly, AR knockout zebrafish with high hepatocyte kras expression have shown a reduction in cell proliferation at early stages compared to wild-type ones; furthermore, androgen treatment induced an acceleration of HCC progression in wild-type zebrafish but not in AR knockout ones, highlighting the AR-androgen role in hepatocellular proliferation [39].

Another mechanism involved is the androgen-independent induction of AR expression, driven by the mammalian target of rapamycin (mTOR) and its associated signaling pathways. The AKT-mTOR signaling pathway is frequently hyperactivated in several malignancies, HCC included [40]. The mTOR complex 1 (mTORC1), an active form of mTOR, phosphorylates AR in the amino-terminal domain, preventing it from degradation.
and upregulating the nuclear translocation of AR. The phosphorylation of AR seems to be an independent predictor of worse prognosis in HCC patients \[37,41\]. At the same time, it is important to notice that AR exert a feedback control on the AKT-mTOR pathway because they induce the transcription of proteins that inhibit AKT by phosphorylation and, by extension, reduce mTORC1 formation. So, AR inhibition could enhance the oncogenic activity of mTOR, and this may explain the poor results of previous trials on anti-androgens strategies for HCC \[37,41–43\].

In addition to the abovementioned mechanisms, androgens and AR enhance the oncogenic activity of chronic hepatitis, mainly HBV. Male HBV carriers have 5- to 7-fold higher incidence of HCC than female carriers \[44\]. The AR-androgen complex enhances the viral transcription and replication by binding with the HBV core promoter region, and at the same time, the hepatitis B virus X protein (HBx) increases AR activity in the liver \[45,46\]. Similarly, HCV core proteins have been shown to have an increased transcriptional activity, mediated by AR \[47\].

Gender differences have also been demonstrated in the expression of microRNA (miRNA), small, noncoding, single-stranded RNAs with post-transcriptional activity that play a role in several complex genetic networks. Under- and overexpression of different miRNA have been associated to several human diseases. In the last two decades, several data on their association with the progression of liver cirrhosis and HCC development were published \[48,49\]. Despite that a closer correlation has been stated with estrogens, the AR–androgen pathway also participates in miRNA transcription changes. In particular, miR-216a is significantly upregulated in HCC cells of male patients, thanks to the presence of an ARE within its promoter region, enhancing early hepatocarcinogenesis \[50\].

Finally, it is important to report that there is growing and strong evidence about the role of other sexual hormones, such as estrogens and progesterone, on liver cell proliferation. Estrogens and their receptors modulate cell proliferation in both healthy and tumoral liver tissue by acting on multiple signaling pathways, leading to a protective effect against liver tumor development. On the other hand, progesterone seems to induce abnormal proliferation and mitosis in liver cells, explaining the correlation between oral contraceptives and HCA development \[9\]. An exhaustive description of these molecular mechanisms is beyond the aim of this review, but this opposite activity compared to androgens stressed further the pivotal role of sex hormones in hepatocellular carcinogenesis \[7,51\].

4. Androgens and Hepatocellular Adenoma

Hepatocellular adenoma (HCA) is a rare benign primary liver tumor with a strong correlation with sex hormones. It is commonly present in women in reproductive age (70–80% of cases) with an incidence of around 3/100,000 cases. HCA incidence in men is even more rare \[52\].

Estrogens and androgens play a role in the mechanisms of the development and progression of the different types of HCA. The main risk factors for developing HCA are oral contraceptives and AAS use, but they tend to develop with a higher incidence in patients with obesity, glycogenosis type 1 and 3, galactosemia, tyrosinemia, and polycystic-ovary syndrome \[53\].

Despite HCA being a benign lesion, a potential malignant transformation to HCC is observed in less than 5% of cases. This risk of transformation is vastly higher in men \[2,54,55\].

HCA are pathologically classified into four main subtypes according to their morphologic and molecular features, following the Bordeaux classification \[56,57\].

