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## **Hepatocellular carcinoma in the setting of alcohol-related liver disease**

Nathalie Ganne-Carrié<sup>1, 2, 3</sup>, Pierre Nahon<sup>1, 2, 3</sup>

<sup>1</sup>AP-HP, Hôpital Jean Verdier, Liver Unit, Bondy, France; <sup>2</sup> University Paris 13, Sorbonne Paris Cité, “équipe labellisée Ligue Contre le Cancer” F-93000 Bobigny; <sup>3</sup> INSERM UMR-1162: Functional Genomics of solid tumours, F-75010, Paris, France.

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## Summary

Alcohol-related liver disease is the most prevalent type of chronic liver disease worldwide and accounts for 30% of HCC and HCC-specific deaths. Alcohol has been associated with an increased risk of several malignancies, this risk starting at doses as low as 10 g/1 unit/day. The carcinogenic process includes direct acetaldehyde toxicity through the formation of protein and DNA adducts, an increased production of reactive oxygen species, changes to lipid peroxidation and metabolism, inflammation and an impaired immune response and modifications to DNA methylation. A high annual incidence of HCC reaching 2.9% has been observed in large European cohorts of patients with alcoholic cirrhosis, with numerous host factors modulating this risk (age, gender, liver failure, genetic polymorphisms affecting oncogenic pathways). Because of impaired surveillance and poor patient compliance, the alcoholic aetiology of underlying chronic liver disease adversely affects the prognosis for HCC through a delayed detection of cancer, frequently achieved outside the context of a surveillance programme and in a setting of advanced liver cirrhosis. This delay results in more advanced HCC and less frequent access to curative therapies, rather than an increased aggressiveness of the cancer or poorer treatment outcomes when compared to non-alcoholic HCC.

**Keywords:** alcohol-related liver disease, compensated cirrhosis, HCC, competing risk analysis.

Worldwide, alcohol accounts for around one third of global incident cases of primary liver cancer with marked variations between countries and regions [1-3]. Alcohol is a recognised carcinogen, the risk of liver cancer-mortality rising with increasing levels of consumption and the level of consumption that minimises health loss being zero [4, 5]. As well as epidemiological data, this paper will focus on the mechanisms underlying alcohol-induced liver carcinogenesis and the characteristics of primary liver cancer that develops in the context of alcohol-related liver disease (ALD).

## **1. Epidemiology**

Primary liver cancer is one of the leading causes of cancer and cancer-related deaths and its incidence has risen markedly during recent decades. Hepatocellular carcinoma (HCC) accounts for the vast majority of primary liver cancers, followed by cholangiocarcinoma and more rarely malignant stromal tumours (sarcoma, hemangioendothelioma, etc.). Almost 90% of cases of HCC develop in a context of an underlying chronic liver disease, usually advanced and accompanied by severe liver fibrosis and cirrhosis. The aetiology of this underlying disease is known in approximatively 90% of cases, and worldwide it is mainly due to hepatitis B and C infections, being less frequently related to alcohol abuse and/or nonalcoholic fatty liver disease. However, the liver cancer burden varies markedly between geographic regions, thus reflecting the variability of exposure to risk factors [6].

### **Alcohol as a risk factor for primary liver cancer**

Alcohol consumption has been associated with an increased risk of several malignancies, this risk starting at doses as low as 10 g/1 unit/day [4, 5] . It is an independent risk factor for the development of HCC, with a relative risk for heavy drinkers compared with non-drinkers and occasional drinkers slightly increased (2.07). However, in the setting of non-fibrotic liver F0/F1, heavy alcohol consumption is no longer associated with an HCC risk after adjustment on smoking habits and metabolic syndrome [7].

Moreover, alcohol synergizes with other risk factors for HCC such as diabetes mellitus and viral hepatitis, with the risks of HCC rising from 2.4 to 9.9 in patients with excessive alcohol consumption over 80g/24h without and with diabetes, and from 19.1 to 53.9 in patients with excessive alcohol consumption without or combined with a viral infection [8]. Obesity has also a synergistic effect with

alcohol because the risk of HCC incidence is 3.1 times higher in alcohol users with a BMI of 30 kg/m<sup>2</sup> or more when compared against non-users with a BMI of less than 30 kg/m<sup>2</sup> [9].

Conversely, alcohol cessation is associated with a risk of HCC that falls by 6%-7% per year, but the detrimental effects of alcohol can remain for decades, a wash-out period of 23 years being necessary to achieve the same incidence of HCC as that seen in abstinent patients. However, these results need to be considered with caution as this analysis only included four studies [10].

### **Incidence and mortality attributable to alcohol**

The epidemiology of HCC remains poorly understood in the context of ALD and is biased because of an absence of comprehensive national registries in many countries (in France, for example, only regional registries are operated and they cover only between 10%-and 50% of the country), disease coding which was changed in 2010 (transition from ICD9 to ICD10), a frequent lack of distinction made between HCC (coded C22.0 in ICD10) and other primary liver cancers (coded C22 in ICD10), and data that are not often accurate and take no account of the cause of associated chronic liver disease.

The epidemiological data available on HCC mainly derive from registries operated under an ensemble modelling approach, national databases on hospitalised patients and cohorts of patients with either HCC or uncomplicated cirrhosis who are prospectively followed up until the onset of HCC.

