Chapter 4
Fatty Liver Disease and Hepatocellular Carcinoma: The Pathologist's View

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Abstract Chronic alcohol misuse and progressed nonalcoholic fatty liver disease (NAFLD) due to the metabolic syndrome and resulting to nonalcoholic steatohepatitis (NASH) are prime causes of hepatocellular carcinoma (HCC) in Western industrialized countries. The incidence of HCC in NASH-cirrhosis is lower than that of HCC occuring in HCV-related or alcoholic cirrhosis. Up to 20% of cases of alcohol-associated HCC may develop in pre-cirrhotic liver while HCC is also increasingly recognised in pre-cirrhotic NASH raising questions on appropriate surveillance measures for these patient populations. The recently described steatohepatitic subtype of HCC presents with higher frequency in NAFLD compared to alcoholic liver disease (ALD) patients. This review will mainly focus on histopathology and summarize current data on the epidemiology, pathogenesis, diagnosis and management of NAFLD- and ALD-related HCC.

Keywords Alcoholic · Nonalcoholic · Fatty Liver Disease · Steatohepatitis · Hepatocellular Carcinoma · Histopathology · Pathogenesis · Diagnosis

4.1 Introduction

Hepatocellular carcinoma (HCC) accounts for 70–85% of the total primary liver cancer burden and it usually arises in a background of chronic liver disease of hepatitis B virus (HBV), hepatitis C virus (HCV) or alcoholic aetiology [43, 46]. It is the fifth most common cancer in men and the ninth most common one in women. In 2012, 782,000 cases were estimated to have occurred globally, with 83% of these

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in less developed regions. HCC was the second most common cause of cancer-related death worldwide, with approximately 746, 000 deaths (9.1% of all cancer deaths in 2012). The prognosis for the majority of those affected with primary liver cancer is poor, with an overall mortality to incidence ratio of 0.95 [21, 46]. Most HCC cases (>75%) occur in Southeast Asia and sub-Saharan Africa, while Southern European countries have intermediate incidence rates and North/South America and Northern Europe present the lowest incidence rates (<5 per 100,000 individuals) [31]. HBV and HCV infections are the main aetiological factors of cirrhosis and HCC in Southeast Asia and sub-Saharan Africa. Chronic alcohol misuse and progressed nonalcoholic fatty liver disease (NAFLD) due to the metabolic syndrome and resulting to nonalcoholic steatohepatitis (NASH) are the prime causes of HCC in Western industrialized countries [69].

4.2 Epidemiology

4.2.1 ALD-related HCC

Hepatocellular carcinoma (HCC) reportedly develops in 5–15% of alcoholic patients with cirrhosis [35]. Alcoholic liver disease (ALD) is the commonest aetiology of HCC in industrialized countries, being responsible for 32–45% of cases [47]. A population-based USA study of nearly 7000 cases of HCC and > 250,000 controls found the risk of HCC (odds ratio – OR) to increase 4-fold in ALD patients and 2.5-fold in patients with NAFLD-associated diabetes and/or obesity [77].

Alcohol is also a recognized potentiating factor for HCC development in patients with chronic HCV-infection [25, 64, 76]. The 10-year cumulative occurrence rate of HCC in HCV-infected patients with alcoholic cirrhosis drinking >120 g alcohol per day is 80.7%, in contrast to 18.5% in alcoholic cirrhotics without evidence of HCV infection and 56.5% in non-drinkers with HCV-related cirrhosis [82]. Chronic alcohol consumption >80 g/day for >10 years increases the risk of HCC almost five-fold; chronic hepatitis C (CHC) patients who drink alcohol have double the risk of developing HCC compared to non-drinking HCV-infected patients [47]. A hospital-based, case-control study led by Hassan et al. [25] and involving 115 HCC patients and 230 non-liver cancer controls showed significant synergy between heavy alcohol consumption and CHC (OR 53.9) and diabetes mellitus (OR 9.9).