(i) HHCA (HNF1\(\alpha\)-inactivated hepatocellular adenoma) is characterized by inactivating mutations of the Hepatocyte Nuclear Factor 1-alpha (NHF1\(\alpha\)) gene that has regulation functions in differentiation and metabolism of hepatocytes. Biallelic inactivating mutations of HNF1\(\alpha\) are found in about 40% of HHCA, while germlinal mutations of HNF1\(\alpha\) predispose individuals to hepatic adenomatosi. HHCA is associated with hepatic steatosis and the absence of an inflammatory infiltrate. Immunohistochem-
ically, a lack of liver fatty-acid binding protein (LFABP) expression, compared to healthy hepatic tissue, is characteristic.

(ii) b-HCA (β-catenin-activated hepatocellular adenoma) is characterized by activating mutations of the CTNNB1 gene, which encodes β-catenin. The β-catenin signaling pathways are involved in maintaining the hepatocellular balance between apoptosis and regeneration. b-HCA is not associated to liver steatosis, and it are histologically very close to well-differentiated HCC, with a high risk of malignant transformation.

(iii) IHCA (inflammatory hepatocellular adenoma) is characterized by the activation of the Janus Kinase/Signal Transducers and Activators of Transcription (JAK/STAT) signaling pathway, generally by gain-of-function mutation of the Interleukin 6 Cytokine Family Signal Transducer (IL6ST) gene. It is the most common HCA subtype (>45%). I-HCA presents inflammatory infiltrates and the overexpression of the acute phase protein (i.e., C-reactive protein and serum amyloid A).

(iv) UHCA (unclassified hepatocellular adenoma) represents a heterogeneous group that does not present the distinctive molecular characteristics of any of the other subtypes and accounts for approximately 5–10% of HCAs. Molecular and genomic studies have proposed a fifth subtype (sh-HCA), characterized by the hyperactivation of the Sonic Hedgehog signaling pathway. It represents about 5% of HCA and has a high risk of histologic hemorrhage and symptomatic bleeding [1,58]. Lastly, the sequencing of the CTNNB1 gene permitted further the subclassifying of b-HCA according to different mutations in exon 3 (bex3HCA) and exon 7 or 8 (bex7,8HCA). Compared to bex7,8HCA, bex3HCA presents a significantly higher risk of HCC transformation due to a stronger activation of the β-catenin signaling pathway [58].

In conclusion, HCA can be subclassified into six major molecular subgroups: HHCA, bex3HCA, bex7,8HCA, IHCA, sh-HCA, and UHCA [1]. Of note, the World Health Organization classification recognizes a mixed subtype (b-IHCA) that presents both inflammatory characteristics and β-catenin pathway activation [59].

Focusing on gender differences, male patients have a high risk of developing b-HCA due to their increased androgen exposure, both endogenous and exogenous. This correlation is even stronger for bex3HCA, justifying the higher rate of HCA malignant transformation in male patients [58]. For this reason, the main international guidelines suggest surgical resection in men regardless of the size or subtype of HCA [53,60,61].

A recent retrospective study of HCA cases in men showed malignant transformation rates lower than expected. In Taiwan, authors reported only four of 26 cases (15%) of malignant transformation. Interestingly, all of these patients presented b-HCA (exon sequencing was not performed) [62].

In another recent retrospective study of HCA in men, the authors reported that 50% of HCA cases presented a significant expression of AR by immunohistochemistry. In the same study, the authors reported an overall concomitant presence of well-differentiated HCC of 15% and no malignant transformation during follow-up, but no subgroup analyses on AR expressing HCA were performed. It is important to notice that in this study, AR tissue expression was not available for all patients and the main subtype was IHCA [63].

The association of oral contraceptives (both estrogen–progestin and progestin-only) with HCA development has been widely demonstrated. Moreover, volume reduction or complete regression of HCA is possible after oral contraceptive discontinuation, and volume increase or recurrence is possible in patients resuming therapy or during pregnancy [53,64–66].

Regarding androgen exposure, the administration of AAS is related to a high risk of HCA development. Several cases of HCA related to AAS use or abuse have been reported in the literature (Table 1) [67–90]. The majority of reported cases referred to patients with hematologic disorders in both the adult and pediatric age groups. The median and mean duration of treatment before HCA detection was 50 and 75 months, respectively. In 69.2%
of cases, multiple lesions were observed. In some cases, radiology and histology confirmed a concomitant diagnosis of hepatic peliosis [75,77,86].