The Global Burden of Disease study in 2015 offers the most up-to-date overview of primary liver cancer and its underlying aetiologies at the global, regional and national levels [5]. The data refer to all types of primary liver cancer (HCC, cholangiocarcinoma and mixed liver cancer) but due to the vast preponderance of HCC, most primary liver cancers can be assimilated to HCC epidemiology. Using vital registration and cancer registry data, mortality was estimated in 195 countries or territories between 1990 and 2015; incidence was derived from mortality estimates and the mortality-to-incidence ratio. By means of a systematic review of the literature, it was possible to identify the proportions of liver cancers due to different risk factors, including alcohol.

This major epidemiological study produced a worldwide estimate of 854,000 new primary liver cancer cases and 810,000 deaths in 2015, 245,000 (30%) of which were attributable to alcohol; age-specific incidence rates rose overall between 1990 and 2015 and there was a marked preponderance of male

patients (204,000; 95%CI: 177,000-240,000). The contribution of an alcoholic aetiology to global incident cases of liver cancer varies markedly between countries and regions, from 6% in the Middle East (Iran) to 14% in North Africa (Morocco) where the leading causes associated with HCC are hepatitis B and C virus (>60%) and up to 50%-60% in Eastern Europe where hepatitis viruses only make a minor contribution to HCC (around 30%). In Europe, marked geographical variations in the contribution of alcohol to primary liver cancer can be seen (Figure 1). Alcohol accounts for 20% cases of HCC in Southern European countries (e.g. Italy or Spain) but up to 63% in Eastern European countries (e.g. Belarus).

In France, the geographical distribution of HCC is also clearly heterogeneous. According to a recently published nationwide epidemiological study [11], the most severely affected regions are Brittany, Burgundy, Upper Normandy and Pays de la Loire, this distribution being superimposed on areas of alcohol production and/or those well known for their excessive alcohol consumption.

In the near future, it is likely that the contribution of alcohol to primary liver cancer will increase in Europe because of improvements to the efficacy of treatments for hepatitis C and hepatitis B viral infections, and rises in several Eastern and Northern European countries of both per capita alcohol consumption and body mass index, conditions associated with an increased risk of both cirrhosis and HCC in the setting of ALD [3].

### **The incidence of HCC in a context of alcohol-related liver disease**

ALD comprises a spectrum of conditions from reversible fatty liver to acute alcoholic hepatitis, chronic fibrosis and cirrhosis, a late stage of disease during which HCC most often develops.

As with other causes of advanced chronic liver disease, it is recommended that patients with alcoholic cirrhosis should be included in HCC surveillance programmes in order to detect any tumour at the earliest possible stage [12, 13]: this strategy has been shown to enable the implementation of curative procedures that can increase survival [14]. Until recently, there was only limited and conflicting data on the incidence of HCC in patients with alcoholic cirrhosis, which triggered controversy regarding the benefits of periodic screening for HCC in these patients.

A retrospective Danish study based on its National Patient Registry [15], and the United Kingdom's General Practice Research Database population-based study [16] both demonstrated a low risk of HCC

(between 0.25% and 0.50% per year and less than 2% at 10 years, respectively) that was far below the 1.5% per year which had been identified as the threshold for the cost-effectiveness of surveillance, thus raising some doubts as to the efficiency of screening for HCC in these patients. However, major selection biases in such studies conducted in hospitalised patients preclude their interpretation in terms of HCC occurrence as they mainly reported the incidence of other liver-related complications constituting risks of death competing with the occurrence of liver cancer in these populations [15, 17]. In this situation, longitudinal studies dealing with the natural history of alcoholic cirrhosis would be more reliable. Such cohort studies are scarce but unlike the registry-based studies previously described they have suggested a high risk of HCC in patients with compensated alcoholic cirrhosis being followed prospectively for the onset of HCC (Table 1). A small but very homogeneous Spanish cohort indicated an annual incidence of HCC of up to 2.6% [18], which suggests that implementing a surveillance programme for the early diagnosis of HCC is warranted in these patients. The recently published results of our French and Belgian CIRRAL prospective cohort of 652 patients with unambiguously biopsy-proven Child-Pugh A alcoholic cirrhosis who were periodically evaluated using liver ultrasonography surveillance for HCC supports this finding, revealing a high annual incidence of HCC (2.9%) and a high percentage of small cancers theoretically eligible for curative treatment, thus emphasising the crucial role of screening for HCC in these patients [19].

However, not all patients with compensated alcoholic cirrhosis are at the same risk of developing HCC. According to Mancebo et al. [18], both platelet count and age can be used to classify in three separate groups those at risk of developing HCC during the next five years. The annual rates of HCC in the group with neither of these factors (age <55 years, platelet count >125,000), with one factor (age >55 years or platelet count <125,000) and with both factors (age >55 years and platelet count <125,000) were 0.3%, 2.6%, and 4.8% ( $p < 0.0001$ ), respectively. Similarly, we determined four variables associated with an increased cause-specific hazard of HCC, namely: old age (HR=1.12, 95%CI: 1.07-1.18;  $p < 0.0001$ ), male gender (HR=2.66 (95%CI: 1.12-6.32;  $p = 0.027$ ), high baseline AFP (HR=1.07, 95%CI: 1.02-1.12;  $p = 0.004$ ) and bilirubin levels (HR=1.06, 95%CI: 1.102-1.096;  $p = 0.005$ ), while high prothrombin time values were associated with a reduced hazard of HCC (HR=0.97, 95%CI: 0.94-0.99;  $p = 0.025$ ) [19]. Unlike previous retrospective [20, 21] or prospective

[22] cohorts, a detrimental effect of environmental factors such as overweight was not observed in these two large European prospective cohorts, probably because of the high percentage of patients with this comorbidity (68% of patients enrolled in the CIRRAL cohort were overweight, including 32.5% obese patients).

