Hepatocellular carcinoma: risk groups, surveillance and outcome

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CHAPTER 1

GENERAL INTRODUCTION AND THESIS OUTLINE

Epidemiology and risk factors

Primary liver cancer is the sixth most common cancer in the world and the second cause of cancer-related death [1]. Hepatocellular carcinoma (HCC) represents more than 90% of primary liver cancers. Incidence rates vary widely between geographical regions and are highest in East Asia and Sub-Saharan Africa, where approximately 85% of all cases occur [1,2]. The high HCC risk in these regions can be explained at least partly by the high prevalence of underlying risk factors, especially chronic hepatitis B virus (HBV) infection and aflatoxin B1 in the diet [2,3]. Nevertheless, also in Western countries with relatively low incidence rates, such as the United States and the Netherlands, incidence has increased in the last decade [4-7]. This increase is probably due to growing impact of chronic liver disease due to hepatitis C virus (HCV) and non-alcoholic fatty liver disease (NAFLD)[8-11].

Currently, major risk factors for HCC development are chronic infections with HBV or HCV, alcohol-related liver disease and NAFLD [9,10,12]. Although HCC generally occurs in patients with underlying cirrhosis, it can also develop in non-cirrhotic livers. Based on previous studies, the proportion of HCC in the absence of cirrhosis varies widely (range: 2%-54%) between geographical regions and underlying risk factors [13].

Hepatitis B

Worldwide, chronic HBV infection accounts for approximately 50% of all new HCC cases [14]. In high endemic regions such as sub-Saharan Africa and East Asia, most patients are infected by vertical transmission from mother to child of which 90% develops a chronic infection. Sexual or parenteral transmission routes are most common in regions with a low incidence of HCC such as the Netherlands. Estimated HCC risk is 15-20 times greater in HBV infected patients than in the uninfected population [2,15]. HCC risk ranges from 0.2-3.7 in 100 persons years in untreated patients and is highest in cirrhotics [16]. Although HBV is a common cause of HCC development in the absence of cirrhosis, the majority of patients with HBV-related HCCs have cirrhosis (70-90%) [17]. In chronic HBV patients, additional risk factors for HCC development are male gender, Asian or African ethnicity, a positive family history of HCC, hepatitis B e antigen (HBeAg) positivity, high viral load (HBV-DNA), longer duration of infection, increased transaminases and/or genotype C [16,18-21]. Co-infections with HCV and/or human immunodeficiency virus (HIV) are also associated with increased HCC risk [15,22].

Primary prevention for HCC can be achieved by hepatitis B vaccination, especially in high endemic countries [23]. Second, antiviral treatment of HBV can reduce HCC risk in patients who achieve viral suppression [24,25]. According to a recent overview [26], HCC risk can be reduced by HBV treatment with currently available highly potent nucleos(t)ide analogs entecavir or tenofovir, but not entirely eliminated (annual HCC incidence range during therapy: from 0.01-1.4% in non-cirrhotic patients and from 0.9-

5.4% in those with cirrhosis, overall risk reduction of approximately 30% in cirrhotics and 80% in non-cirrhotics). The residual risk is probably due to events that may have taken place before treatment initiation or risk factors that are not amenable to change by antiviral therapy [26].

Hepatitis C

HCV is an enveloped, single-stranded RNA virus that is one of the major causes of chronic liver disease worldwide. Transmission occurs through exposure to HCV-infected blood, mainly by contaminated needles in IV drug users or transfusion of unscreened blood products. Chronic HCV infection is the most important risk factor for HCC in Western countries [10]. HCV-infected patients have a 15- to 20-fold increased risk of developing HCC compared to non-infected persons [21,27]. Risk is highest in patients with established cirrhosis (annual risk 3%-5%). Additional risk factors are HCV genotype 1b or 3, older age, male sex, presence of comorbidities such as alcohol abuse, diabetes or obesity and co-infections with HIV and/or HBV [2,15,22,28-30].

There is currently no vaccine for HCV due to its high mutation rate during viral replication, but by reducing HCV transmission the burden of HCC can theoretically decreased: e.g. improvement of hygienic circumstances by the widespread use of disposable syringes and needles, reduction of risky sexual behavior and screening of blood donors and organ donors. Furthermore, achieving a sustained viral response (SVR) after interferon-based HCV treatment is associated with a substantial HCC risk reduction [31,32]. Antiviral therapy for chronic HCV has changed strongly over the past few decades and is nowadays highly effective. Until 2012, combined pegylated (PEG)-interferon plus ribavirin was the standard of care for CHC and resulted in SVR in 40-90% of treatment-naïve patients [33,34]. Nevertheless, interferon-containing antiviral therapy is associated with significant side effects, including deterioration of nutritional status due to fatigue, fever, nausea, depression and/or taste changes, with also significant effects on general functioning. On the other hand, preventive nutritional supports can ameliorate deterioration of nutritional status during interferon-containing therapy and markedly decreases loss of paid labor productivity, physical exercise and performance status during anti-viral therapy [35,36].

Recently, several new generation direct-acting antivirals (DAAs) have been approved. Therefore, interferon-free treatment strategies for HCV are nowadays available, which are more effective and better tolerated [37-39]. However, wide availability is restricted due to excessive costs.

Alcohol

Alcohol-related liver disease includes a wide spectrum of liver injury, ranging from simple steatosis, alcoholic steatohepatitis to cirrhosis. Alcohol is a major cause of liver disease worldwide. Pattern of alcohol intake differs between geographical regions and is highest

in developed countries. Heavy alcohol consumption, defined as daily consumption of three or more alcoholic drinks for prolonged periods, is a clear risk factor for HCC [40]. Furthermore, alcohol intake may have a synergistic effect on HCC risk in patients with HBV, HCV and/or NAFLD by presumably accelerating the progression to cirrhosis [29,41].

Non-alcoholic fatty liver disease

NAFLD is characterized by fatty depositions in the liver in the absence of alcohol abuse and includes a wide spectrum, ranging from simple steatosis to non-alcoholic steatohepatitis (NASH) and is strongly correlated with features of metabolic syndrome [42]. Currently, NAFLD has become one of the leading causes of chronic liver disease and a common risk factor for HCC [10,43]. Increased HCC risk seems to affect especially those with NASH-related cirrhosis. Nevertheless, it has recently been demonstrated that also in non-cirrhotic livers presence of NAFLD and or metabolic syndrome is associated with HCC development [44,45]. Furthermore, steatosis is often present as co-factor in patients with other risk factors for underlying liver disease and is for example associated with an increased HCC risk in HCV-infected patients [46]. NASH-related liver disease can be improved by lifestyle modifications resulting in weight loss [47].

Pathogenesis

In patients with HCV or alcohol-related liver disease, cirrhosis appears to be prerequisite for HCC development. It is thought that HCC may develop as a result of an indirect pathway from chronic inflammation and injury leading to fibrosis with eventual progression to cirrhosis. In case of HCV, the RNA virus does not integrate into the host genome and therefore does not have a direct oncogenic effect. Patients with alcohol abuse develop liver damage through increased oxidative stress with may have an effect on HCC-relevant signaling pathways. On the other hand, HBV could also exert a direct oncogenic effect regardless of presence of cirrhosis. HBV genome integration can lead to host DNA microdeletions and aberrant function of growth regulatory genes in the hepatocyte. In patients with NAFLD, it is thought that oxidative stress (as a result of an increased level of intrahepatic fatty acids) is increased and causes hepatocyte injury and cancer-promoting mutations, which can lead to HCC development [48].
Overall, hepatocarcinogenesis is a complex process, which is not completely understood. It is thought that HCC development is caused by accumulation of genetic changes resulting in altered expression of tumor suppressor and oncogenes. Several molecular deregulated pathways have been described in HCC [49,50].

Surveillance

A potentially important strategy to detect HCC at early stage is regular surveillance of patients at increased risk of HCC development, which is currently advised by several

international and national guidelines [51-54]. The ultimate goal of surveillance is to detect HCC at earlier stages enabling potentially curative therapies with a better outcome and decreased mortality. Nonetheless, evidence for surveillance remains weak and controversial [55,56].

One randomized controlled trial from China in chronic HBV patients compared surveillance with combined ultrasound (US) and alpha–fetoprotein (AFP) at 6-month intervals versus no surveillance and found significantly lower HCC-related mortality rates in the surveillance group [57]. Several important aspects of this study have been criticized [55,58]. Another randomized controlled study in Chinese HBV patients found no benefit from surveillance with AFP alone at 6-month intervals [59]. Based on lower quality evidence, several cohort studies have suggested that survival is improved with HCC surveillance [60,61]. Surveillance could lead to more frequent detection of HCC at early stages enabling curative treatment [60,61].

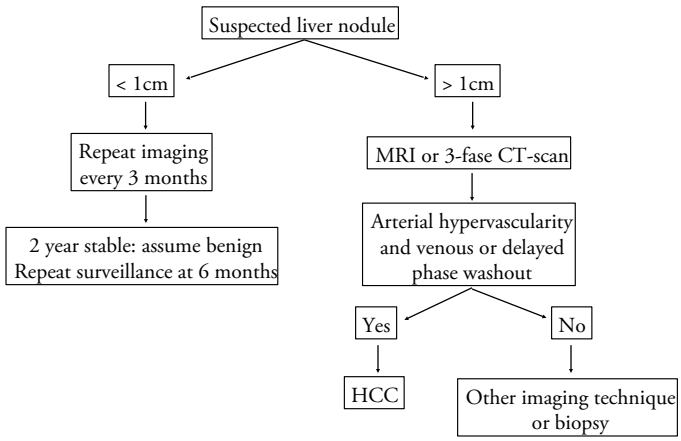
Conventional US and AFP (alone or combined) are most widely used as surveillance modalities, but their sensitivities and specificities are far from perfect [62,63]. Moreover, the optimal interval for surveillance is not clear [64-67]. Current guidelines recommend a surveillance interval of 6 months [51-54].

Finally, selection of the patients eligible for surveillance remains a subject of debate. The decision to start surveillance should depend on the risk of developing HCC, i.e., the incidence of HCC in specific populations. Especially high-risk patients should be included in surveillance programs. However, exact HCC incidence is often unclear. Based on theoretical models, surveillance for HCC is considered to be cost-effective if HCC incidence exceed 1.5%/year in cirrhotics and 0.2%/year in non-cirrhotics [51,68,69]. When HCC incidence rates are higher, increase in life expectancy will be even more substantial. Cost-effectiveness of surveillance largely depends on the proportion of patients eligible to receive potentially curative therapy. Therefore, cut-off for cost-effectiveness is lower in non-cirrhotic patients.

Clinical features

Currently, more HCCs are being diagnosed at early stages, in which most HCCs are still asymptomatic [7]. When symptoms develop, typical manifestations are right upper quadrant abdominal pain, weight loss, early satiety and/or palpable mass. These symptoms often indicate large tumors. Another common presentation of HCC is sudden hepatic decompensation in cirrhotic patients. Tumor rupture, a severe complication, is rare but may occur in large vascular tumors, spontaneously or after minor bunt abdominal trauma. Patients present with severe abdominal pain, vascular collapse and signs of peritoneal irritation [70].

Figure 1: Diagnostic algorithm for suspected hepatocellular carcinoma. MRI= magnetic resonance imaging; CT= computed tomography; HCC=hepatocellular carcinoma.



Diagnostics and staging

In the majority of patients diagnosis of HCC can be made with the aid of noninvasive imaging tests. Once a hepatic nodule has been detected, management depends on its size (Figure 1: Diagnostic algorithm). For lesions with a diameter <1 cm, close follow-up in recommended. When lesions exceeds 1 cm, one or two imaging techniques with typical findings (contrast-enhanced CT or MRI), depending on size and which guideline is used, is required to made the diagnosis. Typical required imaging features of HCCs in cirrhotic livers include enhancement in arterial phase and washout in portal phase compared to the non-affected liver parenchyma. The characteristic pattern of HCC is determined by the dominant arterial blood supply of the tumor compared to the background of liver parenchyma. These non-invasive diagnostic criteria are only valid in patients with cirrhosis. Diagnostic biopsy should be considered for hepatic nodules with atypical imaging features or in absence of cirrhosis [51,52,54,71]. A low risk of tumor seeding along the needle track is reported [72]. In general, diagnosis and managment should be discussed by a multidisciplinary team.

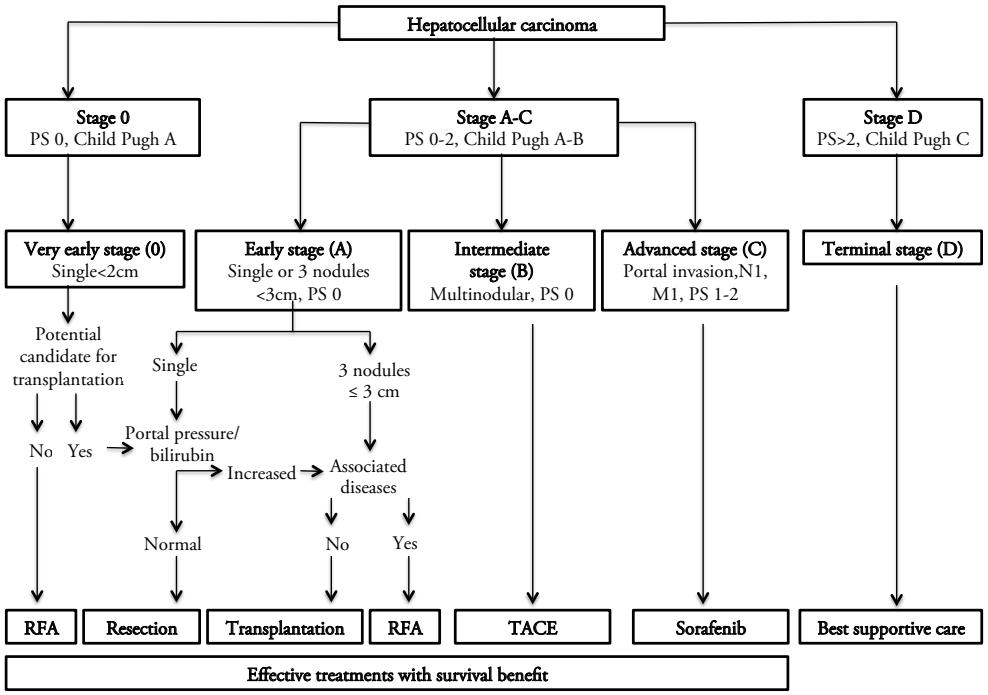
The Barcelona Clinic Liver Cancer (BCLC) Staging System is most widely used for HCC staging and combines tumor burden, hepatic function, and performance status. BCLC tumor stage guides treatment decisions and is strongly related to prognosis [9,73-75]. The system identifies those with (very) early HCC (stage 0 and A) who may benefit from curative therapies, those at intermediate (stage B) or advanced disease stage (stage C) who may benefit from palliative treatments, as well as those at end-stage (stage D) with a very poor life expectancy [51,75].

Treatment

Treatments for HCC can be divided into curative and palliative treatments. Patients should be selected carefully for a treatment that combines high effectiveness with the lowest risk of complications. Treatment decisions should be based on tumor burden, degree of underlying liver dysfunction, comorbidity and performance status (Figure 2). Liver resection and transplantation are the most important curative treatment options for HCC. In non-cirrhotic patients without comorbidity and an (very) early stage HCC, resection is the treatment of choice, because this group of patients tolerates major resections with low morbidity. On the other hand, careful selection is required in cirrhotic patients to diminish risk of postoperative liver failure. Furthermore, due to the persistent underlying liver disease these patients are at risk for developing new HCCs [51,52].