Table 1. Cases of anabolic androgenic steroid-related hepatocellular adenomas.

<table>
<thead>
<tr>
<th>Authors</th>
<th>AAS</th>
<th>Duration (mo)</th>
<th>Indication</th>
<th>Lesion Number</th>
<th>Note</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wang et al. [67]</td>
<td>Stanozolol</td>
<td>48</td>
<td>Aplastic anemia</td>
<td>multiple</td>
<td>β-catenin +</td>
</tr>
<tr>
<td>Nakao et al. [68]</td>
<td>Oxymetholone</td>
<td>72</td>
<td>Aplastic anemia</td>
<td>multiple</td>
<td></td>
</tr>
<tr>
<td>Mulvihill et al. [69]</td>
<td>Oxymetholone</td>
<td>36</td>
<td>Aplastic anemia</td>
<td>multiple</td>
<td></td>
</tr>
<tr>
<td>Montgomery et al. [70]</td>
<td>Oxymetholone</td>
<td>34</td>
<td>Aplastic anemia</td>
<td>multiple</td>
<td></td>
</tr>
<tr>
<td>Sweeney et al. [71]</td>
<td>Multiple a</td>
<td>40</td>
<td>Fanconi’s anemia</td>
<td>multiple</td>
<td></td>
</tr>
<tr>
<td>Bessho et al. [72]</td>
<td>Oxymetholone</td>
<td>48</td>
<td>Fanconi’s anemia</td>
<td>multiple</td>
<td>Concomitant HCC</td>
</tr>
<tr>
<td>LeBrun et al. [73]</td>
<td>Oxymetholone</td>
<td>36</td>
<td>Fanconi’s anemia</td>
<td>multiple</td>
<td>Concomitant HCC</td>
</tr>
<tr>
<td>Touraine et al. [74]</td>
<td>Norethandrolone</td>
<td>54</td>
<td>Fanconi’s anemia</td>
<td>multiple</td>
<td></td>
</tr>
<tr>
<td>Bernstein et al. [75]</td>
<td>Oxymetholone</td>
<td>10</td>
<td>Fanconi’s anemia</td>
<td>single</td>
<td>Hepatic peliosis</td>
</tr>
<tr>
<td>Holder et al. [76]</td>
<td>Multiple b</td>
<td>50</td>
<td>Fanconi’s anemia</td>
<td>single</td>
<td></td>
</tr>
<tr>
<td>Hernandez-Nieto et al. [77]</td>
<td>Methandienone</td>
<td>36</td>
<td>PNH</td>
<td>multiple</td>
<td>Hepatic peliosis</td>
</tr>
<tr>
<td>Boyd et al. [78]</td>
<td>Methyltestosterone</td>
<td>132</td>
<td>Hypogonadism</td>
<td>multiple</td>
<td>Concomitant HCC</td>
</tr>
<tr>
<td>Westaby et al. [79]</td>
<td>Methyltestosterone</td>
<td>120</td>
<td>Hypogonadism</td>
<td>single</td>
<td></td>
</tr>
<tr>
<td>Bork et al. [80]</td>
<td>Danazol</td>
<td>240</td>
<td>HAE</td>
<td>single</td>
<td></td>
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<tr>
<td>Kahn et al. [81]</td>
<td>Danazol</td>
<td>6</td>
<td>Uterine fibroids</td>
<td>multiple</td>
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</tr>
<tr>
<td>Weill et al. [82]</td>
<td>Danazol</td>
<td>48</td>
<td>LES</td>
<td>single</td>
<td>Concomitant HCC</td>
</tr>
<tr>
<td>Bird et al. [83]</td>
<td>Methyltestosterone</td>
<td>84</td>
<td>F-to-M</td>
<td>single</td>
<td></td>
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<td>Coombes et al. [84]</td>
<td>Testosterone</td>
<td>36</td>
<td>F-to-M</td>
<td>single</td>
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<tr>
<td>Kato et al. [85]</td>
<td>Testosterone enanthate</td>
<td>144</td>
<td>F-to-M</td>
<td>multiple</td>
<td>β-catenin +</td>
</tr>
<tr>
<td>Martin et al. [86]</td>
<td>Multiple c</td>
<td>60</td>
<td>Bodybuilding</td>
<td>multiple</td>
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<tr>
<td>Pais-Costa et al. [87]</td>
<td>Multiple c</td>
<td>72</td>
<td>Bodybuilding</td>
<td>multiple</td>
<td></td>
</tr>
<tr>
<td>Socas et al. [88]</td>
<td>Multiple d</td>
<td>180</td>
<td>Bodybuilding</td>
<td>multiple</td>
<td></td>
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<tr>
<td></td>
<td>Multiple e</td>
<td>6</td>
<td>Bodybuilding</td>
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<td>60</td>
<td>Bodybuilding</td>
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<td>Creagh et al. [90]</td>
<td>NA</td>
<td>36</td>
<td>Bodybuilding</td>
<td>multiple</td>
<td></td>
</tr>
</tbody>
</table>