Data from human studies on the association between HBV infection and alcohol consumption are limited. In a French study evaluating the mortality related to HCV and HBV infections in 2001, 95% of HCV-infected patients who died had cirrhosis and 33% had HCC; 35% had reported excessive alcohol consumption. Similarly, in the HBV infection group, 93% and 35% of the individuals had cirrhosis had HCC, respectively and 5% had a history of excessive alcohol use. The mean age at death of both HCV- and HCV-infected patients who drunk excessively was significantly lower compared to non-drinkers or patients who drank modestly. Human immunodeficiency virus (HIV) infection was also a significant co-factor [42]. The risk of HCC development in ALD is increased if iron overload is also present [26].

4.2.2 NAFLD-related HCC

NASH-related cirrhosis may be complicated by HCC [6, 63, 68]. In NAFLD, older age, severity of insulin resistance and diabetes, and iron overload reportedly predispose to HCC [54]. Studies from various geographical areas have shown that the incidence of HCC in NASH-cirrhosis is lower than that of HCC developing in HCV-related or alcoholic cirrhosis [5, 32, 50]. Similarly, the risk of developing HCC is lower in NASH-related cirrhosis (1.3–2.4-fold) compared to that of HCV-cirrhosis (13–19-fold). However, in the last decades, the incidence of NASH-related HCC has been increasing worldwide, possibly as a consequence of the obesity and type 2 diabetes epidemic [57, 68]. In the United States, NAFLD is the fastest growing aetiology of HCC, especially among patients listed for liver transplantation [58, 79], while the number of NAFLD-associated HCC cases increases at a rate of approximately 9% per year [87]. In ALD patients, obesity and the metabolic syndrome appear to increase the incidence and mortality of HCC [14]. Pais et al. [52] have shown that the risk of HCC is higher in patients with alcohol-related cirrhosis in need for liver transplantation who also have NAFLD. In NAFLD patients, moderate alcohol use may potentiate the development of HCC [5].

A study by Marrero et al. [44] stated that NAFLD is the causal aspect of 13–38.2% of patients presenting with HCC not linked to viral infection or alcohol. In Northeast England, the overall incidence of HCC increased 1.8-fold from 2000 to 2010, with the astounding realisation that there was a > 10-fold increase in NAFLD-related HCC, accounting for 34.8% of all the cases in 2010. NAFLD thus became the most common background aetiology in this region [17]. Furthermore, irrespective of the underlying aetiology of HCC associated liver disease, over 50% of patients had type 2 diabetes and two thirds had either type 2 diabetes and/or obesity, defined as a body mass index >30 [17].

The prevalence of HCC is approximately 0.5% in nonalcoholic steatosis and 2.8% in NASH [68]. Studies in Japanese patients indicate that a greater length of follow-up may be necessary for determining the true prevalence of HCC [32]. According to a recent meta-analysis, the annual incidence of HCC in NAFLD patients is 0.44 per 1000 person-years, while in patients with NASH it rises to 5.29 per 1000 person-years [88]. In one of the first follow-up studies of NAFLD patients, HCC was reported in only one patient [55]. Shimada et al. [63] reported that 13 (7.3%) out of 82 cases of biopsy-proven NASH had cirrhosis and six of these (47% of cirrhotics) had HCC. In the same year, Bugianesi et al. [10] found that 44 out of 641 cirrhosis-associated HCC arose in cryptogenic cirrhosis, with cryptogenic cirrhotics having a higher prevalence of obesity, diabetes, markers of insulin resistance and increased triglycerides. This was the first study proposing the inclusion of cryptogenic cirrhosis and HCC in the natural history of NASH. In two subsequent studies, the incidence of HCC in patients with cryptogenic cirrhosis ranged from 18% [44] to 27% [56]. In both these studies, associated clinical features of metabolic syndrome implicated NAFLD as the underlying aetiology of cryptogenic cirrhosis.