Portal hypertension and/or increased bilirubin are associated with a relatively higher risk of postoperative liver decompensation and mortality. According to recommendations of current HCC guidelines, presence of portal hypertension and/or increased bilirubin are absolute contra-indications to perform liver resection. However, these recommendations have been a matter of debate in the literature. Presence of portal hypertension should not be an absolute contra-indication to surgery [76,77]. Instead, it should be more interesting to understand prognostic differences between surgery and other loco-regional

Figure 2: Treatment options in cirrhotic patients according the BCLC staging system.



therapies in HCC patients with significant portal hypertension. Also, large HCCs can be treated successfully by surgical resection in selected patients without cirrhosis [78].
Liver transplantation is the first choice of treatment for patients with a single HCC ≤5 cm or ≤3 nodules all ≤3 cm without vascular invasion or extrahepatic spread (known as the Milan criteria [79]) who are not suitable for resection. Transplantation can cure both the HCC and the underlying cirrhosis. Unfortunately, due to the limited availability of organs the waiting list causes a delay for transplantation with a risk of tumor progression. Therefore, other treatments (mentioned below) are often applied aiming to delay tumor progression during this period.
In the last few years, new local treatment strategies for HCC -most in palliative setting- have been introduced: radiofrequency ablation (RFA), transarterial chemoembolization (TACE) and transarterial radioembolization (TARE). All these therapies induce tumor necrosis by different techniques. In small HCCs, RFA can also be curative. [51,52,54,80].
Finally, the multikinase inhibitor sorafenib leads to improved survival in patients with compensated liver disease (mainly Child-Pugh class A) and advanced HCC. Median survival benefit is approximately three months compared to placebo [81,82].

Prognosis

In the past decades, overall survival of HCC patients has improved [4,7]. However, despite the introduction of HCC surveillance and new treatment strategies in the past decade, most patients still present at relatively late stage, without options for curative treatment. Survival remains poor under these circumstances (median survival: BCLC stage B, 20 months; BCLC stage C, 11 months; BCLC stage D <3 months). Patients with a very early or early stage HCC (BCLC stage 0 and A) have a significant better survival: in patients who underwent curative treatment (resection, liver transplantation or RFA) 5-year survival ranges between 40%-70% [51,52].
Prognosis is influenced by tumor related variables (tumor size, number of nodules, vascular invasion and extrahepatic spread), liver function (defined by Child-Pugh score) and performance status. All these variables are included in BCLC staging system.
Because of the large heterogeneity in patients with intermediate stage disease, a panel of experts proposed a sub-classification of BCLC B patients to facilitate treatment decisions [83]. According to this proposal, BCLC B patients were re-classified into 4 subgroups based on liver function impairment (i.e. Child Pugh score), tumor burden (i.e. beyond Milan and within the "up-to-seven" criteria) and performance status. Based on this sub-classification TACE would not always be the first treatment option. For example, in patients with large lesions TARE can be considered. Furthermore, liver transplantation might be even considered in subgroup of BCLC B patients (with Child Pugh score 8 or 9), since life expectancy may be dominated by the cirrhotic liver dysfunction, rather than by tumor progression [83,84].

Outline of thesis

As outlined above, there are several important issues in current practice that need to be further explored.

PART I IDENTIFICATION OF PATIENTS AT RISK AND SURVEILLANCE FOR HEPATOCELLULAR CARCINOMA

In the first part of this thesis, we focus on high-risk patients for HCC development and HCC surveillance. In **Chapter 2**, we summarize existing evidence including ultrasound, other radiological modalities and various serum biomarkers and current international guideline recommendations on HCC surveillance. Subsequently, we aim to identify specific populations of patients with increased HCC risk (**Chapters 3 and 4**). In **Chapters 5 and 6**, we evaluate effects of surveillance in "real life" clinical practice and patients' perceptions regarding surveillance.

PART II ABSENCE OF CIRRHOSIS IN PATIENTS WITH HEPATOCELLULAR CARCINOMA

Although HCC typically occurs in patients with underlying cirrhosis, HCC can also develop in non-cirrhotic livers. Absence of cirrhosis in HCC patients is the focus of the second part of this thesis. First, we aim to evaluate prevalence of HCC in absence of cirrhosis in the Netherlands, to identify associated risk factors and to compare treatment and survival between cirrhotic and non-cirrhotics patients (**Chapter 7**). In **Chapter 8**, we focus on presence of NAFLD (steatosis versus steatohepatitis) in non-cirrhotic HCC patients.

PART III HEPATOCELLULAR CARCINOMA IN THE NETHERLANDS

In the final part of this thesis, we discuss presence and treatment of HCC in the Netherlands. First, we aim to evaluate trends in diagnostics, treatment and outcome of HCC patients in the Netherlands, to gain more insights in the Dutch situation (**Chapter 9).** In **Chapter 10**, we aim to investigate impact of hospital type and volume on HCC outcomes.

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CHAPTER 2

SURVEILLANCE FOR HEPATOCELLULAR CARCINOMA IN CHRONIC LIVER DISEASE: EVIDENCE AND CONTROVERSIES

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Abstract

Primary liver cancer is the sixth most common cancer in the world and the third cause of cancer-related death. Hepatocellular carcinoma (HCC) represents more than 90% of primary liver cancers and generally occurs in patients with underlying chronic liver disease such as viral hepatitis, hemochromatosis, primary biliary cirrhosis and non-alcoholic steatohepatitis. Especially cirrhotic patients are at risk of HCC and regular surveillance could enable early detection and therapy, with potentially improved outcome. We here summarize existing evidence for surveillance including ultrasound, other radiological modalities and various serum biomarkers, and current international guideline recommendations for surveillance. Ultrasound and alpha–fetoprotein (alone or in combination) are most frequently used for surveillance, but their sensitivities and specificities are still far from perfect, and evidence for surveillance remains weak and controversial. Various other potential surveillance tools have been tested, including serum marker as des-carboxyprothrombin, lectin-bound alpha-fetoprotein, and (most recently) circulating TIE2-expressing monocytes, and radiological investigations such as CT-scan or MRI-scan. Although early results appear promising, these tools have generally been tested in diagnostic rather than surveillance setting, and in most cases, no detailed information is available on their cost-effectiveness. For the near future, it remains important to define those patients with highest risk of HCC and most benefit from surveillance, and to restrict surveillance to these categories.

Introduction

Primary liver cancer is the sixth most common cancer in the world and the third cause of cancer-related death. Hepatocellular carcinoma (HCC) represents more than 90% of primary liver cancers and generally occurs in patients with underlying chronic liver disease. Incidence rates are highest in East Asia and Sub-Saharan Africa, where approximately 85% of all cases occur. The high HCC risk in these regions can be explained at least in part by the high prevalence of underlying risk factors, especially chronic hepatitis B virus (HBV) infection and aflatoxin B1 in the diet [1,2].

Nevertheless, also in countries with relatively low incidence rates such as the USA, incidences have doubled over the last two decades, due to growing impact of chronic liver disease from hepatitis C virus (HCV) and non-alcoholic steatohepatitis (NASH) [3,4]. In Europe, there is a mixed pattern, with increasing or decreasing incidence rates in various countries. These geographical differences are thought to be the consequence of changing patterns of underlying risk factors for HCC, such as viral hepatitis, alcohol abuse and NASH. Although NASH conveys relatively modest risks of HCC, the burden of metabolic syndrome and insulin resistance is expected to have significant further impact on HCC incidence in the Western world in the near future. Also, coexistent metabolic syndrome and obesity further increase HCC risk in patients with other underlying liver diseases [5-7]. Smoking is another cofactor leading to increased HCC risk [7]. Interestingly, the use of cholesterol synthesis inhibitors and metformin and coffee consumption are associated with decreased HCC risk [8-11].

New local treatment options such as radiofrequency ablation (RFA) or transarterial chemo- and radioembolisation (TACE and TARE) have been introduced in recent years. Also, the multikinase inhibitor sorafenib leads to improved survival in patients with advanced stage disease [12]. Nevertheless, most patients present at relatively late stage, without options for curative treatment. Survival remains poor under these circumstances (median survival: Barcelona Clinic Liver Cancer (BCLC) stage B, 15 months; BCLC stage C, 6 months; BCLC stage D, <3 months) [13]. Therefore, new strategies are urgently needed to decrease the burden of HCC. First, primary prevention of HCC can be achieved by hepatitis B vaccination, especially in endemic countries [14]. Second, antiviral therapies appear to be associated with decreased HCC risks in patients with chronic viral hepatitis. Interestingly, according to recent meta-analyses and systematic reviews, interferon-based antiviral treatment for hepatitis B is associated with only modest decrease of HCC risk (relative risk (RR) 0.66, 95% confidence interval (CI): 0.48–0.89). Treatment with the nucleoside analog lamivudine is associated with a more substantial risk reduction (RR 0.22, 95% CI: 0.10–0.50) [15-17]. It is reasonable to expect even better results with the currently available potent nucleos(t)ide analogs tenofovir and entecavir [18].

Third, and focus of the current review, surveillance of patients at increased risk because of underlying chronic liver disease might detect HCC at earlier stages, and could lead to better

outcomes with decreased mortality (5-year survival with curative treatment in BCLC stage 0 or A: 40-70%) [13]. Intuitively, this approach is attractive. Indeed, several international guidelines currently advise surveillance programs, defined as the repeated and systematic administration of a screening test, including registration and patient recall where applicable [19-21]. Nevertheless, surveillance remains controversial because of limited evidence for its efficiency and potential risk of side effects (for example complication from diagnostic biopsy of liver mass detected during surveillance which subsequently proves to be benign) [22,23]. This review offers an overview of current knowledge on HCC surveillance and gives a perspective on current international guidelines.

Biomarkers for HCC surveillance

When introducing a surveillance program, applying the optimal screening modality is essential. Detection of HCC at intermediate or advanced stage (according to Barcelona Clinic Liver Cancer Staging (BCLC) system) by surveillance could have some impact, by allowing TACE or sorafenib therapy. Nevertheless, surveillance should preferably detect (very) early stage HCC (single lesion ≤5 cm or ≤3 lesions each ≤3 cm without vascular involvement or metastasis), allowing potentially curative therapy. Serological biomarkers can be used at relatively low costs, without burden for the patient. Several biomarkers have been investigated for HCC detection, generally in diagnostic setting rather than in surveillance studies.

Alpha-fetoprotein (AFP) is the most frequently used biomarker for HCC worldwide. AFP is a glycoprotein expressed by fetal hepatocytes or poorly differentiated HCC cells. Nevertheless, not all HCC cells secrete AFP into the circulation. Also, serum AFP levels may be elevated in patients with chronic liver disease in the absence of HCC (related to height of transaminases) and in patients with other malignancies [24]. Gupta *et al* [25] published a systematic review with five included studies on diagnostic value of AFP for detecting HCC (all stages combined) in cirrhotic and non-cirrhotic HCV patients. They concluded that even if one assumes best-case estimates of sensitivity and specificity, the case for surveillance by AFP remains weak. By using the usual cut-off point of 20 ng/mL, sensitivities and specificities for detecting all stages of HCC were 41-65% and 80-94%, respectively. Positive and negative likelihood ratios ranged from 3.1 to 6.8 and from 0.4 to 0.6, respectively. In a recent large case-control study with 419 included HCC patients and 417 cirrhotic controls (with various underlying etiologies of liver disease), the performance of AFP in early stage HCC was compared with other biomarkers, such as des-carboxyprothrombin (DCP) and lectin-bound AFP, with more encouraging results [26]. In this study, diagnosis of HCC was based on current criteria for diagnosis, with appropriate blinding and 6-months post-enrollment follow-up for controls to eliminate false negatives. Sensitivity and specificity of AFP for detecting early stage HCC were 53% and 90%, respectively, using the currently recommended clinical cut-off point of 20 ng/mL. When the point in the Receiver Operating Characteristics

(ROC) curve with optimal sensitivity and specificity was used as cut off (10.9 ng/mL), sensitivity was 66% and specificity 82%. This finding would suggest that the usual cut off of 20 ng/mL is too high for optimal performance of AFP surveillance. In a previous small case-control study with 170 HCC patients (68 early stage HCC) and 170 matched cirrhotic controls, the cut off of 20 ng/mL appeared to exhibit optimal sensitivities and specificities [27]. Nevertheless, at this cut off, sensitivities and/or specificities were often insufficient in other studies [28,29].

Des-carboxyprothrombin (DCP) is an abnormal protein generated as a result of an acquired defect in the posttranslational carboxylation of the prothrombin precursor in malignant liver cells [30]. Several studies investigated the performance of DCP as biomarker for early HCC, with unconclusive results [26,31-34]. For example, in a case-control study with 39 HCC patients and 77 matched controls, DCP had a greater accuracy than AFP [34]. DCP testing alone had a sensitivity and specificity of 74% and 86%, respectively, using a cutoff value of 40 mAU/mL. Another study reported much lower sensitivities for DCP with the best performance in chronic viral hepatitis [26].

Another potential biomarker for HCC is lectin-bound AFP: One of the three glycoforms of AFP, based on its reactivity in lectin affinity electrophoresis [35]. In a multicenter prospective study, the diagnostic accuracy of lectin-bound AFP versus AFP was compared in patients with HCV-related cirrhosis [36]. The prevalence of HCC at baseline and during the two years of follow-up was significantly higher in patients with elevated lectin-bound AFP than in those with elevated AFP. The relatively high prognostic value of lectin-bound AFP was even higher in patients with concomitantly elevated AFP levels. This suggests that lectin-bound AFP has some clinical utility as secondary test in HCV patients with mildly elevated AFP levels, by identifying a subgroup with a relatively high likelihood of HCC. Nevertheless, this study has important limitations, such as a relatively short follow-up and potential selection bias. Also, other investigators report less encouraging results. For example, in the previously mentioned case-control study of Marrero *et al* [26], sensitivity of lectin-bound AFP for detecting early HCC was only 37%. Several studies investigated the diagnostic performance of a combination of serum biomarkers. Nevertheless, when combining AFP and DCP, there appeared to be only little or no improvement in sensitivity rates for detecting early stage HCC [26,33,34].

Recently, new serum biomarkers for HCC have been suggested. Tyrosine kinase with Ig and EGF homology domains 2 (TIE2) is a receptor of angiopoietins. TIE2-expressing monocytes (TEMs) were recently reported to be enriched in HCC and other tumors where angiogenesis is known to be important for tumor progression. In a recent publication on 168 HCV infected patients (89 with HCC), frequency of circulating TEMs in periferal blood was significantly higher in case of HCC, and independent of tumor stage [37]. TEMs were also increased in a separate group of non-HCV HCC patients. Performance of TEMs in discriminating HCC from chronic hepatitis or cirrhosis was superior to AFP or DCP (sensitivities 86% and 71% respectively; specificities 81% and 90% respectively.

Nevertheless, another study found increased circulating and intrahepatic TEMs in HCV patients without HCC [38]. Although these findings relate to a relatively small cohort of HCV-infected patients, they raise concern that mobilization and expansion of TEMs may not be strictly HCC-driven, but more generally associated with chronic liver infection [39]. Several other studies investigated the performance of Glypican-3 (GPC3). GPC3 is a surface protein expressed in high percentages of HCCs, whereas it is not detectable in hepatocytes from normal subjects or patients with benign liver disease [40-42]. Another potential marker is Golgi protein 73 (GP73): An amino acid that normally remains in the Golgi complex. Marrero *et al* [43] reported that levels of GP73 are increased in serum of patients with HCC. In this study, sensitivity of GP73 for detecting early HCC was 62%. Also, interleukin-6 (IL-6) has been studied as potential marker for HCC. IL-6 is a cytokine involved in cell growth and differentiation. Serum IL-6 concentrations appeared to be increased in HCC patients (all stages combined) compared to controls [44,45]. Sensitivities of IL-6 for discrimination between HCC patients (all stages combined) and controls ranged from 46% to 73% and specificities from 87% to 95%. Also, levels of squamous cell carcinoma antigen (SCCA: A component of serine protease inhibitors) appeared to be significantly higher in HCC patients than in controls [46,47]. It remains to be seen, whether these new serum biomarkers will provide satisfactory results in the setting of surveillance in clinical practice.

Ultrasonography for HCC surveillance

Currently, ultrasonography (US) is the most widely used method for HCC surveillance. US is not invasive, but time-consuming, relatively expensive and operator-dependent. Also, this investigation is often not suitable in case of overweight. According to a recent meta-analysis with 13 included studies by Singal *et al* [48], pooled sensitivities and specificities for detecting HCC at any stage were both 94%. However, US was less effective for detecting early stage -potentially curable- HCC, with a pooled sensitivity of 63% (95% CI: 49-76).

Another systematic review concluded that US is insufficiently sensitive for HCC surveillance [49]. Sensitivities for detecting HCC (all stages combined) of the 14 included studies ranged from 30% to 100% and specificities from 73% to 100%. Possible explanations for the large variability between studies are differences in operator skills and experience, in tested populations and/or in tumor size.