F-to-M: female to male; HCC: hepatocellular carcinoma; HAE: hereditary angioedema; NA: not available; PNH: paroxysmal nocturnal hemoglobinuria. a: Methyltestosterone, oxymetholone; b: Methandrostenolone, fluxymesterone, oxymetholone, nandrolone; c: Androstenedione, nandrolone; d: Stanozolol, oxymetholone, nandrolone, testosterone, methenolone; e: Stanozolol, oxymetholone, nandrolone, testosterone, boldenone.

As previously mentioned, HCA may transform in malignant tumors (i.e., HCC). Interestingly, in some cases, simultaneous HCA and HCC have been noticed at diagnosis [72,73,78,82]. The hypothesis that some HCA evolved to HCC has been postulated, but it has not yet been demonstrated.

Lesion regression has been observed after treatment discontinuation in some cases, confirming the correlation between AAS and HCA development [91]. So, AAS discontinuation is the cornerstone of the management of these patients, together with the general recommendation of HCA guidelines.
5. Androgens and Hepatocellular Carcinoma

Hepatocellular carcinoma (HCC) is the most common primary form of liver cancer. Sex hormones are involved in HCC development and progression.

Similarly to HCA, several cases of HCC in patients using AAS have been reported in the literature (Table 2) [72,73,78,79,82,92–118]. The majority of patients were taking AAS for hematologic disorders, mainly Fanconi’s anemia. The median and mean duration of treatment was 62 and 76 months, respectively. In 58.3% of cases, multiple lesions were observed, but extrahepatic spread at diagnosis is rare. Of note, in almost all reported cases (96%), patients did not present liver cirrhosis.