4.3 Pathogenesis

4.3.1 ALD-related HCC

As mentioned above, HCC frequently arises in patients with a combination of alcoholic and viral liver disease related to HBV and/or HCV [33], but is also often described in patients without evidence of hepatotropic viral infection [81]. Alcohol is known to cause genetic alterations [34] but it can also act as a co-carcinogen by inducing the hepatic microsomal isoenzyme cytochrome P450 2E1 (CYP2E1), leading to activation of pro-carcinogens present in tobacco smoke, alcoholic beverages and food [22, 24, 69]. Alcohol, tobacco and obesity have been shown to be independent and synergistic risk factors for HCC development in patients with cirrhosis [45].

The mechanisms leading to HCC in either ALD or NAFLD background are notably similar. In ALD, there are several possible mechanisms by which alcohol can drive the development of HCC. These comprise, in addition to CYP2E1 induction that may activate pro-carcinogens as mentioned above, dietary or environmental carcinogens ingested alongside alcoholic drinks, toxicity of acetaldehyde, intensified lipid peroxidation due to reactive oxygen species (ROS), growth factor and cytokine milieu, deregulated immune responses, and DNA lesions caused by oxidative stress by-products [69].

Genetic factors can also play a role regarding predisposition for the development of HCC. A sequence variation within the gene coding for patatin-like phospholipase domain-containing protein 3 (PNPLA3, p.I148M) was shown to modify steatosis, necroinflammation and fibrosis in ALD [70]. Similar to PNPLA3, there may be a role for a transmembrane 6 superfamily member 2 (TM6SF2) gene variant across the ALD spectrum from steatosis, through cirrhosis to HCC [4]. Other polymorphisms implicated in the development of ALD-related HCC include genes involved in ROS formation (myeloperoxidase and superoxide dismutase 2) [49] and in inflammation (CCL5) [13].

Chronic alcohol use can also modulate microRNAs (miRNA/miR) expression influencing ALD progression [71]. For instance, miR-212 is involved in alcohol-induced gut permeability [72], miR-217 is implicated in steatosis via regulation of SIRT1 [86] and miR-199 is associated with an increase in endothelin-1 and hypoxia-inducible factor-1α, which play vital roles in inflammation and steatosis [85]. Several abnormally expressed miRNAs have been reported in HCC, including upregulation of miR-221, miR-21, miR-22 and miR-517a and downregulation of miR-29, miR-24a, miR-26a, miR15-a/b, miR-150, miR-195, miR-122, miR-20 family, miR-124 and let-7 family [71].

4.3.2 NAFLD-related HCC

Recent reviews have focused on mechanisms of hepatocarcinogenesis in NAFLD [43, 57, 89]. Fatty liver shows increased susceptibility to lipid peroxidation with subsequent production of free radicals that may cause DNA mutations. In obesity, fatty liver may be susceptible to carcinogens as a result of impaired ATP production, defective autophagy mechanisms, deregulation of energy and/or hormonal balance, hypoxia and systemic inflammation. In NAFLD, increased susceptibility of the steatotic liver to carcinogenic insults may be related to metabolic derangements related to the 'metabolic syndrome', hyperinsulinemia and the presence of insulin-like growth factor receptors in HCC, the systemic effects of deranged cytokines and adipokines, immune dysregulation and alteration in gut microbiota [43, 57, 89]. Genetic factors may also be responsible for increasing the risk of HCC in NAFLD patients [4]. Carriers of the PNPLA3 p.I148M variant are known to be at increased risk of progressive fibrosis and steatohepatitis of none alcoholic or alcoholic aetiology [59, 67]. Recently, a strong association has emerged between the common PNPLA3 p.I148M variant and the risk of developing HCC in NAFLD patients [38, 39, 65], reporting that the variant increased 3-fold the risk of progression to NASH and, most notably, 12-fold that of developing HCC [38, 39]. Another genetic variant, rs58542926 in *TM6SF2*, was found to be linked with NAFLD-related HCC in univariate analysis [38, 39].