The potential benefit of combining US with AFP for detection of early stage HCC was also explored in the previously mentioned meta-analysis by Singal *et al* [48]. The pooled sensitivities increased from 63% to 69% (95% CI: 53–81), but this was not statistically significant (P=0.65). This result suggests that adding AFP to ultrasound is not very useful for HCC surveillance.

The optimal interval for ultrasonographic surveillance is not known. It should be based on rate of tumor growth up to the limit of its detectability: Available evidence on tumor

growth suggests that the interval from undetectable to a two cm diameter lesion ranges from four to twelve months [50]. According to the meta-analysis by Singal *et al* [48] mentioned above, US sensitivities for detecting early-HCC may be improved by US at 6-month intervals compared to surveillance intervals between six and twelve months (pooled sensitivities: 70% versus 50%, P=0.001). However, confidence intervals of pooled sensitivities were overlapping (95% CI: 55.6–84.6 versus 40.0–59.2).

Two recent retrospective cohort studies from Italy and Korea report that HCC is detected at earlier stage, with curative therapy more often applied and better survival in case of surveillance interval ≤6 months compared to longer surveillance intervals, even after correction for lead time bias [51,52].

A recent randomized control trial investigated whether a 3-month interval of US surveillance was more effective than a 6-month interval [53]. More focal lesions <10 mm were found in the group with 3-months surveillance (5-year cumulative incidence: 41% versus 28%, P=0.002). Nevertheless, 44% of all focal liver lesions detected during surveillance remained indeterminate. No differences in detection rates of small HCCs eligible for curative treatment were observed between the two randomized groups. Inadequate compliance occurred in approximately 10% of both groups. Also, overall 5-year survival rates were similar in both groups (85% versus 86%, P=0.38).

Finally, a recent cluster-randomized trial from Taiwan compared 4-month and 12-month intervals in chronic HBV or HCV patients with thrombopenia. Although tumors were smaller in the group with 4-month intervals, and curative treatment modalities more often applied, 4-year overall survival did not differ [54].

Other imaging techniques for HCC surveillance

Computed tomography (CT) and magnetic resonance (MR) imaging are potential tools for HCC surveillance. Until now, these imaging modalities are mainly used for further evaluation in case of abnormal findings with ultrasonographic surveillance and to determine extent of disease. Test characteristics of CT and MR imaging reported below are therefore all based on diagnostic studies rather than in a setting of surveillance. According to a systematic review of Colli *et al* [49] (studies in the period: 1996-2004 included), spiral CT imaging appeared to offer comparable sensitivities and specificities as US for detecting HCC (all stages) in patients with chronic liver disease. The pooled sensitivities for US and spiral CT imaging were 60% versus 68% and the pooled specificities 97% versus 93%. In the same systematic review, the reported pooled sensitivities (81%) and specificities (85%) of MRI-scan were, respectively, higher and lower than those obtained with US or CT imaging.

However, diagnostic accuracies of CT-scan and MRI-scan have increased in the past decade due to improvement of techniques [55-58]. For example, according to a recent retrospective study, the overall sensitivities of triple-phase multidetector CT (MDCT) imaging for detecting HCC (all stages combined) ranged between various observers

from 78% to 81% [57]. Sensitivity improved with increasing HCC diameter. Also, in another recent study with prospective design, the diagnostic performances of US, MDCT imaging and contrast-enhanced MRI-scan were compared in a population of cirrhotic candidates for liver transplantation [58]. Dynamic MRI-scan with inclusion of the hepatobiliary phase had the highest accuracy with sensitivities for detecting early stage HCC, ranging from 59% to 85%. In contrast to CT imaging, MRI-scan is not associated with radiation exposure. However, MRI-scan is costly and there are no data from clinical practice to support the use of MRI-scan for surveillance.

Surveillance efficiency

The aim of surveillance is to decrease HCC-related mortality. Unfortunately, high-level evidence is limited in this respect. A recent Cochrane review [59] identified only one study with data on mortality: Zhang *et al* [60] performed a large cluster randomized controlled trial in which a policy of surveillance versus no surveillance was compared in 18,816 patients with current or prior HBV infection or a history of chronic hepatitis. There proved to be a survival benefit for the strategy of a 6-monthly surveillance with combined AFP and US compared to the no surveillance strategy. Despite poor adherence to surveillance (58%), HCC-related mortality rates were significantly lower in the surveillance group than in the disease control group (83.2/100,000 and 131.5/100,000, respectively, odds ratio (OR) 0.63). Since this study was performed in chronic HBV patients in China, it is not clear whether its results can be extrapolized to the Western World. Also, according to the Cochrane review, there are some discrepancies between this publication and earlier publications about the same trial [61-63]. For example, numbers of disease controls and total numbers of participants dropped from 9711 and 19,144, respectively, in the initial preliminary publication in 1997 [62] to 9443 and 18,816, respectively, in two later publications in 1999 and 2004, after completion of the trial[60,61]. Also, confidence intervals were computed as if cohorts had been randomly assigned for each individual patient, without taking the cluster randomization into account and statistical significance was claimed with a 95% CI that was only borderline significant (95% CI: 0.41-0.98) [22]. In another large randomized controlled trial from China, the effectiveness of surveillance by 6-monthly AFP measurement in 5581 HBV carriers was investigated [29]. HCC-related mortality rates were not significantly different in the surveillance group compared to the control group (1138/100,000 and 1114/100,000, respectively, P=0.86).

At lower evidence level, several cohort studies suggest that survival is improved with HCC surveillance [64,65]. In a population-based study of Alaska natives, 1487 HBV carriers had surveillance with AFP at 6-month intervals [64]. In case of elevated AFP, US was performed. The long-term survival rate for patients whose HCCs were detected by the surveillance program was compared with a historical disease control group of patients with HCC: Survival rates were significantly higher for patients with HCC

detected by surveillance (5-year survival rates: 42% and 0%, respectively, *P*=0.008; 10-year survival rates: 30% and 0%, respectively, *P*=0.07). Similar results were found in the study of Wong *et al*[65]. In this study, 56 HCC patients were retrospectively divided in three groups according to their initial presentation: Symptomatic patients presenting with abdominal pain, mass, bleeding, or weight loss; asymptomatic patients who had ultrasound for abnormal liver enzyme levels or other (unrelated) indications; and asymptomatic patients with underlying chronic liver diseases in a surveillance program. Patients in the surveillance group survived significantly longer than those in the symptomatic group (median survival of 1300 versus 234 days, *P*=0.009). Median survival of the asymptomatic group without surveillance did not differ significantly from the other groups. However, lead time bias due to disease detection in an early stage could have affected the results of the two previously mentioned studies. Also, length time bias could have biased results by preferential detection of slowly growing lesions that are more likely to remain asymptomatic until late in disease course.

Several studies indicate that surveillance programs could lead to more frequent detection of HCC at early stages, when curative treatment is still possible [60,61,66,67]. In the previously mentioned large randomized controlled trial by Yang *et al* [61], more resectable HCC cases were detected in the surveillance group than in the disease control group during five years follow-up (OR 7.14; 95% CI: 3.53-14.43). In the later publication about the same trial, Zhang *et al* [60] reported similar results. Again, detection rates of small HCC (defined as a tumor diameter <5 cm) were higher in the surveillance group than in the disease control group (45% versus 0%, *P*<0.01).

In the systematic review by Gebo *et al* [66] covering publications in the period: 1996-2002, one prospective cohort study was identified that investigated HCC surveillance in HCV patients. This study by Solmi *et al* [67], compared 360 patients in the surveillance group (combined US and AFP measurements at 6-month intervals) with a disease control group of 2170 patients who received usual care in other hepatology clinics. During a mean follow-up of 56 months, the incidence of HCC in the surveillance group was 6.7% and 5.5% in the disease control group. Of note, in the surveillance group, 75% of the HCC's was unifocal and ≤3 cm in diameter compared to only 16% in the disease control group (*P*=0.000).

Success of a surveillance program depends in general not only on the surveillance modality or target population. Recall strategy and adherence to follow-up are also important factors. In a large retrospective cohort study in the USA, 1873 HCC patients with a prior diagnosis of cirrhosis were identified in the period: 1994-2002 [68]. In the three years before HCC diagnosis, 17% received regular surveillance, 38% received inconsistent surveillance and 45% no surveillance. In the regular surveillance group, 52% received both US and AFP, 46% AFP only and 2% US only. In a subset of 541 patients in whom cirrhosis was diagnosed at least three years prior to HCC, 29% received regular surveillance and 33% inconsistent surveillance. In another prospective

cohort study, 1005 HCV patients were included in a surveillance program with combined US and AFP at 6- to 12-month intervals [69]. During a mean follow-up of 6.1 years, 83 HCC cases were detected: 28% of those were tumors outside Milan criteria. 70% of patients with HCCs outside Milan criteria had experienced consistent surveillance and follow-up, which was not different from the total study group. Only in a minority of patients, absence of surveillance (13%) or follow-up (17%) could explain failure to detect patients at earlier stages. On multivariate analysis, study site was a strong independent predictor of consistent surveillance ($P<0.001$). After correction for study site, also platelet counts >150x106/mL and complete clinic visit adherence were positively associated with consistent surveillance.

Target populations for surveillance

Most HCCs develop in patients with chronic liver diseases. The decision to start surveillance should depend on the chance of developing HCC, i.e. the incidence of HCC in specific populations. Especially high-risk patients should be included in surveillance programs. However, selection of these patients remains a subject of debate. Also, it is important to decide, prior to surveillance, whether the clinical condition of the patient would allow any therapy in case of HCC detection.

Computer analyses in hypothetical cirrhotic patients

*S*everal studies have investigated cost-effectiveness of HCC surveillance in cirrhotic patients based on computer analyses of theoretical models. In general, surveillance is considered cost-effective, if costs are less than $50,000 per life-year or quality-adjusted life-year (QALY) gained. According to Sarasin *et al* [70], surveillance with combined US and AFP measurements at 6-month intervals in patients with compensated cirrhosis results in an increase of life expectancy of three months when HCC incidence is 1.5%/year. However, in this case cost-effectiveness ratio of $55,264 per life year gained exceeds the generally accepted threshold of $50,000 per life year gained. When HCC incidence rates are higher, increase of life expectancy will be even more substantial. Cost-effectiveness ratios of systematic surveillance range between $26,000 (HCC incidence: 6%/year) and $55,000 (HCC incidence: 1.5%/year) for each additional life-year gained, in best case scenarios. Another study, using similar analyses in HCV cirrhotics, suggested that HCC surveillance with combined three-phase CT-scans and AFP measurements at 6-month intervals was more cost-effective than other surveillance strategies [71]. This strategy was associated with an incremental cost-utility ratio of $25,232/QALY compared to no surveillance. These results are based on estimated HCC incidences of 1.4%/year in patients with compensated cirrhosis and 4%/year in patients with decompensated cirrhosis.

Lin *et al* [72] suggested that combined US and AFP measurements at 6-month intervals was the most effective surveillance strategy in HCV patients with compensated cirrhosis

compared to other surveillance strategies (estimated annual HCC incidence: 0.02%-0.1%). However, this strategy entailed higher additional cost per QALY or life-year gained compared to a strategy of annual US and AFP measurements (incremental cost-effectiveness ratio: $106,871/QALY) or no surveillance (incremental cost-effectiveness ratio: $129,915/QALY). In this computerized decision model, surveillance with US at 12-month intervals and AFP measurements at 6-month intervals offered the greatest gain in life-expectancy, while still maintaining a cost-effectiveness ratio <$50,000/QALY or life-year gained, regardless of HCC incidence.

Another study in a hypothetical mixed-etiology cohort of compensated cirrhotic patients suggested that combined AFP measurement and US imaging on a 6-monthly basis is the most effective surveillance strategy [73]. Compared to no surveillance, this strategy is estimated to increase the numbers of HCCs resectable at time of diagnosis more than three-fold with 50% decrease of HCC-related deaths. Significantly more small and medium-sized HCCs (diameter <2 cm or diameter 2-5 cm, respectively) would be identified by surveillance. However, when costs were taken into account, it was doubtful whether US should be routinely offered to those with serum AFP levels ≤20 ng/mL (>$45,000/QALY). Also, cost-effectiveness varied considerably depending on type of underlying chronic liver disease. In this computerized decision model, annual HCC incidence ranged from 1.2% to 4.1%.

HCC incidence in cirrhotic patients

Annual incidence rates of HCC in specific populations with cirrhosis have been investigated extensively. Cirrhotic HBV patients have an increased risk for developing HCC. Studies that included only East Asian cirrhotic HBV patients showed HCC incidence rates around 3.2 per 100 person-years and 5-year cumulative HCC incidence of 15% [74,75]. Studies in European HBV cirrhotics reported lower incidence rates: 2.2 per 100 persons-years and 5-year cumulative HCC incidence of 9% [76-79].

Several studies have investigated the risk of developing HCC in HCV patients [76,80-83]. In a large prospective study of 12,008 Taiwanese males, anti-HCV positive subjects had a 20-fold increased risk of developing HCC in comparison with anti-HCV negative subjects [81]. In this study, the presence or absence of cirrhosis was not evaluated. Other studies reported that especially cirrhotic HCV patients are at increased risk for developing HCC [76,80,83]. In a retrospective cohort study, the annual HCC incidence was 1.4% in European cirrhotic HCV patients [76]. Also, Degos *et al* [83] reported HCC incidence rates of 13.4% after five years follow-up in a cohort of French patients with compensated HCV-related cirrhosis. In another study from Germany, 17 of 838 HCV patients (2%) developed HCC during a mean follow-up of 50 months [80]. All HCCs occurred in cirrhotic livers. Cirrhotic patients had a 20-fold higher risk of developing HCC than HCV patients without cirrhosis at study entry (RR 20.2, 95% CI: 2.4-170.9). Also, Lok *et al* [82] reported that HCC incidence was higher in HCV

Table 1: Characteristics and results of studies on risk of hepatocellular carcinoma in patients with autoimmune hepatitis, alpha 1-antitrypsin deficiency and Wilson's disease.

Ref.	Study design	Country	Study period	Patient no.	Duration follow-up	Results
Autoimmune hepatitis						
Yeoman *et al* [110]	Prospective cohort study	UK	1971-2007	243	median: 11 yrs (range 1-36)	Annual HCC incidence: 1.1% HCC occurred more often in cirrhotic patients (9.3% versus 3.4%, *P*=0.048)
Wang *et al* [111]	Prospective cohort study	USA	Unknown	124	mean: 111 ± 6 months	HCC incidence: 1 per 350 patient-years HCC incidence in cirrhotics: 1 per 182 patient-years
Werner *et al* [112]	Retrospective cohort study	Sweden	1990-2003	473	median: 8.8 yrs (range 1-45)	23-fold increased HCC risk compared to the general population. Only HCC in cirrhotics.
Wong *et al* [113]	Retrospective cohort study	USA	1999-2009	322	mean: 6.25 yrs	HCC incidence all patients: 459 per 100,000 patient-years (0.5%/year) In cirrhotics: 1,920 per 100,000 patient-years (1.9%/year).
Park *et al* [114]	Retrospective cohort study	USA	Unknown	212 (88 cirrhotics)	mean: 123 ± 9 months	HCC incidence in cirrhotics: 1 per 1002 patient-years. (0.1%/year).
Teufel *et al* [115]	Retrospective cohort study	Germany	1970-2009	278 (89 cirrhotics)	mean: 4.8 yrs (in cirrhotic pts)	No HCC observed in 431 cirrhotic patient-years
Alpha 1-antitrypsin deficiency						
Eriksson *et al* [116]	Autopsy study	Sweden	1963-1982	38,250 (17 pts with A1AD)	NA	Increased HCC risk in patients with A1AD compared to controls. (OR 20, 95% CI: 3.5-114.3)
Elzouki *et al* [117]	Autopsy study	Sweden	1963-1994	50,333 (31 pts with A1AD)	NA	Increased HCC risk in patients with A1AD compared to controls (OR 5.0, 95% CI: 1.6-15.8; *P*=0.008). Only significant in males.