### **Prevalence of HCC in liver transplantation candidates with alcoholic liver disease**

Using the US Scientific Registry of Transplant Recipients, trends regarding the prevalence of HCC in liver transplantation candidates were estimated for the principal aetiologies of chronic liver disease. Among the 24,431 adults on the liver transplantation waiting list between 2002 and 2016 with a listing diagnosis of HCC and a known aetiology of underlying chronic liver disease, 2520 patients (10.3%) had ALD alone and 1936 had ALD associated with chronic hepatitis B or C. Between 2002 and 2016, and despite a much lower level, NASH was the fastest growing cause of HCC among candidates on the waiting list (11.5-fold), while the prevalence of HCC among listed patients increased 3.1-fold in the case of ALD alone and 6.4-fold in patients with ALD and virus-related chronic hepatitis [23].

## **2. Pathogenesis and genetics of alcohol-induced liver carcinogenesis**

Chronic alcohol intake alters the architecture and compromises the functional capacity of the liver by triggering steatosis, steatohepatitis and cirrhosis [24]. These pathological events are subsequently sustained and participate in the carcinogenetic process. As well as the development of cirrhosis, which can be considered as a precancerous condition, a number of pathophysiological aspects are specific to hepatic alcohol-mediated carcinogenesis and include: (1) the formation of acetaldehyde and its direct detrimental effects on proteins and DNA; (2) an elevated production of cytochrome P450 2E1 (CYP2E1)- and/or iron-induced reactive oxygen species (ROS), further aggravated by the impairment of antioxidant defences and DNA repair mechanisms; (3) changes to the immune system and the induction of chronic inflammation; (4) interference with methyl group transfer and alterations to gene expression [25]. These pathways may be further affected by host-related genetic heterogeneity that could partly explain the breadth of inter-individual susceptibility to the development of HCC in patients with alcoholic cirrhosis [26]. Figure 2 describes this complex biological interplay, as well as the principal variants reported as



possible genetic markers for a risk of liver cancer in ALD, as a function of their specific interactions with the alcohol-related hepatocarcinogenesis process.

### **Hepatotoxicity of ethanol and tumour promotion**

Ethanol is oxidised into acetaldehyde by the successive effects of alcohol dehydrogenase (ADH) in the cytosol, and aldehyde dehydrogenase (ALDH) in the mitochondria [27]. Acetaldehyde then enters the mitochondria where it is oxidised to acetate by mitochondrial ALDH. Acetaldehyde is a highly reactive and direct mutagenic compound which forms various protein and DNA adducts that promote DNA repair failure, lipid peroxidation and mitochondrial damage, and ultimately favour carcinogenesis. Another major pathway of ethanol metabolism includes its oxidation in microsomes by CYP2E1 enzyme, a step that requires nicotinamide adenine dinucleotide phosphate (NADPH) rather than nicotinamide adenine dinucleotide (NAD<sup>+</sup>), as for ADH. In parallel, ROS are formed due to the metabolism of alcohol by CYP2E1 and the re-oxidation of NADH in the mitochondria [28].

Genetic variations in the alcohol-metabolising enzymes that modulate the resulting amounts of carcinogenic acetaldehyde have been largely explored as potential inherited markers of alcohol-induced cancers, including those of the liver. [29]. In particular, ALD patients who are homozygous for the ADH1C\*1 allele (thought to confer higher enzymatic activity) appear to be more frequently exposed to the onset of HCC [30]. Similarly, the weakly active ALDH2\*2-conferring allele has been found to be associated with liver cancer in excessive drinkers. [31, 32].

### **Alcohol-induced oxidative stress and iron metabolism**

A pivotal mechanism implicated in alcohol-related hepatocarcinogenesis is oxidative stress that is secondary to ROS derived from alcohol metabolism, inflammation and increased iron storage. Indeed, ROS promote damage to cellular macromolecules and participate in the progression of liver carcinogenesis through the formation of lipid peroxides such as 4-hydroxy-nonenal [33]. An accumulation of ROS causes structural and functional alterations to DNA that leads to cell cycle arrest or apoptosis. This damage severely affects gene functions such as replication and transcription, and plays a major role in cancer initiation and promotion [34]. ROS accumulation also induces the production of various cytokines, the activation of immune cells and the upregulation of angiogenesis and the metastatic process [35].