In experimental models of hepatocarcinogenesis steatosis alone is not sufficient for HCC development [57]. Additional inflammation may be necessary as shown in mice under prolonged choline-deficient high fat diet that developed spontaneous HCC [78]. Alterations in the pro-inflammatory nuclear factor kappa B (NF-κB) signalling may play a significant role [57]. In a non-obese inbred mouse model with spontaneous fatty liver and steatohepatitis, hepatocellular adenomas and HCC emerge with time in up to 40% of male mice but <10% of female mice supporting gender predilection in HCC development [66].

4.4 Diagnosis

HCC is the only major cancer in which diagnosis and indication for treatment are not regularly established by histology. If a liver mass/nodule is detected on ultrasound then the most trustworthy imaging diagnostic tools for the detection of HCC are four-phase computed tomography (CT) and/or dynamic contrast enhanced magnetic resonance imaging (MRI). According to current guidelines [18], diagnosis of HCC in cirrhotic liver of any aetiology can be established if a mass > 2 cm shows specific imaging pattern (arterial hyper-enhancement followed by contrast washout in the venous/delayed phase) with one of the above mentioned techniques (positive predictive value and specificity >90–95%); in masses measuring 1–2 cm use of two imaging techniques (CT and MRI) is required and the accuracy of non-invasive

diagnosis is 73–88% [41]; a focal hepatic mass with atypical imaging characteristics, or a focal hepatic mass detected in a non-cirrhotic liver, should undergo biopsy [15, 20]. However, imaging diagnostic quality outside tertiary centres may not be as expertly assessed as the data which formed the basis of the guidelines, which was generated in carefully controlled and supervised multicentre trials. Although many cases of suspected HCC are referred to specialist centres for expert review, one recent study suggested that approximately 20% of presumed HCC nodules are incorrectly diagnosed by non-invasive techniques [51]. α-fetoprotein (AFP) serum levels >200 ng/mL have high specificity for HCC diagnosis in patients with cirrhosis and radiologic evidence of focal hepatic lesions, although AFP serum testing is not universally advocated owing to poor sensitivity – particularly in cases with early stage disease. Combination serum tests have been suggested, but have yet to impact clinical practice [7, 8, 29].

The specificity of liver biopsy for HCC diagnosis is 100%, with a superior overall sensitivity of 86–93%, although in nodules <1 cm the sensitivity falls to 83%. In approximately 2–11% of the cases a diagnosis cannot be made because of specimen inadequacy [48]. Immunohistochemical staining for glypican-3, heat-shock protein-70 and glutamine synthetase may aid diagnosis when conventional histology is not conclusive [15]. Liver biopsy in suspected HCC offers in addition to superior diagnostic sensitivity and specificity, precise histological typing/subtyping, prognostic and predictive information, and data for molecular classification. Molecular signatures from gene expression profiling based on HCC tissue material may be used in the future as biomarkers for prognosis and/or treatment stratification in HCC following careful validation [16, 40, 75].

The diagnosis of HCC frequently occurs at a late stage, a fact that may be explained by underutilisation of surveillance, delayed follow-up and suboptimal effectiveness of surveillance tests [31].

4.5 Histopathology

4.5.1 ALD-related HCC

HCC in ALD usually develops in a background of macronodular cirrhosis [35] (Fig. 4.1). However, up to 20% of cases of alcohol-associated HCC may also develop in precirrhotic liver disease [26]. Generally, HCC has acinar, pseudoglandular, trabecular and/or compact growth patterns, frequently with multiple histological patterns seen within a single tumour. Intracellular Mallory-Denk bodies (MDB) are frequent within ALD-related HCC and the incidence of HCC is significantly higher in ALD-cirrhosis with MDBs than without [69]. MDB contain p62 and other proteins, including keratins 8 and 18 and ubiquitin. p62 is an autophagy substrate and its overexpression is considered a marker of impaired autophagy implicated in the development of human HCC [2]. In the background surrounding liver, ALD