Propst *et al* [118]	Retrospective cohort study	Austria	1990-1992	Group 1: 240 cirrhotics with different etiologies (25% A1AD) Group 2: 130 non-cirrhotic A1AD pts	Unknown	No significant differences in HCC prevalence between cirrhotic A1AD patients and cirrhotic subjects due to other causes. No HCC in non-cirrhotic A1AD patients
Wilson's disease						
Walshe *et al* [119]	Retrospective cohort study	UK & Sweden	1955-1987 1987-2000 1966-2002	159	range: 10-45 yrs	9 patients (6%) developed abdominal malignancies (2x HCC). Higher incidence compared to the general population
Thattil *et al* [120]	Case report & review	Switzer-land	No limitation	NA	NA	19 published case reports of HCC in patients with Wilson's disease

AIH = autoimmune hepatitis, HCC= hepatocellular carcinoma, A1AD= Alpha 1-antitrypsin deficiency, OR= odds ratio, CI= confidence interval, NA= not applicable, yrs= years, pts=patients

patients with cirrhosis than in those with bridging fibrosis (annual HCC incidence rates: 1.45% versus 0.8%, *P* for trend=0.08). This study was performed in the USA.
In patients with cirrhosis due to causes other than viral hepatitis, HCC incidence rates are generally not precisely known. Nevertheless, alcoholic liver disease is a clear risk factor for HCC. In a meta-analysis of Bagnardi *et al* [84], the consumption of alcohol was associated with an increased risk for developing liver cancer (RR 1.86, 95% CI: 1.53-2.27 for alcohol consumption of 100 gram/day compared to no alcohol). The presence or absence of cirrhosis was not evaluated in this meta-analysis. In a Danish nationwide cohort study, 169 (2%) of 8482 patients with alcoholic cirrhosis developed HCC during a median follow-up of 4.1 years [85]. Five-years cumulative HCC risk was 1.0% (95% CI: 0.8-1.3). Kuper *et al*[86] reported a relative risk of HCC of 2.4 for alcoholism alone and 22.4 for alcoholic cirrhosis compared to the general population. Several other studies also reported increased HCC risk in cirrhotic patients with alcohol as primary cause [87-91]. In a case-control study, heavy alcohol consumption contributed to a significant part of the 115 included HCC cases (32%), independent of other known risk factors [92].
Patients with cirrhosis due to genetic hemochromatosis also are at increased risk of developing HCC. According to a meta-analysis, patients with genetic hemochromatosis who are homozygous for the C282Y mutation have a 11-fold increased HCC risk compared to controls (OR=11, 95% CI: 3.7-34) [93]. In a Swedish population-based cohort study, patients with genetic hemochromatosis had a 20-fold increased risk for developing primary liver cancer compared to the general population (standardized incidence ratio: 21, 95% CI: 17–28) [94]. In both studies, the presence or absence of cirrhosis was not evaluated. Several other studies reported similar results [95-98].
Primary biliary cirrhosis (PBC) is another important risk factor for HCC development. In a recent meta-analysis, PBC patients had a 19-fold higher risk to develop HCC compared to the general population [99]. In a large Japanese cohort of PBC patients, HCC incidence was 2.4% during a mean follow up of 80 months. HCC incidence was higher in males than in females (5.1% versus 2.0%) [100]. Caballeria *et al* [101] compared HCC incidence in 140 PBC patients and a group of cirrhotic HCV patients. Cumulative HCC incidence in PBC patients was 3.6%, during a mean follow-up of 5.6 years. Incidence rates were much higher (11.1%), when considering only those patients with late stages of disease. PBC patients in stages III and IV appeared to have comparable risks for HCC as cirrhotic HCV patients. Other studies reported similar results [102-105].
The exact incidence of HCC in patients with cirrhosis due to non-alcoholic fatty liver disease (NAFLD) is not known. Nevertheless, a recent systematic review of White *et al* [106] reported that patients with NASH-cirrhosis had a consistently higher risk of HCC. Cumulative incidence rates ranged from 2.4% over 7 years to 12.8% over 3 years. In cohorts with non-cirrhotic patients with NAFLD and NASH the risk of developing

HCC was minimal: cumulative HCC mortality ranged from 0%-3% for study periods ranging from 6 to 21 years. Ascha *et al* [107] reported that annual cumulative HCC incidence in patients with NASH-cirrhosis was 2.6%, compared to 4.0% in patients with HCV cirrhosis (P=0.09). Besides, in several studies features suggestive of NAFLD were occasionally observed in HCC patients without a well-defined other cause of chronic liver disease [108,109].
Cirrhotics patients with autoimmune hepatitis (AIH) [110-115] or alpha 1-antitrypsin deficiency [116-118] seem to have an increased HCC risk. However, the HCC incidence is lower than in cirrhotics with viral hepatitis. HCC in patients with Wilson's disease seems to be rare [119,120] (Table 1). Only case-reports are available for HCC in patients with cystic fibrosis [121-123].

HCC incidence in non-cirrhotic patients

The previously mentioned cost-effectiveness analyses were all restricted to cirrhotic patients and cannot be extrapolized to non-cirrhotic patients. However, also in some subgroups of HBV carriers without cirrhosis, HCC surveillance with US and AFP could be cost-effective [19]. HCC incidence should exceed 0.2%/year under these circumstances to allow cost-effective surveillance.
A prospective population study in 22,707 Taiwanese male HBV carriers without cirrhosis showed that annual HCC incidence rate was 0.5% [124]. Annual rates increased with age. A Japanese study also reported a relatively high incidence of HCC in HBV carriers (0.4%/year) [125]. Both studies only included East Asian patients. In this population, with in general early HBV infection through vertical transmission, annual HCC incidence in non-cirrhotic carriers depends on age and starts to exceed the threshold of 0.2%/year around the age of 40 years [126]. In two large community-based prospective cohort studies from Taiwan, predictors of progressive disease in chronic hepatitis B were evaluated [127,128]. High transaminases, HBeAg positive status and high serum HBV DNA levels were risk factors for developing HCC. Nevertheless, non-Western HBV carriers remain at significant risk for developing HCC, regardless viral replication status [127,129,130]. Although HBsAg loss leads to a pronounced reduction of HCC risk, incidence remains higher that in the general population due to HBV DNA integration in the liver cell [124,131,132].
In contrast, uncontrolled prospective cohort studies in North America have indicated that the HCC incidence in HBV carriers could vary considerably [133,134]. Annual HCC incidence rates ranged between 0.06% and 0.46%. Non-Asian HBV carriers without cirrhosis are generally infected in adulthood by horizontal transmission and often exhibit low level of viral replication. They appear to be at limited risk of developing HCC [135-137]. However, HBV carriers with HCV or HIV co-infection or with a first degree relative with HCC are at increased risk for developing HCC [138-140]. Chronic HBV patients from sub-Saharan Africa often develop HCC at young age [129,141].

The HCC incidence in HCV patients without cirrhosis is not clear. A Japanese study investigated HCC incidence in HCV patients and reported that annual HCC incidences ranged from 0.5% to 7.9% in untreated chronic HCV patients with mild fibrosis and cirrhosis, respectively[142]. However, Lok *et al* [82] reported that annual HCC incidence in HCV patients with bridging fibrosis was only 0.8%. The risk of developing HCC in non-cirrhotic patients with chronic liver disease due to other causes than viral hepatitis, is not exactly known.

Recommendations of guidelines

In the last decade, several guidelines for management of HCC have been published worldwide. Most relevant are the practice guidelines of the American Association for Study of Liver Disease (AASLD) [19], the EASL–EORTC Clinical Practice Guidelines on the management of hepatocellular carcinoma [20] and the Asian Pacific Association for the Study of the Liver (APASL) consensus recommendations on hepatocellular carcinoma [21] (Table 2). The AASLD guideline recommends surveillance for selected groups of cirrhotics (viral hepatitis, alcohol, PBC, genetic hemochromatosis, alpha-1 antitrypsin deficiency) and high-risk HBV patients without cirrhosis [19]. It is stated that there is insufficient evidence to recommend surveillance in HCV patients with advanced fibrosis or non-cirrhotic NAFLD. The EASL guideline advises surveillance in cirrhotics (regardless underlying cause)[20]. Also for HCV patients with advanced fibrosis and non-cirrhotic HBV carriers with active hepatitis or family history of HCC, surveillance is recommended. Of note, according to the EASL guideline, the presence of advanced (Child-Pugh C) cirrhosis prevents potentially curative therapies to be employed, and surveillance is not cost-effective under these circumstances, except for patients on the waiting list for transplantation. In fact, Child-Pugh C cirrhosis will also exclude radiologic interventions or sorafenib in palliative setting. According to the APASL recommendations, cirrhotic patients with HBV and/or HCV should undergo surveillance [21]. Regarding modality of surveillance, the AASLD and EASL guidelines both recommend surveillance by US [19,20]. According to the APASL guideline, surveillance should be performed by combined US and AFP measurements [21]. All three guidelines recommend a surveillance interval of six months [19-21].

Conclusion

In the last decade, there has been a marked increase in therapeutic options for HCC. Nevertheless, curative options are only feasible in case of early detection. Although regular surveillance could be beneficial in this respect, there is only limited evidence for its effectiveness in clinical practice. US at 6-month intervals appears the most promising tool for surveillance, but the debate on serum tumor markers such as AFP has not yet ended. Defining those high-risk subgroups who will benefit most from surveillance remains an important research goal for the near future.

Table 2: Comparison of recommendations regarding hepatocellular carcinoma surveillance in AASLD, EASL and the APASL guidelines.

Characteristics	**AASLD guideline** [19]	**EASL guideline** [20]	**APASL guideline** [21]
Recommended target population	Cirrhotic HBV and HCV patients Alcoholic cirrhosis Stage 4 primary biliary cirrhosis Cirrhosis due to genetic hemochromatosis Cirrhosis due to alpha 1-antitrypsin deficiency HBV carriers of Asian origin (male >40 years, female >50 years) African/North American Blacks with hepatitis B HBV carriers with family history of HCC	Cirrhotic patients with Child-Pugh stage A and B Cirrhotic patients with Child-Pugh stage C awaiting liver transplantation Non-cirrhotic HBV carriers with active hepatitis or family history of HCC Non-cirrhotic patients with chronic hepatitis C and advanced liver fibrosis F3	Cirrhotic HBV and HCV patients
Surveillance benefit uncertain	HBV carriers younger than 40 (males) or 50 (females) Hepatitis C and stage 3 fibrosis Non-cirrhotic NAFLD		
Surveillance modality	US	US	US and AFP
Interval	6 months	6 months	6 months

HBV=hepatitis B virus; HCV= hepatitis C virus; NAFLD= non-alcoholic fatty liver disease; US=ultrasound, AFP= Alpha-fetoprotein; HCC=hepatocellular carcinoma

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CHAPTER 3

NO INCREASED RISK OF HEPATOCELLULAR CARCINOMA IN CIRRHOSIS DUE TO WILSON DISEASE DURING LONG-TERM FOLLOW-UP

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Abstract

Background and aims: Data on risk of hepatocellular carcinoma (HCC) in patients with Wilson disease are scarce. We determine HCC risk in a well-defined cohort of Wilson patients**.**

Methods: All patients with a confirmed diagnosis of Wilson disease (Leipzig score ≥ 4) in three Dutch university referral hospitals were included in this retrospective cohort study. End of follow-up was defined as date of diagnosis of HCC, liver transplantation, death or last available hospital visit. Also, a meta-analysis was performed to determine incidence and mortality rate of HCC in Wilson disease based on all published cohorts.

Results: In total, 130 patients with Wilson disease were followed during a median follow-up of 15 years (range 0.1-51.2). At baseline, cirrhosis was present in 74 patients (57% of total: 64% compensated and 36% decompensated). At end of follow-up, liver disease severity was improved, stable or deteriorated in 20%, 46% and 24% of all cases (10% unknown), respectively. Two patients developed HCC (one despite excellent decoppering after 50 years follow-up, the other with newly diagnosed Wilson disease). Estimated annual HCC risk for all patients was 0.09% (95% confidence interval (CI): 0.01-0.28). Subgroup analysis in cirrhotic patients revealed an annual HCC risk of 0.14% (95% CI: 0.02-0.46). The meta-analysis showed an annual HCC risk of 0.04% (95% CI: 0.01-0.10) and HCC mortality rate of 2.6/10,000 person-years (95% CI: 0.7-7.0).

Conclusions: Even in case of cirrhosis, HCC risk is low in Wilson disease. Our data do not support regular HCC surveillance in Wilson disease.

Introduction

Wilson disease (WD) is a rare autosomal recessive disorder of copper transport. Mutations in the ATP7B gene (encoding for a copper-transporting P-type ATPase (ATP7B) that is mainly expressed in hepatocytes and functions as a transmembrane copper transporter) lead to imperfect formation of holoceruloplasmin and decreased copper secretion into bile. As a result, there is accumulation of copper in the liver, brain and other organs. Clinical presentation varies widely. In symptomatic patients hepatic and/or neurologic disease are most common. Asymptomatic patients are most often detected by family screening.

In general, patients with cirrhosis are at significant risk for hepatocellular carcinoma (HCC). Therefore, the European Association for the Study of the Liver (EASL) guideline advises HCC surveillance by bi-annual ultrasound, regardless the underlying cause of cirrhosis [1].

There is substantial evidence favoring surveillance in case of hepatitis B or C, and to a lesser extent, in case of cirrhosis due to alcohol, primary biliary cirrhosis or hemochromatosis [2]. However, strong evidence is lacking for many other cirrhotic conditions. The decision to start surveillance should depend on risk of developing HCC. Cost-effectiveness studies have indicated that HCC surveillance becomes cost-effective in patients with cirrhosis when HCC incidence exceeds 1.5%/year [3,4].

Although liver cirrhosis is an important complication in patients with WD, published data on the risk of HCC in these patients are scare. In the current study we therefore determined the risk of HCC in a well-defined cohort of patients with WD and related HCC risk to severity of liver disease and the efficacy of decoppering treatment.

Patients and methods

All consecutive adult patients with a diagnosis of WD in the database of the three university hospitals (University Medical Center Utrecht, Erasmus Medical Center Rotterdam and University Medical Center Groningen) with referral function and caring for the large majority of WD patients in the Netherlands were included in this retrospective study. Patients generally visited the out-patient clinic at 6-month intervals, and more often if clinically indicated. Although no formal HCC surveillance was provided, radiological imaging (ultrasound, MRI) was performed often at one-year interval in cirrhotic patients. The database does not include patients under current care of pediatricians. Diagnostic criteria for WD according to Leipzig criteria [5], endorsed by the recent EASL guideline on diagnostics and treatment of Wilson disease [6], were evaluated for all patients. These diagnostic criteria include the presence of Kayser-Fleischer rings, neurologic symptoms, a low ceruloplasmin level (<0.2 g/L), Coombs-negative hemolytic anemia, an increased basal urinary copper excretion level (>100 µg/24 hours before start of decoppering therapy), increased hepatic copper concentration (>250 µg/g of dry weight) and mutations related to WD on both or one chromosome(s).

End of follow-up was defined as date of HCC diagnosis, liver transplantation, death or last available hospital visit before June 2013. All collected data were obtained from (electronic) medical records. Diagnosis of HCC was based on EASL guideline criteria.1 Potential relation of liver disease severity or efficiency of decoppering and HCC risk was explored.

Data on initial clinical presentation (i.e. hepatic, neurologic, combined, asymptomatic) were reported for each patient. Also, data on liver disease severity were collected at the time of WD diagnosis and at the end of follow-up. As previously published [7], liver disease severity was classified as: (1) mild (disturbed liver biochemistry only); (2) moderate (disturbed liver biochemistry combined with discrete abnormalities on ultrasound, computed tomography (CT) scan or magnetic resonance imaging (MRI), and/or, if performed, slight or moderate fibrosis on liver biopsy but without any laboratory, radiologic, or histologic abnormalities suggesting cirrhosis); (3) compensated end-stage liver disease (cirrhosis evident by clinical, laboratory, radiologic, or histologic findings, classified as stage A according to the Child-Pugh score); or (4) decompensated end-stage liver disease (cirrhosis evident by clinical, laboratory, radiologic, or histologic findings, classified as stage B or C according to the Child-Pugh score). At the end of follow-up, liver disease severity was classified as improved (a decrease in liver disease severity score as defined above7 from baseline), stable (liver disease severity score did not change during follow-up) or deteriorated (an increase in liver disease severity score from baseline). Performance of liver transplantation was also reported.