**Table 2. Cases of anabolic androgenic steroid-related hepatocellular carcinomas.**

<table>
<thead>
<tr>
<th>Authors</th>
<th>AAS</th>
<th>Duration (mo)</th>
<th>Indication</th>
<th>Lesion Number</th>
<th>Note</th>
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<td>8</td>
<td>Aplastic anemia</td>
<td>multiple</td>
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<tr>
<td>Kosaka et al. [93]</td>
<td>Oxymetholone</td>
<td>36</td>
<td>Aplastic anemia</td>
<td>multiple</td>
<td></td>
</tr>
<tr>
<td>Guy et al. [94]</td>
<td>NA</td>
<td>4</td>
<td>Aplastic anemia</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>Henderson et al. [95]</td>
<td>Multiple</td>
<td>90</td>
<td>Aplastic anemia</td>
<td>multiple</td>
<td></td>
</tr>
<tr>
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<td>Oxymetholone</td>
<td>15</td>
<td>Aplastic anemia</td>
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<td>Linares et al. [97]</td>
<td>Oxymetholone</td>
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<td>Fanconi’s anemia</td>
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<td></td>
</tr>
<tr>
<td>Shapiro et al. [98]</td>
<td>Multiple</td>
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<td>Fanconi’s anemia</td>
<td>multiple</td>
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</tr>
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<td>Hepatic peliosis</td>
<td></td>
</tr>
<tr>
<td>Kew et al. [99]</td>
<td>Multiple</td>
<td>84</td>
<td>Fanconi’s anemia</td>
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<tr>
<td>Bessho et al. [72]</td>
<td>Oxymetholone</td>
<td>48</td>
<td>Fanconi’s anemia</td>
<td>multiple</td>
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<tr>
<td></td>
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<td>Fanconi’s anemia</td>
<td>multiple</td>
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<tr>
<td></td>
<td>Methyltestosterone</td>
<td>51</td>
<td>Fanconi’s anemia</td>
<td>multiple</td>
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<tr>
<td>LeBrun et al. [73]</td>
<td>Oxymetholone</td>
<td>36</td>
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<td>139</td>
<td>DBA</td>
<td>multiple</td>
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<td>de Planque et al. [103]</td>
<td>NA</td>
<td>24</td>
<td>PNH</td>
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<td>PNH</td>
<td>multiple</td>
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</tr>
<tr>
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<td>Methyltestosterone</td>
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<td>Hypopituitarism</td>
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<tr>
<td></td>
<td>Methandienone</td>
<td>96</td>
<td>Cryptorchidism</td>
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<tr>
<td>Christopherson et al. [105]</td>
<td>Testosterone</td>
<td>NA</td>
<td>80</td>
<td>Hypogonadism</td>
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<td>Gleeson et al. [106]</td>
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<td>Boyd et al. [78]</td>
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<tr>
<td>Westaby et al. [79]</td>
<td>Methyltestosterone</td>
<td>204</td>
<td>Hypogonadism</td>
<td>single</td>
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</tr>
<tr>
<td>Thoufeeq et al. [107]</td>
<td>Danazol</td>
<td>228</td>
<td>HAE</td>
<td>multiple</td>
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</tr>
<tr>
<td>Crampon et al. [108]</td>
<td>Danazol</td>
<td>156</td>
<td>HAE</td>
<td>single</td>
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</tr>
<tr>
<td>Berkel et al. [109]</td>
<td>Danazol</td>
<td>NA</td>
<td>HAE</td>
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<td>Weill et al. [82]</td>
<td>Danazol</td>
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<td>SLE</td>
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</tr>
<tr>
<td>Middleton et al. [110]</td>
<td>Danazol</td>
<td>36</td>
<td>Endometriosis</td>
<td>single</td>
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<td>Buamah [111]</td>
<td>Danazol</td>
<td>24</td>
<td>Pituitary adenoma</td>
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<td></td>
</tr>
<tr>
<td>Lin et al. [112]</td>
<td>Testosterone cypionate</td>
<td>14</td>
<td>F-to-M</td>
<td>multiple</td>
<td></td>
</tr>
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</table>

AR +
### Table 2. Cont.

<table>
<thead>
<tr>
<th>Authors</th>
<th>AAS</th>
<th>Duration (mo)</th>
<th>Indication</th>
<th>Lesion Number</th>
<th>Note</th>
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<td>Kesler et al. [113]</td>
<td>Testosterone cypionate</td>
<td>84</td>
<td>Bodybuilding</td>
<td>multiple</td>
<td>β-catenin +</td>
</tr>
<tr>
<td>Solbach et al. [114]</td>
<td>Multiple f</td>
<td>72</td>
<td>Bodybuilding</td>
<td>multiple</td>
<td>β-catenin and AR +</td>
</tr>
<tr>
<td>Woodward et al. [115](2 cases)</td>
<td>NA</td>
<td>NA</td>
<td>Bodybuilding</td>
<td>single</td>
<td></td>
</tr>
<tr>
<td>Gorayski et al. [116]</td>
<td>Multiple g</td>
<td>26</td>
<td>Bodybuilding</td>
<td>single</td>
<td>β-catenin + (weak)</td>
</tr>
<tr>
<td>Hardt et al. [117]</td>
<td>Multiple h</td>
<td>60</td>
<td>Bodybuilding</td>
<td>single</td>
<td></td>
</tr>
<tr>
<td>Overly et al. [118]</td>
<td>Multiple i</td>
<td>48</td>
<td>Bodybuilding</td>
<td>single</td>
<td></td>
</tr>
</tbody>
</table>