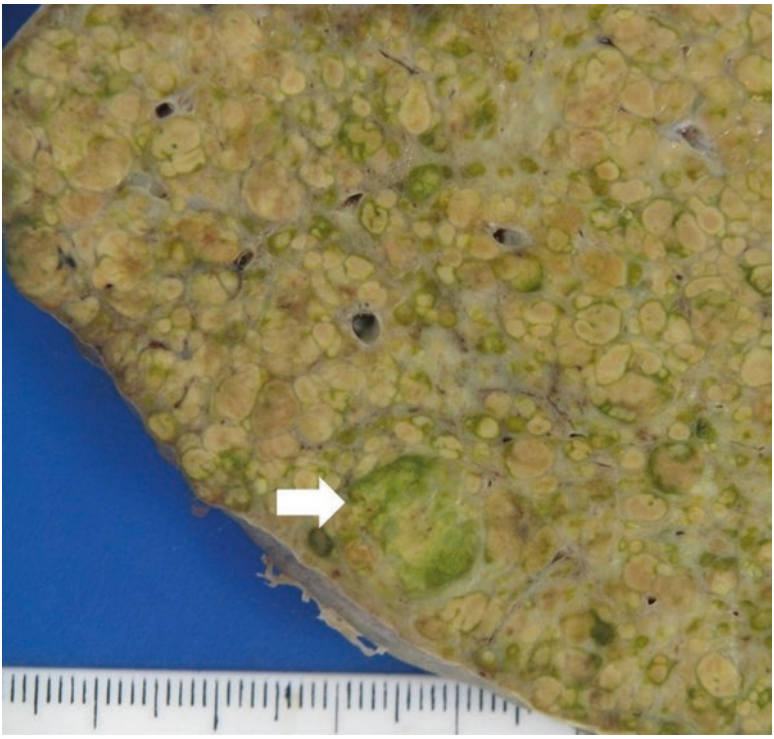

Fig. 4.1 Gross appearance of hepatocellular carcinoma (*arrow*) in segment VII of an explant liver with mixed micronodular and macronodular alcoholic cirrhosis

shares several histological features with NAFLD, such as steatosis and mixed parenchymal inflammation, and in the presence of steatohepatitis hepatocyte ballooning with or without Mallory-Denk bodies (MDB) and sinudoidal/pericellular fibrosis with a perivenular predominance [31, 74].

Dysplastic nodules in cirrhosis may be the precursor lesion for HCC in the multistage process of hepatocarcinogenesis [37, 73]. Dysplastic features, such as increased nuclear density ratio of >1.5, small-cell change and clear cell change have been associated with increased risk of progression of image-detected hepatic nodules to HCC in a prospective liver biopsy study [73].

The presence of large cell change, referring to groups of enlarged hepatocytes with mild nuclear pleomorphism, hyperchromasia and/or multinucleation, has been reported as an independent risk factor for HCC, with an estimated odds ratio of 3.3 [36]. However, in non-HBV related chronic liver disease, large cell change is not thought to be a dysplastic lesion and therefore a direct precursor of HCC; it is rather indicative of an increased risk for tumour development [30, 36, 37]. Histopathology reports of specimens from cirrhotic livers should always include a comment on the presence (or absence) of both small cell and large cell change [74].

Intrahepatic cholangiocarcinoma may occur in cirrhotic liver and its incidence is increasing [12]. Biliary intraepithelial neoplasia, a precursor of cholangiocarcinoma, has been described in the liver of patients with alcoholic cirrhosis, supporting its role in the development of biliary malignancy in this background [80].

4.5.2 NAFLD-related HCC

It is increasingly recognized that HCC may develop in non-cirrhotic liver in patients with NAFLD [11, 74] (Fig. 4.2). The first three cases were reported in 2008, in patients with features of the metabolic syndrome and steatosis without NASH or significant fibrosis [23]. In 2009, Paradis et al. reported 31 HCC arising in non-cirrhotic NAFLD patients with metabolic syndrome; of these, only one patient had NASH and 65% had mild fibrosis. Alexander et al. [3] have studied 157 patients with non-cirrhotic HCC and showed a strong association with the presence of NAFLD in the background liver. Compared to HCC in NAFLD-cirrhosis, tumours in non-cirrhotic NAFLD are usually larger and of lower histologic grade [53]. Male gender is a risk factor for HCC in non-cirrhotic NASH-related HCC in cohorts from France [53] and Japan [83, 84]. Other risk factors include type 2 diabetes and pre-existing hepatocellular adenoma [53]. In Japan, 21–28% of NAFLD-related HCC developed in patients without advanced fibrosis. Currently, screening and surveillance of patients with advanced NAFLD are reserved only for those with cirrhosis,