For each patient, data regarding treatment for WD during the entire follow-up were obtained. In patients who were treated for at least one year, efficacy of decoppering was determined at the end of follow-up based on values of serum non-ceruloplasmin-bound copper concentration and 24-hour-urinary copper excretion. Efficacy of decoppering was classified as: (1) excellent (serum non-ceruloplasmin-bound copper concentration <10 μg/dL and 24-hour urinary copper excretion <100 μg); (2) moderate (serum non-ceruloplasmin-bound copper concentration <10 μg/dL or 24-hour urinary copper excretion <100 μg); or (3) poor (serum non-ceruloplasmin-bound copper concentration >10 μg/dL and 24-hour urinary copper excretion >100 μg). In case of treatment with chelators, 24-hour-urinary copper excretion was determined with continued chelator treatment and should not exceed 500 μg to be qualified as appropriate.7 When urine aliquots were used for follow-up data on urinary copper excretion, we estimated 24-hour urinary copper excretion, assuming 24-hour urinary volumes of 1500 mL.

Furthermore, a comprehensive search of the literature was performed to identify studies that investigated long-term outcomes, including occurrence and/or mortality of HCC, in patients with WD. The electronic database of PubMed was searched for articles published in the English language. The search term comprised synonyms for “Wilson disease” as domain and “hepatocellular carcinoma” or “long-term follow up” as outcome. Studies were eligible for inclusion if they investigated long-term outcomes of WD

patients and reported total number of patients that developed or died from HCC and person-years of follow-up. When total number of person-years was not mentioned, it was estimated, if possible, based on mean duration of follow-up and number of patients. In order to evaluate whether our search included all published studies, we checked the reference lists of the included articles.

Statistical Analysis

Values are given as means and ranges or in case of a non-Gaussian distribution, as medians and ranges. Differences in values between two time points (i.e. baseline and end of follow-up) and between subgroups were analyzed for significance with Pearson's Chi-square test or Fisher's Exact test, as appropriate. A two-sided p-value <0.05 was considered statistically significant. A sample size weighted pooled analysis, based on data of the included studies and the current report, was conducted to estimate annual HCC risk and HCC mortality rate in patients with WD. Estimated HCC incidences and mortality rates for the total group, the subgroup of cirrhotics and the data of the meta-analysis were calculated with the Mid-P exact test, based on the total number of patients who developed or died from HCC and person-years of follow-up.

Results

Baseline characteristics

A total of 130 patients with WD were identified. Baseline characteristics are given in Table 1. More than half of all patients displayed Kayser-Fleischer rings at baseline (59%). Patients with neurologic symptoms were more likely to have Kayser-Fleischer rings than patients with exclusive hepatic presentation (88% versus 43%). Serum ceruloplasmin levels were reduced in 82% of all patients, whereas 24-hour urinary copper excretion was elevated in 72% of all patients. Coombs-negative hemolytic anemia was present in 19% of patients at the time of WD diagnosis. Quantitative hepatic copper content was determined in 37 patients. The median copper content was 652 µg/g dry liver weight (range 44-1600 µg/g). In 32 of these patients (86%) hepatic copper content was above 250 µg/g. Mutations related to WD were detected on both or one chromosome(s) in 68 (90%) and 5 (5%) of the 76 patients in whom mutation analysis was performed. The p.H1069Q mutation was present in 44 patients (58%: 18% homozygous and 40% heterozygous, respectively). Overall, median Leipzig score was 8 points (range 4-13).
Median age at the time of diagnosis of WD was 16 years (range 0-43). In two infant patients, WD diagnosis was based on mutations related to WD on both chromosomes. Male to female ratio was nearly equal. Presentation was exclusively hepatic in more than half of the patients (55%). Almost one third of patients (30%) had both neurologic and hepatic presentation at the time of diagnosis of WD, whereas a small percentage of patients presented with only neurologic symptoms (9%) or were asymptomatic (4%). In

Table 1: Baseline characteristics of 130 patients with Wilson disease

Patient no.	**130**
Presence of diagnostic criteria of Wilson disease (n (%))	
Kayser-Fleischer rings	77 (59)
Neurologic symptoms	50 (38)
Serum ceruloplasmin <0.2 g/L	106 (82)
Coombs-negative hemolytic anemia	25 (19)
24-hour urinary copper excretion >100 µg	94 (72)
Liver biopsy (Cu > 250 µg/g dry weight)	32/37 (86)†
Positive genetic testing	
Mutation(s) on both chromosomes	68/76 (90)†
Mutation(s) on 1 chromosome	5/76 (5)†
Leipzig score (median and range)	8 (4-13)
Age at diagnosis (median and range, in years)	16 (0-43)
Male gender (n (%))	65 (50)
Presentation (n (%))	
Hepatic	72 (55)
Neurologic	11 (9)
Combination	39 (30)
Asymptomatic	5 (4)
Unknown	3 (2)
Duration of follow-up (median and range, in years)	14.8 (0.1-51.2)

Results indicate numbers and, between brackets, percentages; †percentages are calculated based on number of total patients in whom quantitative hepatic copper content was determined (n=37) respectively mutation analysis was performed (n=76).

three patients baseline data were not available and mode of presentation was unknown. Median duration from WD diagnosis to end of follow-up was 15 years (range 0.1-51.2 years) (Table 1). Total follow-up duration in this study was 2336 patient years.

Liver disease severity

At baseline, cirrhosis was present in 74 patients (57% of total: 64% compensated and 36% decompensated). Distribution of severity of liver disease changed significantly over time (p=0.009), mainly due to progression from compensated to decompensated cirrhosis (Table 2). At the end of follow-up, liver disease severity was improved, stable and deteriorated in 20%, 46% and 24% of patients, respectively. Data on liver disease severity were incomplete in 13 patients (10%). Twenty-eight patients (22% of all patients) received a liver transplant. Liver transplantation was performed within one month of WD diagnosis in seven cases. Overall, median duration from WD diagnosis to liver transplantation was 1.4 years (range 0.1-38.5 years). Five patients died due to complications of their liver disease and two deaths were related to liver transplantation.

Treatment and efficiency of decoppering

For patients who were treated for at least one year (n=111), prescribed medication for WD during the entire disease course and efficiency of decoppering at the end of follow-

Table 2: Severity of liver disease7 at baseline and end of follow-up in 130 patients with Wilson disease

	At baseline	At end of follow-up	P-value
	n (%)	n (%)	0.009
Normal	12(9)	20 (15)	
Mild	10 (8)	12 (9)	
Moderate	23 (18)	28 (22)	
Compensated cirrhosis	47 (36)	29 (22)	
Decompensated cirrhosis	27 (21)	39 (30)†	
Unknown	11 (8)	2 (2)	

†28 patients (22% of all patients) underwent liver transplantation

up are given in Table 3. Median duration of follow-up in this group of patients was 17.1 years (range 1.5-51.2 years). Zinc was frequently prescribed, with 92% of patients being treated with zinc alone, sequentially or combined with chelators. Penicillamine treatment was initially started in 59% of patients, whereas 69% received penicillamine at any time during the disease course. Trientine was prescribed in 14% of patients. Efficiency of decoppering was determined at the end of follow-up based on values of serum non-ceruloplasmin-bound copper concentration and 24-hour urinary copper excretion. Excellent decoppering was present in 34% of patients. Nevertheless, most patients (42%) had moderate efficiency of decoppering. In 13% of patients efficiency was poor and in 11% data on serum non-ceruloplasmin-bound copper concentration copper and 24-hour urinary copper excretion at the end of follow-up were not available.

HCC development

Two patients were diagnosed with HCC. The first HCC case occurred in a 39-year-old male patient. He presented with decompensated cirrhosis in combination with HCC

Table 3: Efficiency of decoppering during treatment in 130 patients with Wilson disease

	n (%)†
Treatment‡	
Zinc	102 (92)
Penicillamine	77 (69)
Trientine	16 (14)
Efficiency of decoppering at end of follow-up	
Excellent	38 (34)
Moderate	47 (42)
Poor	14 (13)
Unknown	12 (11)
Duration of follow-up (median and range, in years)	17.1 (1.5-51.2)

†Total number of patients is 111 (only patients with ≥ 1 year therapy included); ‡Alone, sequentially or combined

(tumor size: six cm, Barcelona Clinic Liver Cancer (BCLC) stage D, alpha-fetoprotein (AFP) level: 2295 µg/L). There were no additional risk factors for HCC development, such as other metabolic liver diseases, viral hepatitis or alcohol abuse. WD diagnosis was based on the presence of Kayser-Fleischer rings, a low ceruloplasmin level (0.13 g/L), increased 24-hour urinary copper excretion (584 µg) and mutations related to WD on both chromosomes (Leipzig score: 9 points). Treatment for HCC was not performed. He died four months after presentation. The second patient was a 63-year-old female with unequivocal WD diagnosed 50 years earlier. WD diagnosis was based on the presence of Kayser-Fleischer rings, a low ceruloplasmin level (0.05 g/L), increased 24-hour urinary copper excretion (345 µg) and mutations related to WD on both chromosomes (Leipzig score: 10 points). Despite excellent decoppering at the end of follow-up after initial penicillamine treatment and subsequent maintenance therapy with zinc acetate (serum non-ceruloplasmin-bound copper: <10 µg/dL and 24-hour urinary copper excretion: 34 µg), she progressed to decompensated cirrhosis in which an HCC developed (BCLC stage D). AFP level at time of diagnosis was 120 µg/L. No additional risk factors for liver disease were present in this patient. Treatment for HCC was not performed. She died six months after HCC diagnosis.

Estimated annual HCC risk for all patients was 0.09% (95% confidence interval (CI): 0.01-0.28). Subgroup analysis in cirrhotic patients indicated an annual HCC risk of 0.14% (95% CI: 0.02-0.46). Both patients died due to their HCC and therefore incidence and mortality rates did not differ (8.6/10,000 persons-years for all patients and 13.9/10,000 persons-years in cirrhotic patients, respectively).

Our literature search resulted in the identification of nine other studies that investigated outcomes, including HCC occurrence, in WD patients during long-term follow-up [8-16]. After exclusion of four studies because of missing data on duration of follow-up [10,11,14,16,17], data of the current report and five other studies [8,9,12,13,15] were included in the meta-analysis. Study characteristics and outcomes of the included studies are given in Table 4. Based on data of the current study and three other studies

Table 4: Details of studies investigating long-term follow-up, including occurrence or mortality of hepatocellular carcinoma, in patients with Wilson disease

First author (year)	Follow-up time (person-years)	No. of patients who developed HCC	No. of patients who died of HCC
Beinhardt (2014) [8]	3116	0	0
Bruha (2011) [9]	1761†	-	1
Merle (2007) [12]	2476	0	0
Svetel (2009) [13]	1576†	-	0
Lowette (2010) [15]	375†	1	0
Current report	2336	2	2

†Calculated based on mean follow-up and number of patients; HCC=hepatocellular carcinoma

[8,12,15], the pooled annual HCC risk was 0.04% (95% CI: 0.01-0.10). Based on data of the current study and five other studies that reported HCC mortality [1,8,9,12-15], the pooled mortality rate was 2.6/10,000 person-years (95% CI: 0.7-7.0).

Discussion

In the EASL guideline HCC surveillance is generally recommended in all patients with cirrhosis, regardless of underlying cause [1]. Nevertheless, it remains to be seen whether this approach is cost-effective. The main finding of the current study is that even with advanced cirrhosis, HCC risk appears to be low in patients with WD.

Previous publications on HCC risk in WD are limited. The review by Thattil *et al* [18] summarizes 28 published case reports. Almost all HCC cases occurred in cirrhotic livers with a predominance of males. Walshe *et al* [11] performed a retrospective study to assess the frequency of abdominal malignancies in general in WD. Two cohorts of patients (n=363) from two specialist WD clinics (Cambridge/London: n=310 in the period: 1955-2000 and Uppsala: n=53 in the period:1966-2002) were included in this study. Of all patients who were followed for 10 years or more (n=159), nine patients developed abdominal malignancies (including two HCCs). Incidences rates of hepatic, biliary and pancreatic cancers were claimed to be significantly higher in the study population than in the general population of Sweden in 2000. Detailed information on WD diagnosis and treatment was not provided for this cohort, and other co-existent causes of liver disease were not rigorously excluded, although patients came from diverse locations including Egypt, Greece, Serbia, Italy and the UK. Furthermore, several other studies describing clinical presentation and outcomes of large cohorts of WD patients from Austria, Czech, Denmark and Germany mentioned only one or none HCC case(s) during long-term follow-up [8-10,12].

Based on our findings, annual HCC incidence in WD patients (0.09% and 0.14% in all patients and cirrhotics, respectively) is far below the accepted threshold incidence for HCC surveillance to be cost-effective (1.5%/year for cirrhotics) [3,4]. Results of our meta-analysis confirmed the low HCC incidence as well as HCC-related mortality rate in WD patients. Therefore, our data do not support regular surveillance in patients with WD, even in case of cirrhosis, unless additional risk factors for HCC (e.g. hepatitis B or C, alcohol) are present. Additionally, none of our patients developed a cholangiocarcinoma, despite the increased risk for this tumour in cirrhotic livers in general.

The reason for the low HCC incidence in WD patients compared to other chronic liver diseases, remains unclear. Based on earlier animal studies, it has previously been suggested that excessive copper accumulation might have a protective effect on hepatocarcinogenesis [19-22]. On the other hand, in the Long-Evans Cinnamon rat model for WD, long and persistent copper accumulation resulted in an increased risk of HCC which could be prevented by administration of D-penicillamine [23]. In this

model, carcinogenesis is thought to be the result of liver injury leading to chronic inflammation and cirrhosis due to chronic copper accumulation. Relation between HCC risk and efficacy of decoppering treatment could not be analyzed in detail in the current study due to the relatively small number of HCC cases. Nevertheless, most WD patients exhibited moderate or poor decoppering without developing HCC. Also, the second HCC case occurred in a patient despite excellent decoppering.

Our study has some strengths and limitations. Most important, a diagnosis of WD was unequivocally established in all cases (Leipzig score ≥ 4), and detailed information on WD treatment as well as effectiveness of decoppering was available. Also, the long follow-up (median 15 years: up to 51 years) in this cohort allowed a reasonable reliable estimation of HCC risk. On the other hand, the study design is retrospective. Moreover, patients could have been missed as a result of death before diagnosis of HCC or WD. Nevertheless, our results are in accordance with the clinical observation that HCC in patients with WD is rare. This contention is supported by the fact that during the period 2003-2013 no liver transplant was performed in the Netherlands for HCC in WD patients. In this period, nine liver transplantations were performed in WD patients with decompensated liver cirrhosis, all without HCC at pathological examination of the explant. The total number of transplantations for HCC in the Netherlands was 165 in this period (data from the Eurotransplant database). Furthermore, majority of WD patients in the Netherlands is treated in one of the three university hospitals that participated in this study.

In conclusion, we found that the risk of HCC development in WD patients on long-term follow-up was low. Our data therefore do not support regular HCC surveillance in patients with WD in absence of other risk factors.

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CHAPTER 4

CLINICAL IMPACT OF FIVE LARGE-SCALE SCREENING PROJECTS FOR CHRONIC HEPATITIS B IN CHINESE MIGRANTS IN THE NETHERLANDS

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Abstract

Background and aims

In low endemic countries it is debated whether first generation migrants should be screened for chronic hepatitis B infection. We describe the clinical impact of five large-scale Dutch screening projects for hepatitis B in first generation Chinese migrants.

Methods

Between 2009 and 2013 five independent outreach screening projects for hepatitis B targeting first generation Chinese migrants were conducted in five main Dutch regions. To explore the relevance of our screening we defined clinical impact as the presence of an indication for: 1. antiviral therapy, 2. strict follow-up because of high hepatitis B DNA levels and/or 3. surveillance for hepatocellular carcinoma.

Results

In total, 4423 persons participated in the projects of whom 6.0% (n=264) were HBsAg positive. 129 newly diagnosed HBsAg positive patients were analysed in specialist care. Among these patients prevalence of cirrhosis was 6.9% and antiviral therapy for hepatitis B was started in 32 patients (25%). In patients without a treatment indication, strict follow-up because of high hepatitis B DNA levels and/or surveillance for hepatocellular carcinoma was considered indicated in 64 patients (50%).

Conclusions

In our screening project in first generation Chinese migrants, antiviral treatment, strict follow-up because of high hepatitis B DNA levels and/or surveillance for hepatocellular carcinoma were considered indicated in three out of four analysed HBsAg positive patients. These data show that detection of hepatitis B in Chinese migrants can have considerable impact on patient care.