ROS include hydroxyethyl, superoxide anion, hydroxyl radicals and numerous free radicals that accumulate following the successive actions of pro- and anti-oxidant enzymes [36]. While antioxidant defences are impaired by ethanol consumption, genetic variants affecting the enzymes that regulate the production and detoxification of ROS have been shown to modulate the outcome of ALD. In particular, myeloperoxidase (MPO) catalyses the reaction between  $\text{H}_2\text{O}_2$  and  $\text{Cl}^-$  to form the highly reactive hypochlorous acid (HOCl) and anion ( $\text{OCl}^-$ ) [37] and is affected by a G to A base exchange at position -463 involving its promoter [38]. In addition, manganese superoxide dismutase (SOD2) generates  $\text{H}_2\text{O}_2$  within the mitochondria [39], leading to the formation of highly reactive hypochlorous acid. A genetic dimorphism substitutes either alanine (Ala) or valine (Val) in the mitochondrial targeting sequence of SOD2 [40] and results in higher mitochondrial activity for the Ala-SOD2 variant. In large prospective cohorts of alcoholic cirrhotic patients, it was shown that the carriage of two G-MPO alleles and/or the possession of at least one Ala-SOD2 allele alone were independent risk factors for the onset of HCC [41]. These variants were also associated with liver iron overload, possibly through enhanced mitochondrial hydrogen peroxide production [42], a condition which has also been reported to be associated with a risk of HCC in these patients, along with usual HFE gene mutations [43, 44].

### **Activation of innate immunity, cytokine and chemokine systems**

Alcohol interacts with the immune system and affects tumour immune surveillance, both mechanisms which may favour tumour development and progression. Indeed, the innate immune response aims to identify cancerous clones in order to inactivate transforming cells. This response is promoted by inflammatory mediators (chemokines and cytokines) which are produced by various immune cells [45]. Alcohol consumption increases gut permeability and the translocation of bacteria-derived LPS from the gut to the liver [46]. In Kupffer cells, LPS interacts with TLR4, which leads to the production of pro-inflammatory cytokines such as interleukin 6 (IL-6) and  $\text{TNF}\alpha$ . The IL-6/STAT3 and  $\text{TNF}\alpha/\text{NF-}\kappa\text{B}$  axes have been highlighted as major biological pathways involved in hepatocarcinogenesis [47]. Although the precise mechanisms by which pro-inflammatory cytokines promote liver cancerisation are not fully understood, their signals regulate gene expression through the STAT3 and NF $\kappa$ B transcription factors. NF- $\kappa$ B, one of the main transcriptional regulators of the inflammatory response, is activated during ALD [48] and increases the production of various pro-inflammatory mediators which include  $\text{TNF}\alpha$ , IL-1, IL6,

EGF and TLRs [49]. The latter promote ROS accumulation and activate STAT3, thus participating in the cancerisation process [50]. Finally, alcohol abuse promotes immunosuppression through the reduced recruitment of the CD8<sup>+</sup> cells that participate in the anti-tumour response [51].

A genetic heterogeneity affecting the expression of TNF $\alpha$ , IL1 $\beta$ , IL6 or EGF has been reported to modify the risk of liver cancer, particularly in patients infected with HBV or HCV [52]. In ALD patients, the highly productive cytokine IL6-174G allele is associated with HCC [53]. Epidermal growth factor (EGF) promotes cancer growth and invasiveness [54] and is subject to functional polymorphism involving an A to G exchange in the 5' untranslated region of the *EGF* gene [55]. The G allele, resulting in higher transcription levels, has been associated with the presence of HCC in Caucasian populations affected by alcohol- or HCV-related liver diseases [56], a finding that was further confirmed by a recent meta-analysis [57].

The role of chemokines and their receptors in tumorigenesis has been widely reported, particularly regarding lymphocyte recruitment in the livers of patients with HCC [58]. In ALD patients, TNF $\alpha$  induces the expression of various chemokines such as RANTES, a T-cell chemoattractant and immunoregulatory molecule that may play a pivotal role in the migration of inflammatory cells to the liver [59]. A *RANTES* promoter polymorphism (*G-403A*) results in the increased RANTES expression of *A-403* alleles and favours the development of various malignancies [60]. This finding was replicated in a prospective study conducted in Caucasian patients with cirrhosis, where alcoholic cirrhotic patients carrying two *G-403* alleles presented a higher risk of developing liver cancer during follow-up [61].

### **Changes to folate metabolism**

Epigenetic regulation also involves various chemical modifications, including DNA methylation [62]. Oxidative metabolites such as acetaldehyde and ROS generated by means of alcohol metabolism can induce epigenetic changes through alterations to folate metabolism, an essential component of DNA synthesis and methylation [63]. Polymorphisms in the methylene tetrahydrofolate reductase (*MTHFR*) gene, leading to changes in folate metabolism, have been reported to be associated with HCC development [64], including in patients with ALD [65, 66].

### **Modulation of lipid metabolism**

Alcohol abuse is characterised by an accumulation of fat (mainly triglycerides, phospholipids and cholesterol esters) in hepatocytes. Initially revealed by GWAs studies, an SNP (rs738409 C>G for I148M) in the adiponutrin/patatin-like phospholipase-3 (PNPLA3) protein sequence has rapidly become a well-established genetic factor associated with steatosis and fibrosis in ALD patients [67-69]. This genetic variation is considered a loss-of-function mutation that promotes an intracellular triglyceride accumulation and lipotoxicity in hepatocytes by impairing their ability to hydrolyse triglycerides from lipid droplets [70]. The rs738409 (G) allele is associated with steatosis, advanced fibrosis and cirrhosis in ALD [71, 72] and has been repeatedly highlighted by numerous European research groups in the context of both large case-control [72-77] and prospective studies [78] and then subsequent meta-analyses [79, 80] (including one based on individual participant data [81]) as a major genetic driver of liver cancer development in patients with alcoholic cirrhosis).