AR: androgens receptors; DBA: Diamond–Blackfan anemia; F-to-M: female to male; HCA: hepatocellular adenoma; HAE: hereditary angioedema; NA: not available; PNH: paroxysmal nocturnal hemoglobinuria; SLE: Systemic lupus erythematosus. a: Methyltestosterone, norethandrolone, stanozolol, oxymetholone; b: Testosterone propionate, oxymetholone; c: Oxymetholone, methyltestosterone; d: Methylandrostenolone, oxymetholone; e: Methyltestosterone, testosterone; f: Nandrolone, testosterone isocaproate, methandienone, stanozolol; g: Testosterone, stanozolol, nandrolone; h: Testosterone, trenbolone acetate, androstanediol, boldenone, methandriol, letrozole, oxymetholone, methandienone; i: Methylandrostenolone, oxandrolone, stanozolol, nandrolone deconoate, methenolone.

Laboratory alterations, mainly the mild to moderate elevation of serum bilirubin, AST, ALT, and GGT values, were reported in almost all cases. It is impossible to extrapolate if these alterations were related to the cancer or to an underlying hepatotoxicity.

Interestingly, only in three cases was serum alpha-fetoprotein elevated, so its sensitivity is extremely low in this cohort of patients [95,112,113].

In terms of immunohistochemistry analyses, a β-catenin test was performed in only three cases, and the authors reported two strong and one weak positive results [113,114,117]. Tumoral AR expression was tested in only three cases and two of them resulted in AR overexpression [112,114,117].

Survival data are lacking in the majority of reported cases. Notably, these data would have been scarcely informative since HCC therapeutical scenarios and prognosis have significantly changed over the last years.

The role of the AR expression and its activity in the prognosis of HCC remains inconclusive. Recent studies have shown a linear regression of AR expression among histological grades with different gene transcription profiles. So, AR expression and activity are not strictly related in hepatocarcinogenesis. AR play a role in defining prognosis that is qualitatively different among HCC grades. High expression of AR seems to be related to a better prognosis in early stages, but AR activity seems to be associated with worse prognosis in high-grade HCC [119]. Despite the failure of the first trials on androgen-related pathway-inhibiting therapies for HCC, new insights on the AR–androgen mechanism in liver cancer development and progression are motivating researchers to continue investigating the potential anti-cancer effect of anti-androgens, AR inhibitors, AR-degrading molecules, and selective AR modulators [120].

Some of these drugs have shown promising results in vitro, but further studies are needed.

### 6. Conclusions

It is abundantly clear that sex hormones, both androgens and estrogens, play a complex role in hepatocarcinogenesis, and that they significantly influence the pathogenesis and the development of HCA and HCC. Their mutual interaction has shown profound effects on the development of these neoplasms, suggesting the presence of endocrine mechanisms that have been only partially understood. Given this complexity, it is essential to expand the knowledge of the role of sex hormones in the hepatocellular environment. A better and deeper comprehension of androgen regulation in hepatocellular proliferation...
could provide new insights into the development and progression of HCC; it might also lead to the identification of highly specific therapeutic targets and increase our actual therapeutic strategies.

Giving the rising incidence of AAS use for a non-medical purposes, it is crucial that physicians are aware of the increased risk of liver neoplasms in patients that are generally young and without the usual risk factors of chronic hepatic disease.

The available literature on this topic is quite old; thus, any prognosis assumption is hampered by the significant progresses made in both the diagnostic and the therapeutic fields for HCA and HCC during the last years.

In conclusion, it is mandatory to continue investigating and defining the involvement of sex hormones, particularly androgens, in hepatocarcinogenesis, in order to optimize the diagnosis and treatment of male patients and AAS users.

Author Contributions: Conceptualization, L.I.; writing—original draft preparation, L.I., E.F. and N.R.; writing—review and editing, L.I.; supervision, M.D. and F.G.F. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Conflicts of Interest: The authors declare no conflicts of interest.

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