Introduction

Chronic hepatitis B virus (HBV) infection represents a global public health problem resulting in significant morbidity and mortality. Worldwide approximately 240 million people are chronically HBV-infected [1]. Annually more than 780,000 patients die of HBV-associated liver disease [2].

Chronic HBV patients are at significant risk of developing liver cirrhosis, hepatic decompensation and hepatocellular carcinoma (HCC). Overall, HBV is responsible for 30% of all cirrhotic cases and for 53% of all new HCC cases worldwide [3,4]. Although cirrhosis is a strong risk factor for HCC, 30%-50% of HCCs associated with HBV occur in the absence of cirrhosis [5]. Several factors, such as male gender, Asian ethnicity, hepatitis B e antigen (HBeAg) positivity, high HBV viral load, increased transaminases and possibly genotype C have been suggested to be independently associated with elevated HCC risk [6,7].

In high endemic regions primary prevention using HBV vaccination has led to a significant decrease in HBV-related morbidity and mortality [2,8]. Moreover, antiviral therapy for HBV and adequate surveillance for HCC in high risk patients reduce the incidence of HBV-related morbidity and mortality significantly [9-12].

In the Netherlands the prevalence of chronic HBV infection is low and concentrated in migrants from intermediate and high endemic countries [13]. In the Dutch general population the prevalence of chronic HBV is estimated to range between 0.2% and 0.5% [14]. In first generation migrants the estimated prevalence ranges between 1.1 and 3.8% [13,14], but may be higher in migrants from specific countries. Surveillance data revealed that 77% of chronic HBV patients in the Netherlands were born abroad, almost all in intermediate or high endemic countries [15]. First generation migrants from countries with high viral hepatitis prevalence could therefore be an important target group for HBV screening. China is with a HBV prevalence of approximately 10% a high endemic region [16] and Chinese form the sixth largest non-Western migrant population in the Netherlands. Therefore, the number of migrants with chronic HBV was estimated to be large.

Currently in the Netherlands, screening for HBV is mainly performed as a part of primary prevention programmes aiming at preventing transmission. However, secondary prevention aimed at reducing HBV-related morbidity and mortality may also be relevant.

In the current report, we describe the clinical outcome of five large-scale Dutch screening programmes that offered targeted HBV screening to first generation Chinese migrants aiming at secondary prevention.

Patients and methods

Between 2009 and 2013 five independent outreach screening projects for hepatitis B targeting first generation Chinese migrants were conducted in the cities of Rotterdam

(2009) [17], The Hague (2010), Utrecht (2011), Arnhem/Nijmegen region (2013) and Eindhoven (2013). The campaigns combined disease awareness activities with free HBV testing. Furthermore, the campaigns were organised in collaboration with the hospitals and direct referral pathways were established. In this study we focus on clinical impact after referral to specialist care. The details of the preparation and campaigning phase and participation rate are therefore beyond the scope of this article but have been described for the campaign in Rotterdam [17]. We here provide a brief summary.

Community based Chinese organizations and Chinese key figures were engaged in the campaign and outreach activities took place where people were tested in several community centers in for example Chinese schools and churches. The campaign was started together with the celebration of the Chinese New Year in order to create as much awareness as possible. Information about the campaign was also printed in Chinese Newspapers, folders and a special website. In three campaigns (Utrecht, Arnhem/Nijmegen and Eindhoven) registered Chinese people were also contacted by letter. During 9 months after starting the campaign people could be tested at the Municipal Public Health Service. Testing was offered free of charge.

In the screening phase participants were offered free HBV testing at outreach locations. All blood samples were tested for hepatitis B core antigen (anti-HBc). When tested positive for anti-HBc, hepatitis B surface antigen (HBsAg) was determined on the same blood sample. After positive HBsAg confirmation testing, samples were further tested for antibodies to HBsAg (anti-HBs) and to hepatitis B e antigen (anti-HBe), HBeAg and alanine aminotransferase (ALT) level.

Anti-HBc, HBsAg, HBeAg, and anti-HBe testing was performed with microparticle enzyme immunoassay (MEIA). HBsAg-positive sera were retested with a confirmation assay. For anti-HBs testing, a quantitative MEIA was used. Assessment of the HBV viral load was performed using a real-time polymerase chain reaction (PCR) assay.

Patients who tested positive for HBsAg were contacted by phone by a Chinese speaking employee of the Municipal Public Health Service and were invited to the local Municipal Public Health Service for counselling and for both source and contact investigation according to the current national primary prevention programmes aiming at preventing transmission. Participants found to be susceptible for HBV infection were advised to get vaccinated.

In Rotterdam and The Hague the following referral guideline was used: patients were referred to a medical specialist (internal medicine or gastroenterology) when either HBeAg testing was positive or ALT was above the upper limit of normal. Persons who were HBeAg negative and had normal ALT levels were referred to the general practitioner with the advice to check ALT levels yearly for the next three years. Veldhuijzen et al. showed that HBeAg status and ALT level can accurately predict high HBV DNA load in patients with chronic HBV. They concluded that a referral guideline using HBeAg status and ALT level can be used to select patients for referral to specialist care [18]. In

the remaining cities all HBsAg positive patients were referred for further evaluation to a medical specialist.

In referred HBsAg positive patients, HBV DNA and ALT levels were determined. Furthermore, abdominal ultrasounds with or without additional liver stiffness measurements with the aid of Fibroscan® (Echosens, Paris, France) or liver biopsies were performed to assess whether cirrhosis was present. Cirrhosis was defined as presence of surface nodularity of the liver, segmental atrophy or signs of portal hypertension on ultrasound and/or grade F4 measured with Fibroscan® (>15 kPa) and/or grade Metavir 4 on liver biopsy. Patients were monitored at 3 to 6 month intervals according to the Dutch and American Association for the Study of Liver Diseases (AASLD) Chronic Hepatitis B guidelines [19,20].

Indication for antiviral treatment and follow-up in patients not initially considered for treatment was assessed according to the Dutch and American Association for the Study of Liver Diseases (AASLD) Chronic Hepatitis B guidelines [19,20]. Both guidelines use the same criteria. Strict follow-up is needed in patients at increased risk for hepatitis B flare. These include HBeAg positive and negative patients with normal or slightly elevated ALT and a viral load >20,000 IU/ml [19,20]. According to the AASLD HCC and Dutch Chronic Hepatitis B guidelines, HCC surveillance is indicated not only for cirrhotic patients but also for east-Asian male HBV carriers over 40 years, east-Asian female HBV carriers over 50 years of age in absence of cirrhosis and HBV carriers with a family history of HCC because of a high risk for developing HCC [20,21].

Antiviral therapy for HBV and adequate surveillance for HCC in high risk patients reduce the incidence of HBV-related morbidity and mortality significantly [9-12]. Therefore, identifying patients with an indication for: 1. start of antiviral treatment, 2. strict follow-up because of high HBV DNA levels (at increased risk for hepatitis B flare with a consequent treatment indication) (HBV DNA>20,000 IU/ml) (both according to Dutch and AASLD Chronic Hepatitis B guidelines) and/or 3. surveillance for HCC according to AASLD HCC guideline [19-21], is essential to determine the relevance of screening programmes. In this study we explored the relevance of our screening programmes, by identifying these patient groups. Persons already being within specialist care for HBV were excluded from our analysis.

Statistical Analysis

Continuous data are expressed as medians and ranges, and discrete variables as absolute and relative frequencies. The Mann-Whitney U or Kruskal-Wallis tests were applied to compare continuous data between the five regions. Categorical variables were compared with Pearson's Chi-square or Fisher's exact tests. A two-sided p-value <0.05 was considered statistically significant.

Results

One third of the Chinese population in the Netherlands lives in the cities of Rotterdam, The Hague, Utrecht, Arnhem/Nijmegen and Eindhoven (approximately 29,000 people). In total, 4423 persons participated in the screening programmes of whom 6.0% (n=264) were HBsAg positive (Table 1). The percentage of HBsAg positive patients varied between 3.9 and 8.6 between the five regions (P<0.001), with the highest prevalence in Rotterdam.

Of the 264 identified HBsAg positive patients, 61% (n=160) were referred for specialist care. In Rotterdam and The Hague the use of a referral guideline resulted in a lower referral rate (37% and 33%, respectively) than in Utrecht, Arnhem/Nijmegen region and Eindhoven (93%, 100% and 100%, respectively). In Utrecht 3 patients refused to be referred to specialist care or were not reached by the Municipal Public Health Service resulting in a referral rate of 93%. According to the referral guideline HBeAg negative patients with normal ALT were not referred to a specialist in Rotterdam and The Hague. One of the HBsAg positive patients evaluated by a specialist in another region with a treatment indication within one year of follow-up (initially normal ALT) was HBeAg negative.

In total, 129 newly diagnosed HBsAg positive patients were analysed in specialist care (49% of all HBsAg positive patients, 81% of all referred patients). The remaining HBV patients turned out to be already under specialist care (n=10), were lost to follow-up (n=14) or data of the patients were missing because of referral to other regions patients and patients could not be traced (n=7) (Table 1). Of these newly diagnosed HBsAg positive patients 32 were HBeAg positive (25%) and 96 (75%) were HBeAg negative (HBeAg status was missing in 1 patient). There were differences between the five regions with the highest percentages of HBeAg negative patients in The Hague, Utrecht and Arnhem/Nijmegen region (80%, 91% and 82% respectively). In Rotterdam and Eindhoven respectively 56% and 63% of the patients were HBeAg negative (data not shown in tables).

Table 2 gives an overview of patient characteristics and results of the clinical referral of the newly diagnosed HBsAg positive patients. The median age of the newly diagnosed HBsAg positive patients analysed in specialist care was 45 years (range: 20-81) and most patients were male (64%). Age and gender did not differ significantly between five regions.

At time of presentation, 9 of the 129 (6.9%) newly diagnosed HBsAg positive patients had cirrhosis based on evaluation on ultrasound with or without additional Fibroscan® (F4) and/or liver biopsy (Table 2).

Within one year of initial consultation, antiviral therapy for chronic HBV was started in 32 patients (12% of the 264 HBsAg positive patients, 25% of the 129 newly diagnosed HBsAg positive patients analysed in referral centers). There were significant differences between the five regions (P=0.040): the highest percentage of patients with a treatment

Table 1: Prevalence of chronic hepatitis B in 4423 Chinese migrants in the Netherlands: results of a screening program.

	Total	Rotterdam	The Hague	Utrecht	Arnhem/ Nijmegen	Eindhoven	P-value
Total patients screened	n=4423	n=1090	n=1296	n=613	n=747	n=677	
Anti-HBc negative*	2524 (57)	556 (51)	777 (60)	366 (60)	454 (61)	371 (55)	
HBsAg positive (and anti-HBc+)*	264 (6.0)	94 (8.6)	63 (4.9)	44 (7.2)	29 (3.9)	34 (5.0)	<0.001
HBsAg positive patients referred to specialist care**	160 (61)	35# (37)	21# (33)	41(93) $	29 (100)	34 (100)	
New HBsAg positive patients analysed in specialist care	129	32	15	34	28	20	
Already within specialist care	10	3	0	3	0	4	
Lost to follow-up ***	14	0	6	4	0	4	
Missing data	7	0	0	0	1	6	

Values are numbers with percentages in parentheses; P-value applies to differences between the five regions; HBV = hepatitis B virus; NA = Not assessed; * Percentages of total patients screened; ** Percentages of all HBsAg positive patients; #Referral guideline used; *** Lost to follow-up between local Municipal Public Health Service and specialist care; $ 3 patients refused to be referred to specialist care or were not reached by the Municipal Public Health Service.

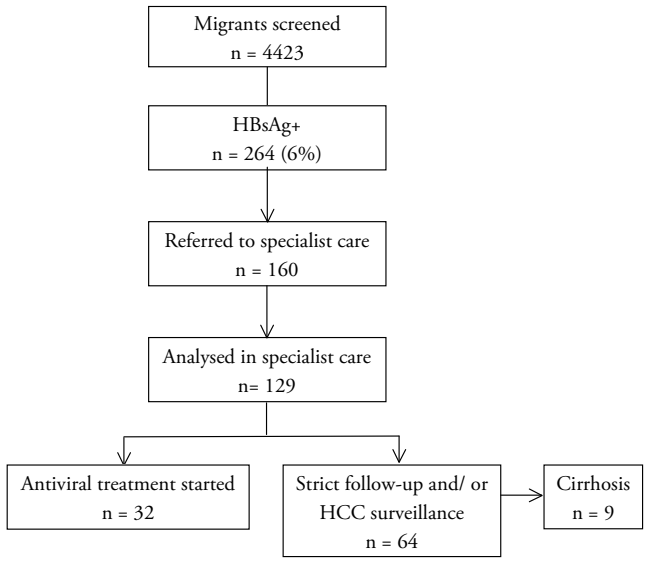
Table 2: Outcome of clinical follow-up in 130 newly diagnosed chronic hepatitis B patients analysed in specialist care

	Total	Rotterdam	The Hague	Utrecht	Arnhem/ Nijmegen	Eindhoven	P-value
New HBsAg positive patients analysed in specialist care	129	32	15	34	28	20	
Median age (range) in years	45 (20-81)	38 (22-75)	37 (20-81)	44 (27-65)	51 (21-70)	50 (28-71)	0.126
Male gender	83 (64)	20 (63)	8 (53)	22 (65)	19 (68)	14 (70)	0.866
Presence of cirrhosis*	9 (6.9)	5 (16)	1 (6.7)	1 (2.9)	1 (3.6)	1 (5.0)	NA
Antiviral therapy started within one year of initial consultation	32 (25)	15 (47)	3 (20)	5 (15)	7 (25)	2 (10)	0.040
Treatment indication:							
– Cirrhosis and HBV DNA ≥2000 IU/mL	6 (4.7)	3 (9.4)	1 (6.7)	0 (0)	1 (3.6)	1 (5.0)	
– HBeAg+/ HBeAg-, HBV DNA>20,000 IU/ml, ALT ≥2 ULN	20 (15)	9 (28)	2 (13)	2 (5.9)	6 (21)	1 (5.0)	
– HBeAg+/ HBeAg-, HBV DNA >20,000 IU/ml, ALT < 2 ULN, significant fibrosis/ moderate-severe necroinflammation	4 (3.1)	1 (3.1)	0 (0)	3 (8.8)	0 (0)	0 (0)	NA
– HBeAg-, HBV DNA ≥2000 and ≤20,000 IU/ml, ALT ≥2 ULN	2 (1.6)	2 (6.2)	0 (0)	0 (0)	0 (0)	0 (0)	
Strict follow-up and/or HCC surveillance	64 (50)	10 (31)	7 (46)	18 (53)	14 (50)	15 (75)	0.506
– Cirrhosis (without treatment indication)	2 (1.6)	1 (3.1)	0 (0)	1 (2.9)	0 (0)	0 (0)	
– HBV DNA >20,000 IU/mL	18 (14)	5 (16)	5 (33)	2 (5.9)	3 (11)	3 (15)	NA
– ♂> 40 years/♀> 50 years of age**	30/14 (34)	1/3 (13)	1/1 (13)	12/3 (44)	8/3 (39)	8/4 (60)	

Values are numbers and, in parentheses percentages of all newly analysed HBsAg positive patients, unless otherwise indicated. P-value applies to differences between the five regions; HBV = hepatitis B virus; ULN=upper limit of normal; HCC = hepatocellular carcinoma; *based on liver biopsy, Fibroscan® and/or abdominal ultrasound; **Not included in the other categories with an indication for strict follow-up or HCC surveillance (no cirrhosis and HBV<20,000IU/mL).

indication was found in Rotterdam (47%), whereas only 10% 36 of the patients in Eindhoven had a treatment indication. In most patients (20 of 32 patients) antiviral treatment was started because of high HBV DNA levels (HBV DNA>20,000 IU/ml) in combination with persistently and markedly elevated ALT levels (ALT ≥2 ULN). In six patients antiviral treatment was started because of cirrhosis in presence of significant HBV DNA levels (HBV DNA>2000 IU/ml), and in four patients because of high HBV DNA levels (HBV DNA>20,000 IU/ml), mildly elevated ALT levels (ALT <2 ULN) and the presence of significant fibrosis (F2 or F3, 8.5-15 kPA) or moderate-severe necro-inflammation on liver biopsy. Finally, in two HBeAg-negative patients antiviral therapy was necessary due to significant HBV DNA levels (HBV DNA ≥2000 and ≤20,000 IU/ml) in combination with markedly elevated ALT levels (ALT ≥2 ULN) (Table 2).
In patients without a treatment indication within one year of follow-up, strict follow-up because of high HBV DNA levels (HBV DNA>20,000 IU/ml) and/or HCC surveillance was considered indicated in 64 newly diagnosed HBsAg positive patients (24% of the 264 HBsAg positive patients, 50% of all analysed, newly diagnosed HBsAg positive patients) (Table 2). The percentage of patients with an indication for follow-up did not differ significantly between the five regions (P=0.506). In two patients without antiviral treatment indication, HCC surveillance was indicated because of the presence of cirrhosis despite undetectable HBV DNA levels. The main reason for HCC surveillance in absence of cirrhosis was age >40 years (n=30) in males and >50 years in females (n=14). In 18 other patients without antiviral treatment indication within one

Figure 1: Outcome of hepatitis B screening in 4423 Chinese migrants in the Netherlands.



year of follow-up, strict follow-up was considered indicated because of the presence of high HBV DNA (HBV DNA >20,000 IU/mL) (Table 2).
Overall, 96 (36%) of all HBsAg positive patients, (75% of all new HBsAg positive patients analysed in referral centers) needed antiviral treatment within one year of follow-up or were thought to need strict follow-up because of high HBV DNA levels or HCC surveillance (Figure 1).