Other polymorphisms affecting genes involved in lipid metabolism have also been proposed as potential genetic risk markers of ALD-related liver carcinogenesis. A variant in NCAN, an extracellular matrix proteoglycan that was initially identified as a modifier of steatosis during GWAs performed in patients with NASH [82], was subsequently also associated with the presence of HCC in European patients with ALD [83]. Interestingly, the authors reported a higher rate of HCC among carriers of both the at-risk NCAN-T and PNPLA3-G variants when compared to those with only one or neither of these genetic traits. Detailed mapping of this region subsequently identified TM6SF2 rs58542926 as the causal variant responsible for all the associations previously reported on the NCAN locus [84]. Also characterised by a loss-of-function of the TM6SF2 protein, the polymorphic variant of the *TM6SF2* gene impairs VLDL export and increases intracellular lipids, which explains its association with steatosis and fibrosis in ALD [85]. Similar to the NCAN variant, the combination of TM6SF2 polymorphism with PNPLA3 genotypes was recently associated with a higher rate of liver cancer in a European case-control cohort of alcoholic cirrhotic patients [86]. However, apart from their direct effect on steatosis, which alone is not recognised as a risk factor for HCC development in ALD, the potential biological explanation linking these genetic traits with the liver cancerisation process is as yet unknown.

### **3. HCC screening, tumour characteristics, access to curative treatment and survival in patients with HCC that has developed in the setting of ALD**

Several publications have reported a positive impact of HCC screening on diagnosis at an early-stage, eligibility for curative treatment and overall survival. However, despite international guidelines on HCC screening in patients with cirrhosis, periodic surveillance is not optimal in clinical practice in a target population. In the USA and Europe, fewer than 30% of HCC cases are diagnosed by surveillance and surveillance is indicated but missed in more than one third of patients. Moreover, in eligible patients under surveillance, the intervals between screening ultrasounds are often longer than 6 months [87-89]. In addition, cases of ALD or non-alcoholic fatty liver disease are more likely to be associated with deficient surveillance than those with hepatitis C virus-related cirrhosis [90, 91]. Lastly, the large number of undiagnosed alcohol-related cirrhosis contributes to the low rate of HCC surveillance and late diagnosis.

These delays result in a large tumour size at diagnosis and a poor outcome in patients with ALD [89, 90]. Conversely, when diagnosed in the context of a strict surveillance programme, HCC has a prognosis independent of the aetiology of the underlying cirrhosis which is dictated by liver function, oncologic features and treatment, suggesting that ALD per se is not a detrimental factor in terms of outcome.

For descriptive purposes, Table 2 reports the characteristics of HCC and patients together with crude survival rates as a function of the aetiology of underlying chronic liver disease [89, 90, 92, 93]. The impact of an alcoholic aetiology on the clinical presentation, treatment and outcome of HCC, as well as on each Barcelona Clinic Liver Cancer (BCLC) stage, was compared with the aetiology of HCV during two major studies. In the Italian Liver Cancer (ITA.LI.CA) database [90], 1642 HCV and 573 alcoholic patients (daily alcohol intake >80 g for men and 60 g for women over more than 10 years, without other known causes of liver disease) who were diagnosed with HCC between January 2000 and December 2012 were analysed retrospectively. When compared to HCV-related HCC, cases of alcohol-related HCC were more frequently diagnosed outside surveillance, in patients with more severe liver function impairments and presenting at a more advanced stage with larger tumours that

were more frequently multifocal or infiltrating/massive and associated with portal vein thrombosis. After adjustment for the lead-time, median overall survival was significantly reduced among patients with alcohol-related HCC (27.4 months versus 33.6 months in HCV patients,  $p = 0.021$ ). The prognostic importance of a patient's underlying aetiology disappeared when survival was assessed at each BCLC stage and using Cox multivariate regression models.

In France, a recent prospective study issued a caution regarding the worse cancer presentation in patients with alcohol-related HCC [93]. Among 894 patients with newly diagnosed HCC included prospectively between May 2008 and October 2009 in a French database and then followed prospectively every year for five years, a comparison was made between 582 (65.1%) with alcohol-related HCC and 312 (34.9%) with non-alcoholic related HCC. Alcohol-related HCC was more likely to be diffuse and detected in patients with a poorer performance status and liver function. After adjustments for lead-time bias, median overall survival was significantly reduced in alcohol-related HCC patients (9.7 months versus 5.7 months in HCV patients;  $p = 0.0002$ ). The prognostic importance of alcohol disappeared when survival was assessed at each Barcelona Clinic Liver Cancer (BCLC) stage. Patients with HCC detected in the context of a cirrhosis follow-up programme ( $n = 199$  [22.3% of the whole cohort]) had a better lead time-adjusted median overall survival versus patients with HCC diagnosed incidentally (11.7 vs 5.4 months;  $P < 0.0001$ ). Thus, by comparison with patients with non-alcohol-related HCC, patients with alcohol-related HCC have reduced overall survival, mainly because of poorer liver function and tumour characteristics at diagnosis, as attested by their similar survival within each BCLC stage.

These studies have led to the conclusion that an alcoholic aetiology adversely affects the prognosis for HCC because of the delay in detecting the cancer, frequently achieved outside any surveillance programme and in a setting of advanced liver cirrhosis. As a result, the HCC stage is more advanced and less frequently amenable to curative therapies. These features, rather than greater cancer aggressiveness or worse treatment outcomes, justify the poorer prognosis of Western alcoholic patients when compared to HCV-infected patients.