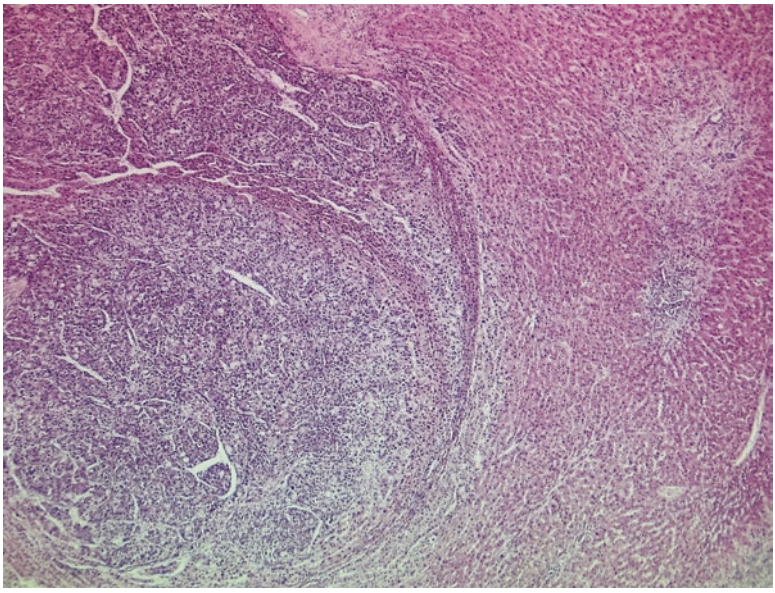

Fig. 4.2 Hepatocellular carcinoma (*left*) in non-cirrhotic liver (H-E, x100)

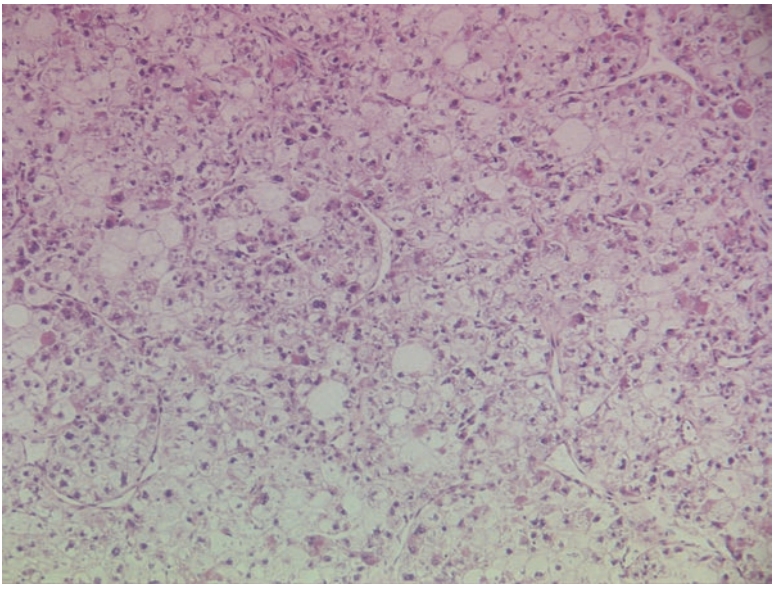

Fig. 4.3 Steatohepatitic hepatocellular carcinoma: many tumour cells are steatotic and some are ballooned and contain Mallory-Denk bodies (H-E, x200)

raising concern over the management of non-cirrhotic patients [6, 17, 27]. The insufficiency of early detection screening methods in non-cirrhotic NAFLD may explain the fact that most NAFLD-related HCC present at an advanced stage [79].