Discussion

This is the first large-scale screening project for chronic HBV offered to first generation Chinese migrants aiming at secondary prevention. Previous studies in which screening for chronic HBV was offered to migrants from high endemic countries tested fewer patients and mainly focussed on determination of prevalence of chronic HBV, participation rates in screening or cost-effectiveness [14,17,22,23].
Since China is a high endemic region for HBV and Chinese form a large migrant population in the Netherlands, the estimated number of migrants with chronic HBV is large. We therefore chose Chinese migrants as the target population in this study. Moreover, after the campaign in Rotterdam described by Veldhuijzen et al. [17] showed that Chinese migrants can be reached with an outreach campaign and that on site testing was well accepted we decided to expand the campaign to other regions with high number of Chinese migrants and determine the clinical impact of the screening. However, it is likely that this campaign could also be expanded to other migrant groups from high endemic HBV regions.
This study documents that among Chinese migrants, 12% of all HBsAg positive patients (25% all chronic new HBV patients analysed in referral centers) received an indication for antiviral treatment within one year of initial consultation. In patients without a treatment indication, strict follow-up because of high HBV DNA levels and/or HCC surveillance were considered indicated in 24% of all HBsAg positive patients (50% of all analysed, newly diagnosed HBsAg positive patients). These findings can probably be extrapolated to countries with similar Chinese migrant populations.
Since chronic HBV infections are mostly asymptomatic and treatment has become highly efficient, secondary prevention aiming at early detection and initiation of treatment is essential for reducing HBV-related morbidity and mortality. Moreover, in patients without a treatment indication, strict follow-up is needed when at increased risk for hepatitis B flare and when at risk for developing HCC. This especially holds true for the Southeast Asian population because of the higher risk of developing HCC [21].
The percentage of HBsAg positive patients varied between 3.9% and 8.6%, with the highest prevalence in Rotterdam. It should be mentioned that prevalence data from screening campaigns may not reflect the true population prevalence, as for example persons already known with chronic HBV might not take part in the screening.

Furthermore, based on previous studies it remains controversial whether the prevalence of chronic HBV in migrants reflects the prevalence in the country of origin [24].
In three regions (Utrecht, Arnhem/ Nijmegen and Eindhoven) hepatitis C screening was also offered. The prevalence of HCV infection was low: only two patients were newly diagnosed with chronic hepatitis C (data not shown). Although the number of chronic HCV patients in this screened population is low, we found one patient who had already developed a HCC. However, because of the low HCV prevalence no recommendation regarding HCV screening or surveillance can be made based on our data.
For a screening programme aiming at secondary prevention to be effective, appropriate referral from primary tot secondary care is essential. Referral to specialist care was very successful in this study. 81% of all HBsAg positive patients with an indication for referral were further evaluated by a medical specialist. The amount of patients who received consultation by a medical specialist is probably so high in this study because the campaigns were organised in collaboration with the hospitals and direct referral pathways were established. Furthermore, referral rates are probably also high because of the outreach design of the screening in which awareness activities were offered and people voluntarily participated.
The indication for antiviral treatment, strict follow-up and HCC surveillance were assessed according to the Dutch and AASLD Chronic Hepatitis B guidelines and AASLD HCC guideline [19-21]. These guidelines differ from the European Association for the Study of the Liver (EASL) chronic hepatitis B guideline [25]. The EASL guideline recommends that all patients with serum HBV DNA levels >2,000 IU/ml and persistent elevated ALT should be considered for treatment, regardless of HBeAg status. According to the Dutch and AASLD Chronic Hepatitis B guidelines, treatment of HBeAg positive patients is recommended at higher HBV DNA levels (i.e. >20,000 IU/ml). When treatment indication would be assessed according to the EASL guideline, the percentage of patients indicated for antiviral treatment would increase. However, it would not influence the outcome because the patients would shift from the follow-up group to the treatment group.
A discrepancy in international guidelines that would influence the outcome is the additional recommendation in the AASLD hepatitis B guideline that any carrier over 40 years with persistent or intermittent ALT elevation and/or high HBV DNA >2,000 IU/mL should be screened for HCC. In this study we used the AASLD HCC guideline that excludes this group. Including this group of patients for HCC surveillance in our study would result in an even higher amount of patients needing HCC surveillance.
It should be mentioned that surveillance for HCC and the indication for strict follow-up based on a high HBV DNA levels remain controversial because of limited evidence for its efficiency [26,27]. However, studies have shown that HCC incidence should exceed 0.2% per year in non-cirrhotics to allow cost-effective HCC surveillance, which holds

true for Asian HBV carriers. Despite the controversies we chose to apply the current guidelines for determining the indication for strict follow-up and HCC surveillance.
This study was not designed to address the question whether a national screenings programme for HBV in first generation Chinese migrant groups should be implemented.
This study has several limitations. The first limitation is the outreach design of the screening campaigns in which the percentage of reached chronic HBV patients is unknown. We do know that overall approximately 29,000 Chinese people live in the cities of Rotterdam, The Hague, Utrecht, Arnhem/Nijmegen and Eindhoven (one third of the Chinese population in the Netherlands). The actual number might be higher because a large number of Chinese migrants are not officially registered. In total, 4423 persons (15% of the Chinese population in the five regions) participated in the screening programmes. Veldhuijzen et al. [17] showed that Chinese migrants can be reached with an outreach campaign and that on site testing was well accepted.
Secondly, this is an observational study including five independent screening programmes with different patient characteristics resulting in heterogeneous groups. However, age and gender did not differ significantly between five regions and organisation of the campaigns, referral pathways and clinical follow-up was similar in all the five programmes. Furthermore, the numbers and follow-up of patients referred to specialist care are too small to make any long term conclusions. However, this is so far the largest screening project offered to first generation Chinese migrants aiming at secondary prevention described in English literature. Finally, this study provides no data on cost-effectiveness of the screening. However, a recent review of cost-effectiveness of screening for chronic HBV suggests that screening of migrant populations is cost-effective [23,28]. Although no recommendations regarding HBV screening in Chinese migrants can be made based on the data of this study, the high amount of patients who needed antiviral treatment within one year of follow-up or were thought to need strict follow-up because of high HBV DNA levels or HCC surveillance stresses the need for future research to compare costs and benefits of various screening strategies for chronic HBV in migrants.
In conclusion, in our screening project in first generation Chinese migrants, in 36% of all HBsAg positive patients (75% of all positive HBsAg patients analysed in referral centers), antiviral treatment, strict follow-up because of high HBV DNA levels and/or HCC surveillance were considered indicated. These data show that detection of HBV infection in Chinese migrants can have considerable impact on patient care.

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CHAPTER 5

SURVEILLANCE FOR HEPATOCELLULAR CARCINOMA IS ASSOCIATED WITH INCREASED SURVIVAL: RESULTS FROM A LARGE COHORT IN THE NETHERLANDS

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ABSTRACT

Background and aims: Effectiveness of surveillance for hepatocellular carcinoma is controversial. We here explore its effects in "real life" clinical practice.

Methods: Patients with hepatocellular carcinoma diagnosed in the period 2005-2012 in five Dutch academic centers were evaluated. Surveillance was defined as ≥2 screening tests during 3 preceding years and at least one radiologic imaging test within 18 months before diagnosis.

Results: 295 (27%) of 1074 cases underwent surveillance. Median time interval between last negative radiologic imaging and hepatocellular carcinoma diagnosis was 7.5 months. In the surveillance group, cirrhosis (97% vs. 60%, P<0.001) and viral hepatitis were more frequent and non-alcoholic fatty liver disease or absence of risk factors less frequent.
In case of surveillance, tumor size was significantly smaller (2.7 vs. 6.0 cm), with lower alpha–fetoprotein levels (16 vs. 44 µg/L), earlier tumor stage (BCLC 0 and A combined: 61% vs. 21%) and resection/transplantation (34% vs. 25%) or radiofrequency ablation (23% vs. 7%) more often applied, with significantly higher 1-, 3- and 5-year survival rates.
Survival benefit by surveillance remained significant after adjustment for lead-time bias based on assumed tumor doubling time of 90 days, but not with doubling time of ≥120 days. In multivariate analysis, surveillance was an independent predictor for mortality (for interval ≤9 respectively >9 months: adjusted hazard ratios 0.51 and 0.50, 95% confidence intervals: 0.39-0.67 and 0.37-0.69).

Conclusions: Surveillance for hepatocellular carcinoma was associated with smaller tumor size, earlier tumor stage, with impact on therapeutic strategy and was an independent predictor of survival.

Introduction

Liver cancer is the sixth most common cancer in the world and the third cause of cancer-related death [1]. Hepatocellular carcinoma (HCC) represents more than 90% of primary liver cancers and generally occurs in patients with underlying chronic liver disease. In Western countries with a relatively low incidence rate such as the Netherlands, incidence has increased in the last decade [2,3].

Currently, several international guidelines advise regular surveillance of patients at increased HCC risk [4-6]. The goal is to detect HCC at earlier stages enabling curative therapies with a better outcome and decreased mortality. Nonetheless, surveillance is controversial [7]. One randomized controlled trial from China in chronic hepatitis B (HBV) patients compared surveillance with combined ultrasound (US) and alpha–fetoprotein (AFP) at 6-month intervals vs. no surveillance and found significantly lower HCC-related mortality rates in the surveillance group [8]. Several aspects of this study have been criticized [7,9]. Another randomized controlled study in Chinese HBV patients found no benefit from surveillance with AFP alone at 6-month intervals [10]. Based on lower quality evidence, several cohort studies suggest improved survival with surveillance (studies summarized in references [11,12]).

Conventional US and AFP (alone or combined) are most widely used as surveillance modalities, but their sensitivities and specificities are far from perfect [13,14]. Moreover, the optimal surveillance interval is a matter of debate [15-18].

In the present study we investigate the contribution of surveillance in a large group of HCC patients in "real life" clinical practice in the Netherlands.

Patients and methods

All patients with an HCC diagnosis in the period 2005-2012 in five Dutch academic centers were evaluated. Diagnosis of HCC was based on AASLD 2005 and 2011 guideline criteria [4,19]. Collected data were obtained from (electronic) medical records. Extensive efforts were done to clarify all missing data, e.g. by contacting patients, referring hospital or general practitioners.

Tumor characteristics, such as number of tumor lesions, maximum diameter of the largest tumor lesion and tumor stage according to Barcelona Clinic Liver Cancer (BCLC) staging system, and laboratory values at time of diagnosis were obtained for each patient. Also, data on ECOG performance status [20], clinical symptoms such as right upper quadrant abdominal pain and weight loss (≥3 kg), presence of cirrhosis (based on clinical, laboratory, radiologic and histologic findings) and cause of underlying liver disease (including HBV and HCV, hemochromatosis, alcohol-related liver disease (defined as ≥3 alcoholic drinks/day [21]) and NAFLD (defined as steatosis or steatohepatitis on liver biopsy or presence of metabolic syndrome in the absence of other risk factors for chronic liver disease such as alcohol abuse) were obtained. Severity of liver disease was estimated by calculating the MELD score [22].

Patients were categorized as receiving surveillance (defined as at least two screenings tests (AFP and/or imaging test) during the three years before HCC diagnosis and at least one radiologic imaging test within 18 months before diagnosis) or no surveillance. Additionally, time interval (i.e. time between last negative radiological surveillance imaging and date of HCC diagnosis) for patients in the surveillance group was determined. Patients in the surveillance groups were divided into two subgroups based on this time interval: ≤9 months and >9 months.
Patients were categorized into treatment groups based on application of surgical therapy (resection or transplantation), radiofrequency ablation (RFA), transarterial chemoembolization (TACE) or transarterial radioembolization (TARE), sorafenib or best supportive care. Patients undergoing sequential therapy appertaining to ≥2 treatment groups were included in the treatment group presumed to have most impact on outcome. In case of RFA and subsequently TACE, with at least a 1-month interval, patients were included in the RFA group. When combined RFA and TACE were performed within a 1-month interval, patients were included in the TACE group.
This study was in agreement with the Declaration of Helsinki and analyses were performed with institutional medical ethical consent, in an anonymized database.

Statistical analysis

Continuous data are expressed as medians and ranges, and discrete variables as absolute and relative frequencies. Mann-Whitney U or Kruskal-Wallis tests were applied to compare continuous data in various groups. Categorical variables were compared with Pearson's Chi-square or Fisher's exact tests.
Survival time was calculated from date of diagnosis to date of death or end of follow-up (latest: end of study 1-1-2013). To deal with lead-time bias, which represents the apparently improved survival caused by the earlier diagnosis in the course of the disease, we calculated lead-times for all patients who underwent surveillance prior to HCC diagnosis. Lead-time was calculated by using the parametric model proposed by Duffy *et al.* [23] assuming an exponential distribution of the HCC sojourn time. Calculated lead-time for patients in the surveillance group was subtracted from their survival time. Kaplan-Meier survival curves and log-rank tests were used to compare survival rates between various groups. Apart from HCC surveillance, sex, age, etiology of underlying liver disease, presence of cirrhosis, pain symptoms and weight loss, PS, year of HCC diagnosis and MELD score were tested as possible predictors for overall mortality by univariate Cox proportional hazard regression analysis. Factors with a p-value <0.05 in univariate analyses were included in subsequent multivariate analyses. In a separate uni- and multivariate analysis, BCLC stage and applied treatment were also included. Subgroup analyses were performed in cirrhotics and in patients in whom surveillance is recommended according to AASLD guidelines [4]. A two-sided p-value <0.05 was

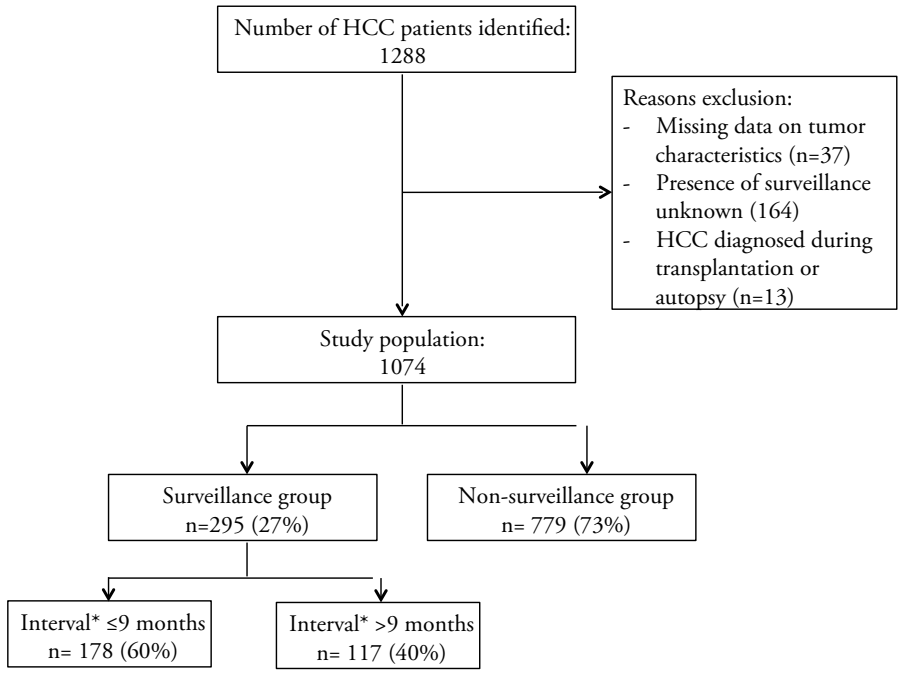
considered statistically significant. Statistical analysis was performed using SPSS (version 20).