## **Conclusion/perspectives**

Alcohol is a recognized carcinogen for several malignancies, the risk starting at low doses (10 g/1 unit/day). The contribution of an alcoholic aetiology to global incident cases of HCC - estimated at around 30% in 2015 - is expected to increase in the years to come, concurrent with both the decline in virus-induced HCC because of more effective viral eradication [94] and control [95], and the rising alcohol consumption observed in developing countries [96]. Because of impaired surveillance and poor patient compliance, an alcoholic aetiology of underlying chronic liver disease adversely affects the prognosis for HCC due to delayed cancer detection, frequently achieved outside any surveillance programme and in a setting of advanced liver cirrhosis, leading to less frequent access to curative therapies.

As in other causes of advanced chronic liver disease, it is recommended that patients with alcoholic cirrhosis should be included in HCC surveillance programmes so that any tumour can be detected at the earliest possible stage: this strategy has been shown to enable the implementation of curative procedures that can increase survival [14]. In this respect, it is pivotal to identify among millions of excessive drinkers those who are susceptible to developing HCC (i.e. primarily cirrhotic patients) and among them to select those especially prone to developing liver cancer [97]. Indeed, the limitations of surveillance procedures call for a refinement of these costly programmes in order to identify those populations in whom surveillance may be futile and/or cost-ineffective [98], or by contrast those where surveillance should be intensified. Several genetic variants have been reported to be associated with a higher risk of HCC in alcoholic cirrhotic patients and might offer an opportunity for the individualised management of patients as a function of their genetic backgrounds [97]. The constitution of large multicentre prospective cohorts of patients with alcoholic cirrhosis and involving sequential biobanking is an unmet need in hepatology. The challenge is to understand how combining several genetic variants and dynamic changes to serum or plasma biomarkers with clinical data might help to refine the stratification of alcoholic cirrhotic patients as a function of different HCC risk classes, as well as facilitating early HCC detection [99]. These advances will enable the adaptation of screening and preventive measures and the criteria for early diagnosis, and hopefully will optimise the allocation of limited resources in these difficult-to-manage but numerous patients [100].

## Legends to figures

**Figure 1: Geographical distribution of the contribution of alcohol to absolute primary liver cancer deaths in Europe for both genders** (adapted from Akinyemiju T et al. JAMA Oncol 2017;3: 1683–1691, Supplementary Table 10e)

**Figure 2: Biological pathways involved in alcohol-mediated liver carcinogenesis.** Ethanol is first metabolised by alcohol dehydrogenase (ADH) into carcinogenic acetaldehyde, which causes major cell damage by forming DNA and protein adducts. CYP2E1 also triggers the formation of acetaldehyde and reactive oxygen species (ROS). Oxidative stress secondary to ROS accumulation damages cell components through lipid peroxidation and DNA mutagenesis via the formation of adducts and the impairment of repair mechanisms. The effects of iron and lipid accumulations usually observed in ALD also participate in ROS accumulation, and further aggravate the deleterious effects of oxidative stress. In addition, alcohol promotes inflammation via the translocation of gut bacteria and LPS which triggers the production of pro-inflammatory cytokines and chemokines implicated in both the anti-tumour immune response and ROS formation. Acetaldehyde also alters methyl transfer, leading to DNA hypomethylation associated with modifications to gene expression (oncogenes and tumour suppressor genes). The principal variants reported as being potential genetic modifiers of the liver cancer risk in ALD patients are depicted in Figure 2 as a function of their specific interactions with these molecular pathways.