A special HCC subgroup with steatohepatitic morphology (SH-HCC) has recently been described (Fig. 4.3) [60, 61], presenting a higher frequency in NAFLD compared to ALD [28, 62]. In SH-HCC, >5% of tumour cells contain fat, there is widespread ballooning with or without MDB, interstitial fibrosis and foci of mixed inflammation, including neutrophils. Steatohepatitic features have been reported in 13.5–36% of HCC and are more commonly seen in tumours from patients with metabolic risk factors and steatosis or steatohepatitis in the surrounding non-neoplastic liver [3, 28, 60–62]. The presence of steatohepatitic morphology does not affect HCC prognosis [28, 62].

4.6 Management

Management decisions for HCC, independent of aetiology, are widely based on the Barcelona Clinic for Liver Cancer (BCLC) staging system - which incorporates an assessment of patient liver function and performance status in addition to tumour burden, linked to a treatment algorithm [18]. BCLC staging enables stratification of patients to those fit for potentially curative treatments (resection, transplantation and ablation) and those better served by transarterial chemoembolization or systemic

therapies such as [31]. Nowadays, curative therapies can improve survival in patients diagnosed at an early stage and provide a possible long-term cure. Surgical resection is the first-line option for patients with solitary HCC without clinically relevant portal hypertension; patients with portal hypertension and early stage HCC defined as within Milan criteria (1 lesion <5 cm or 3 lesions <3 cm) are considered for transplantation. Patients with intermediate stage HCC may benefit from hepatic arterial therapies delivering treatment preferentially to the tumour rather than non-tumour tissues - typically an embolising agent in combination with doxorubicin or cisplatin. Longer term follow up of these patients has yet to be reported and presently, the lack of tissue assessment pre-transplant has hampered the identification and validation of predictive biomarkers with the potential to identify patients with downstaged disease who are more likely to benefit from transplantation. Patients diagnosed at advanced stages may benefit from sorafenib, a multikinase inhibitor with antiangiogenic and anti-proliferative effects [15]. Sorafenib was the first and still is the only approved systemic therapy targeting pathways involved in hepatocarcinogenesis as the majority of subsequent phase III randomised trials have with molecular inhibitors have failed [16]. The tide may have turned recently, as a sorafenib-like multikinase inhibitor has been shown to have benefit 2nd in patients whose tumours progress on sorafenib [9]. Enrichment trials stratifying patients expressing c-MET on liver biopsy to treatment or not with a MET inhibitor are awaited (www.clinicaltrials.gov). Furthermore, early studies with immune checkpoint blockade, targeting the tumour inhibition of cytotoxic T cell responses, have shown promise in some patients with HCC [1, 19]. The need to identify biomarkers that will aid patient stratification to one therapy over another may yet drive the need for tumour biopsy in the not too distant future.

4.7 Epilogue

HCC in the setting of NAFLD is increasing and associations with the metabolic syndrome, diabetes and obesity are well established. The recently described steatohepatitic subtype of HCC presents with higher frequency in NAFLD compared to ALD patients. In contrast to ALD, HCC in NAFLD frequently develops in non-cirrhotic liver raising questions on appropriate surveillance measures for this population. On the other hand, surveillance for HCC in alcoholics with cirrhosis is less effective because of socioeconomic reasons. Patient and tumor characteristics in NAFLD-associated HCC are different compared to HCC of other aetiology, with older age and cardiovascular disease posing problems in therapeutic decisions and limiting available treatment choice. However, early stage HCC in NAFLD-patients has an excellent outcome and curative therapy should be applied in suitable patients. Prevention of obesity and underlying metabolic conditions, early HCC diagnosis through targeted surveillance programs and more effective treatment modalities are clearly needed for reducing the burden and improving the outcome of NAFLD-related HCC. The burden of ALD-related HCC is entirely preventable by reducing the prevalence of harmful and/or hazardous alcohol use.

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