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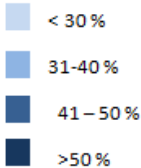
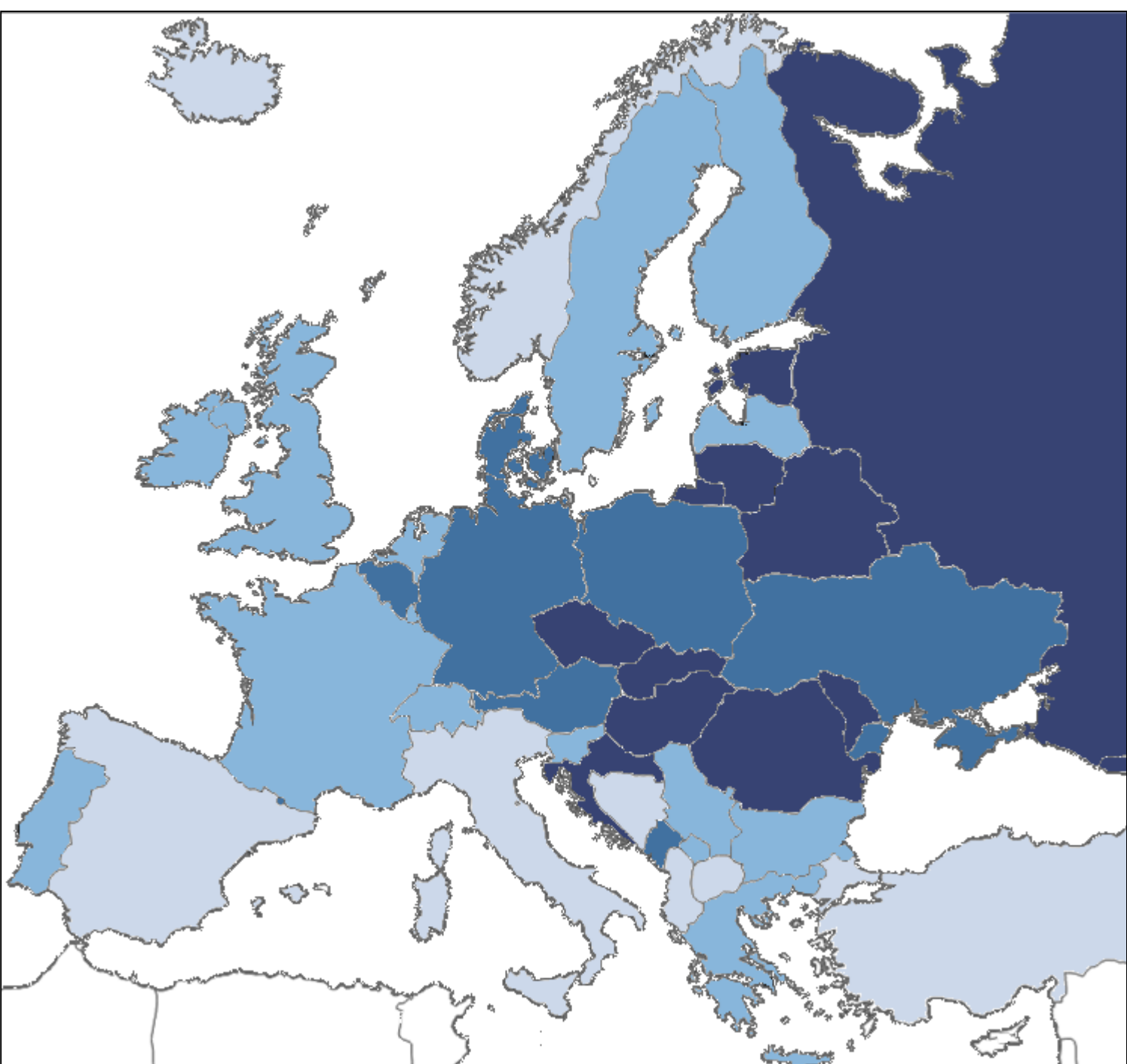
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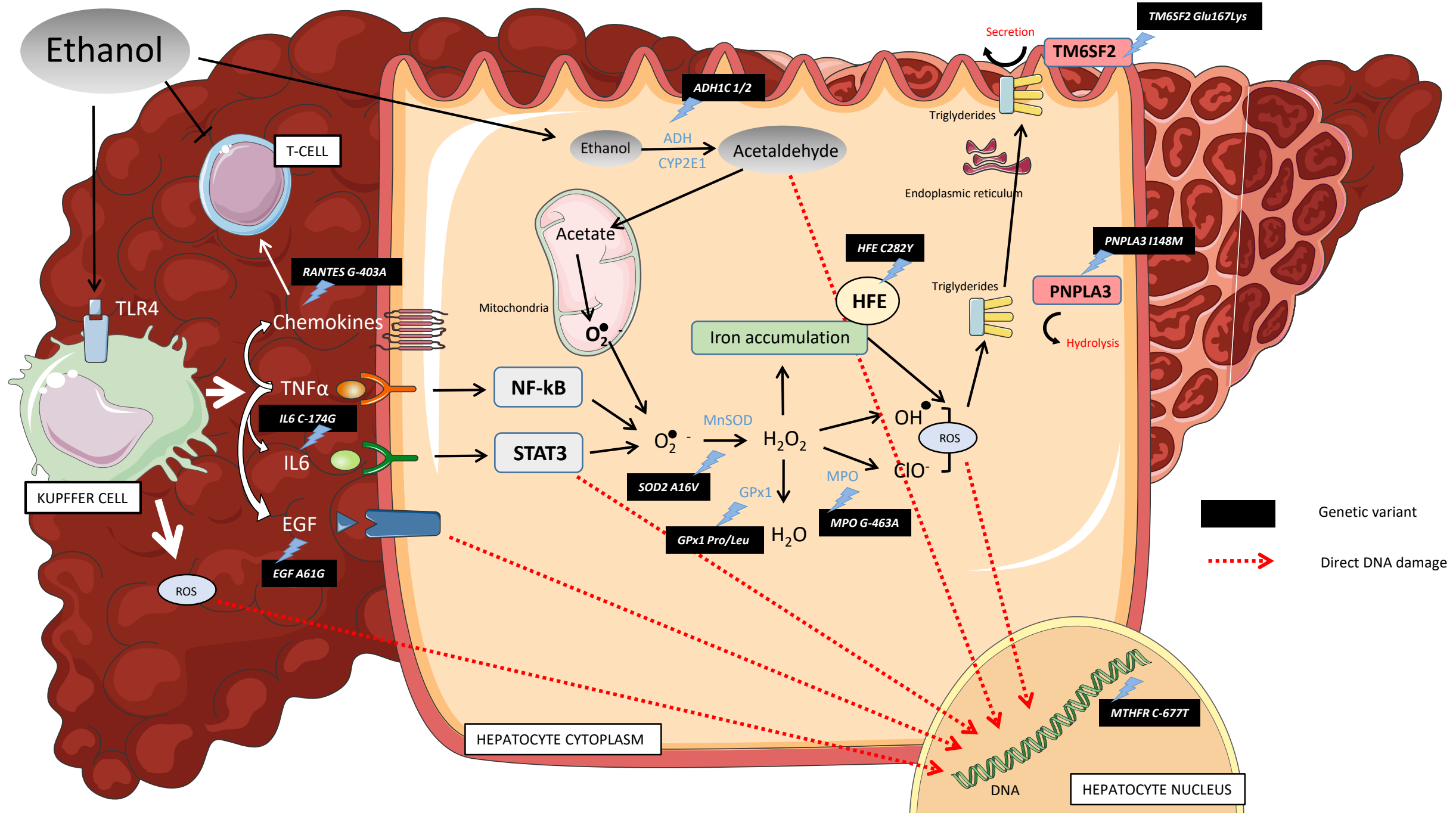
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Nation	%
Albania	20 %
Andorra	47 %
Austria	49 %
Belgium	48 %
Biélorussie	63 %
Bosnia and Herzegovina	29 %
Bulgaria	40 %
Croatia	54 %
Cyprus	32 %
Czech Republic	51 %
Denmark	49 %
England	36 %
Estonia	61 %
Finland	37 %
France	37 %
Germany	44 %
Greece	31 %
Hungary	52 %
Iceland	26 %
Ireland	36 %
Italy	20 %
Latvia	34 %
Lithuana	59 %
Luxembourg	38 %
Macedonia	29 %
Malta	24 %
Moldova	61 %
Montenegro	41 %
Netherland	35 %
Northern Ireland	34 %
Norway	25 %
Poland	42 %
Portugal	37 %
Romania	54 %
Russia	53 %
Scotland	36 %
Serbia	38 %
Slovakia	51 %
Slovenia	51 %
Spain	21 %
Sweden	33 %
Switzerland	39 %
Turkey	19 %
Ukraine	47 %
Wales	37 %





**Table 1. Incidence of HCC and risk factors in longitudinal cohorts of patients with alcoholic cirrhosis**

Author (year)	Patients (n)	Country	FU (yrs.)	HCC (n)	Annual incidence (%)	Host risk factors	Environmental risk factors
N'Kontchou [22] (2006)	478	France	4.2	108	5.6	Age Gender	BMI Diabetes
Torisu [21] * (2007)	47	Japan	6.8	9	2.1	Age	Diabetes
Kodama [20]* (2013)	85	Japan	3.0	6	2.5	Age	Diabetes
Mancebo [18] (2013)	450	Spain	3.5	62	2.6	Age Platelets	-
Ganne-Carrié [19] (2018) <i>CIRRAL cohort (NCT 01213927)</i>	652	France Belgium	2.4	43	2.9	Age Gender AFP Bilirubin TP	-

\*Retrospective Cohorts

**Table 2. Tumour characteristics, access to curative treatment and survival of patients with alcohol-related HCC compared to those with non-alcohol-related HCC (crude comparison)**

Author (year)	Patients (n) According to HCC aetiology	Single nodule (%)	Main nodule size (cm)	BCLC A/B/C/D (%)	Child-Pugh A/ B/ C %	Curative treatment (TH, surgery, RF; %)	Overall survival (median, months)
<b>Trevisiani</b> <sup>1</sup> [92] (2007) <i>ITA.LI.CA</i>	- Alcohol 59 - HBV 87 - HCV ..... 461 - Mixed .....135	67.8 50.6 59.9 51.6	3.0 3.2 3.0 3.3	NA	67.8/ 23.7/ 8.5 61.4/ 30.2/ 8.4 69.7/ 24.3/ 6.0 65.2/ 27.4/ 7.4	47.4 <sup>a</sup> 48.6 44.4 37.3 <sup>b</sup>	32 (19.3– 44.7) 33 (19.6– 46.4) 36 (31.7– 40.3) 29 (25.0– 33.0)
<b>Schütte</b> [89] (2012)	- Alcohol 302 - HBV/HCV 91	NA	NA	14.6/ 30.5/ 43.7/ 11.3 22.0/ 35.2/ 34.1/ 8.0	49.3/ 31.1/ 9.6 59.3/ 23.1/ 8.8	NA	14.7 (NA) 9.8 (NA)
<b>Bucci</b> [90] (2016) <i>ITA.LI.CA</i>	- Alcohol 573 - HCV 1642	43.3* 57.9	4.1* 3.3	33.5/ 20.2/ 34.4/ 11.9* 47.2/ 13.9/ 31.1/ 7.9	55.5/ 35.8/ 8.7* 68.5/ 26.4/ 5.1	37.9* 57.0	32.4 (26.6– 38.3) <sup>\$</sup> 40.6 (37.7– 43.5)
<b>Costentin</b> [93] (2018) <i>CHANGH</i>	- Alcohol 582 - Other 312	33.5 <sup>£</sup> 42.3	53.0* 63.0	12.5/ 5.7/ 46.0/ 26.8 <sup>^</sup> 22.0/ 5.1/ 47.4/ 34.8	39.3/ 39.5/ 21.2* 66.0/ 23.7/ 10.3	16.3* 27.1	5.7 (1.57-13.34) * 9.7 (2.43-14.33)

1; HCC diagnosed in the context of a surveillance programme; a- HBV patients underwent liver transplantation (10.7%, p = 0.012) and alcoholic patients underwent hepatic resection (18.6%, p = 0.045) more frequently than expected (4.4% and 11.6%, respectively)

\* ≤ 0.001 \*\*p = 0.003 \$ p = 0.002 £ p = 0.009 ^0.06