Results

Patient characteristics

In the period January 2005- December 2012, 1288 HCC patients were under care in the five participating hospitals (60% of all Dutch HCC patients in this period [3]). After exclusion of 214 patients because of missing data, 1074 (83%) were included in this study (Figure 1). Of all included patients, 27% (n=295) underwent HCC surveillance, without change during the study period. Surveillance was performed by radiological investigations (ultrasound, CT or MR imaging) alone in 17% and in combination with AFP in 83%. Median number of surveillance tests in the three years before HCC diagnosis was 7 (range 2-23: 4 (range 1-9) by radiological investigations and 3 (range 0-15) by AFP). Median time interval between last negative radiologic imaging and HCC diagnosis was 7.5 months (range: 0.2-18 months) (≤9 months in 60% of patients and >9 months in 40%).

Figure 1: Flow chart of patient inclusion and details of surveillance



*Time between date of HCC diagnosis and last negative radiologic imaging

Patient characteristics of the total group and separately for the surveillance and non-surveillance groups are given in Table 1. Viral hepatitis was the underlying cause for liver disease in 37%, alcohol abuse in 28%, NAFLD in 16%, hemochromatosis in 2%, and other liver diseases in 3%. Fourteen percent had no risk factors for underlying liver disease, despite extensive investigation for potential causes. Viral hepatitis was more common in the surveillance group (61% vs. 27%), whereas NAFLD (7% vs. 20%) or absence of cause(s) for underlying liver disease (3% vs. 18%) were more common in the non-surveillance group. Cirrhosis was present in 97% of the surveillance and in 60% of the non-surveillance groups (P<0.001). In the whole group, 93% of all HCV patients exhibited cirrhotic liver, which was the case in 75% of HBV patients and in 24% of patients with unknown cause for underlying liver disease. Of the patients without risk factors for underling liver disease (n=146), 27% had grade ≤2 fibrosis (based on histology), 4% grade 3 fibrosis (based on histology), 24% cirrhosis (based on histological and/or radiological findings) and 45% no signs of cirrhosis (based on clinical, laboratory and radiological findings in absence of available histology). Furthermore, proportion of patients >75 years old was significantly higher in the non-surveillance group than in the surveillance group (17% vs. 8%, P<0.001).

Clinical and tumor characteristics

As indicated in Table 2, most patients had a single tumor (56% and 50% in surveillance and non-surveillance groups, respectively). Nevertheless, patients who did not receive surveillance more often exhibited multifocal or diffuse HCC (35% vs. 20%, respectively). In the surveillance group, tumor size was significantly smaller (2.7 vs. 6.0 cm; P<0.001) and HCC was detected in an earlier tumor stage (BCLC 0 and A combined: 61% vs. 21%) than in the non-surveillance group (Table 2).

Performance scores did not differ between surveillance and non-surveillance groups. In the non-surveillance group, right upper quadrant abdominal pain (36% vs. 12%, P<0.001) and weight loss (25% vs. 7%, P<0.001) at time of diagnosis were significantly more common than in the surveillance group (Table 2).

In the surveillance group, median AFP (16 µg/L vs. 44 µg/L; P<0.001) and ALT levels (44 U/L vs. 49 U/L; P=0.074) were lower than in the non-surveillance group. In contrast, patients who underwent surveillance had higher MELD scores than those in the non-surveillance group (10 vs. 9; P<0.001) (Table 2). Tumor size and BCLC stage increased with longer surveillance interval (Table 2).

Treatment

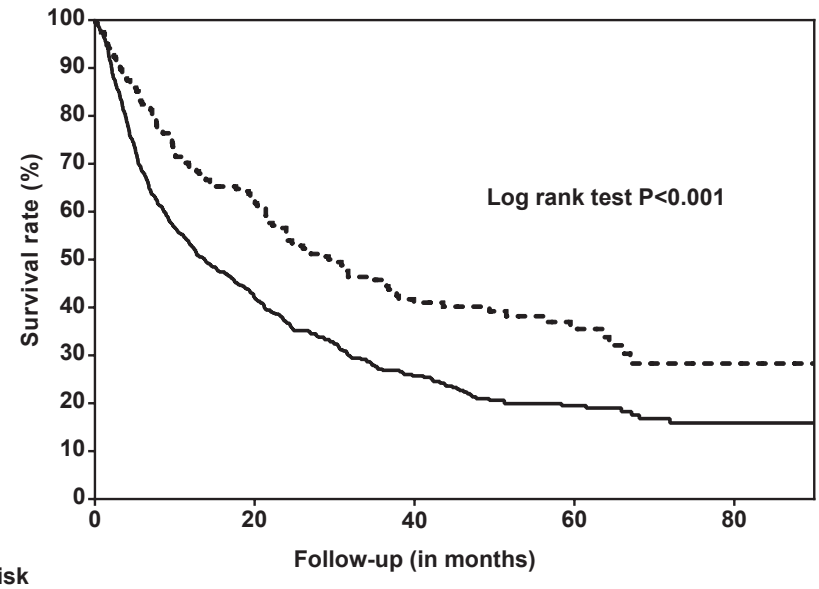
In total, 28% of all patients received surgical treatment (resection/transplantation), 11% RFA, 17% TACE/TARE, 11% sorafenib and 29% best supportive care. Details of treatment were not available in 4%. In the surveillance group, surgical treatment (34% vs. 25%) and RFA (23% vs. 7%) were more often performed than in patients

in the non-surveillance group. In total, 16% and 17% of patients in the surveillance respectively non-surveillance groups received TACE/TARE. Proportion of patients who received sorafenib was lower in the surveillance group (3% vs. 14%). Applied treatments did not differ between the two interval groups (Table 2).

Survival

Data on vital status were available in 999 patients (93% of all included patients). The median follow-up after HCC diagnosis was 11 months (range 0.1-95) and was significantly longer in the surveillance group (15 months vs. 10 months). In total, 58% of all patients (n=623) died during follow-up. Observed 1-, 3- and 5-year survival rates were significantly higher in the surveillance group than in the non-surveillance group (68%, 47% and 39% vs. 55%, 29% and 22%, respectively) (Figure 2, log rank test P<0.001). When evaluating the survival rates of the two time interval groups separately, both groups had survival benefit compared to the non-surveillance group (hazard ratio (HR) 0.64, 95% CI 0.51-0.81 for interval ≤9 months and HR 0.55, 95% CI 0.42-0.73 for interval >9 months). However, there was no significant survival benefit in patients >75 years old in the surveillance group compared to the non-surveillance group (HR 0.85, 95% CI 0.48-1.50).

Figure 2: Observed survival of patients with hepatocellular carcinoma in the surveillance group (dotted line) and non-surveillance group (solid line) (P<0.001)



Number at risk					
Surveillance:	279	123	57	24	6
Non-surveillance:	720	216	86	43	8

Table 1: Patient characteristics of 1074 patients with hepatocellular carcinoma with or without previous surveillance.

	Total group	Surveillance group	Interval ≤9 months	Interval >9 months	P-value *	Non-surveillance group	P-value **
Patient nr	1074 (100)	295 (27)	178 (60)	117 (40)		779 (73)	
Male gender	814 (76)	229 (78)	139 (78)	90 (77)	0.814	585 (75)	0.387
Age at HCC diagnosis (median, range)	63 (8-91)	60 (19-90)	60 (19-90)	61 (29-85)	0.375	64 (8-91)	<0.001
Etiology					0.348		<0.001
Chronic viral hepatitis							
Hepatitis B	171 (16)	58 (20)	29 (16)	29 (25)		113 (14)	
Hepatitis C	206 (19)	113 (38)	76 (43)	37 (32)		93 (12)	
Co-infection	16 (2)	8 (3)	4 (2)	4 (3)		8 (1)	
Hemochromatosis	20 (2)	2 (1)	1 (<1)	1 (1)		18 (2)	
Alcohol	306 (28)	71 (24)	42 (24)	29 (25)		235 (30)	
NAFLD	176 (16)	22 (7)	11 (6)	11 (9)		154 (20)	
Others	33 (3)	13 (4)	10 (6)	3 (3)		20 (3)	
No risk factors known	146 (14)	8 (3)	5 (3)	3 (2)		138 (18)	
Presence of cirrhosis	756 (70)	286 (97)	176 (99)	110 (94)	0.018	470 (60)	<0.001

Results indicate nrs and, between brackets, percentages. *P-value applies to differences between the two surveillance interval groups; **P-value applies to differences between the surveillance versus non-surveillance groups; HCC=hepatocellular carcinoma; co-infection=hepatitis B+C infection. NAFLD= non-alcoholic fatty liver disease.

Table 2: Clinical and tumor characteristics of 1074 patients with hepatocellular carcinoma subdivided according to presence of surveillance prior to HCC diagnosis

	Total group	Surveillance group	Interval ≤9 months	Interval >9 months	P-value *	Non-surveillance group	P-value **
Patient no.	1074 (100)	295 (27)	178 (60)	117 (40)		779 (73)	
No. of lesions					0.856		<0.001
1	551 (51)	165 (56)	97 (55)	68 (58)		386 (50)	
2	125 (12)	52 (18)	33 (18)	19 (16)		73 (9)	
3	65 (6)	18 (6)	10 (6)	8 (7)		47 (6)	
Multifocal/diffuse	333 (31)	60 (20)	38 (21)	22 (19)		273 (35)	
Tumor size (cm)	5.0 (0.8-26.0)	2.7 (0.8-17.0)	2.5 (0.8-17.0)	3.0 (0.8-12.0)	0.025	6.0 (0.9-26.0)	<0.001
Performance score					0.831		0.277
0	382 (36)	108 (37)	65 (37)	43 (37)		274 (35)	
1	354 (33)	99 (34)	60 (34)	39 (33)		255 (33)	
2	115 (11)	24 (8)	13 (7)	11 (9)		91 (11)	
>2	57 (5)	12 (4)	9 (5)	3 (3)		45 (6)	
Unknown	166 (15)	52 (17)	31 (17)	21 (18)		114 (15)	
Right upper quadrant abdominal pain							
Yes	318 (30)	35 (12)	23 (13)	12 (10)	0.489	283 (36)	<0.001
No	756 (70)	260 (88)	155 (87)	105 (90)		496 (64)	
Weight loss							
Yes	219 (20)	21 (7)	13 (7)	8 (7)	0.879	198 (25)	<0.001
No	885 (80)	274 (93)	165 (93)	109 (93)		581 (75)	
BCLC stage					0.183		<0.001
0	65 (6)	43 (15)	33 (19)	10 (9)		22 (3)	
A	277 (26)	136 (46)	77 (43)	59 (50)		141 (18)	
B	378 (35)	61 (21)	34 (19)	27 (23)		317 (41)	
C	273 (26)	37 (12)	23 (13)	14 (12)		236 (30)	
D	81 (7)	18 (6)	11 (6)	7 (6)		63 (8)	

ALT (U/L)	47 (4-1158)	44 (4-445)	45 (4-282)	44 (14-445)	0.651	49 (7-1158)	0.074
AFP (μg/L)	28 (1-2.7x10^6)	16 (1-2.9x10^5)	19 (1-2.9x10^5)	12 (1-1.9x10^4)	0.092	44 (1-2.7x10^6)	<0.001
MELD score	9 (6-33)	10 (6-28)	10 (6-28)	9 (6-20)	0.087	9 (6-33)	<0.001
Treatments					0.452		<0.001
Surgical therapy	300 (28)	101 (34)	57 (32)	44 (38)		199 (25)	
Resection	191 (18)	31 (10)	13 (7)	18 (15)		160 (20)	
Transplantation	104 (10)	67 (23)	44 (25)	23 (20)		37 (5)	
Both	5 (<1)	3 (1)	0 (0)	3 (3)		2 (<1)	
RFA$^{\$}$	120 (11)	66 (23)	37 (21)	29 (25)		54 (7)	
TACE/TARE^	177 (17)	48 (16)	33 (19)	15 (13)		130 (17)	
Sorafenib	115 (11)	10 (3)	8 (4)	2 (2)		105 (14)	
Best supportive care	313 (29)	62 (21)	29 (22)	23 (19)		250 (32)	
Unknown	49 (4)	8 (3)	4 (2)	4 (3)		41 (5)	

Results indicate nrs and, between brackets, percentages; Continuous variables reported as medians and ranges; $^{\$}$9 patients received RFA and subsequently TACE with more than 1-month interval; ^in 28 patients combined TACE and RFA within a 1-month interval was performed as initial therapy; *P-value applies to differences between the two surveillance interval groups; **P-value applies to differences between the surveillance versus non-surveillance groups; BCLC stage=tumor stage according Barcelona Clinic Liver Cancer staging system; ALT=alanine aminotransferase; AFP=alpha-fetoprotein; RFA=radiofrequency ablation; TACE=transarterial chemoembolization; TARE=transarterial radioembolization.

Based on assumed HCC median tumor doubling times of 60 days or 90 days, survival benefit for the surveillance group remained significant after adjustment for lead-time bias based on the approach of Duffy *et al.* [23] (HR 0.74, 95% CI 0.61-0.90 and HR 0.79, 95% CI 0.65-0.95). However when HCC tumor doubling time ≥120 days was assumed, survival benefit disappeared (HR 0.83, 95% CI 0.69-1.01).
In multivariate analysis, surveillance was an independent predictor for lower overall mortality (adjusted HR 0.51, 95% CI 0.39-0.67 for interval ≤9 months and adjusted HR 0.50, 95% CI 0.37-0.69 for interval >9 months) after adjusting for age, cause of underlying liver disease, presence of cirrhosis, right upper quadrant abdominal pain symptoms and weight loss, PS and MELD score (Table 3). Nevertheless, when also adjusted for BCLC stage and applied treatment, surveillance was no longer an independent predictor for overall mortality (Supplementary table 1). Analysis in the subgroup of cirrhotics (adjusted HR 0.49, 95% CI 0.37-0.65 for interval ≤9 months and adjusted HR 0.48, 95% CI 0.35-0.67 for interval >9 months) and in the subgroup of patients in whom surveillance is recommended according to AASLD guidelines (adjusted HR 0.49, 95% CI 0.37-0.65 for interval ≤9 months and adjusted HR 0.49, 95% CI 0.36-0.68 for interval >9 months) yielded similar results. On the other hand, >62 years of age, HCV as cause of underlying liver disease, presence of cirrhosis, a worse PS, right upper quadrant abdominal pain, weight loss and a higher MELD score were independent predictors for higher overall mortality (Table 3).

Discussion

This "real life" study showed that HCC patients who underwent surveillance prior to diagnosis had a smaller tumor size, earlier BCLC tumor stage and lower AFP levels, received more often surgical therapy and had a better overall survival than patients without surveillance. Also, in multivariate analysis, HCC surveillance was found to be an independent predictor of better survival. The findings are in line with previously published retrospective cohort studies, meta-analyses and systematic reviews [11,12]. After additional adjustment for BCLC stage and applied treatment, surveillance was no longer an independent predictor for overall mortality. This interesting finding suggests that surveillance allows better survival through HCC detection in earlier BCLC stage, enabling more effective therapy. Since the two available randomized controlled trials [8,10] on HCC surveillance yielded contradictory results [9,11], lower level of evidence from observational studies could contribute to advises in guidelines [11,13,24]. Potential limitations of observational studies are length-time bias (i.e. detection of indolent tumors by surveillance) and/or lead-time bias (i.e. detection of tumors in earlier stage by surveillance). Statistical techniques to adjust for lead-time bias were performed in five earlier observational studies [25-29]: in three studies [26-28] the formula of Schwartz *et al.* [30] was used, whereas the remaining studies [25,29] used the approach of Duffy

Table 3: Relation between patient/tumor characteristics and mortality in HCC patients in the Netherlands: univariate and multivariate Cox proportional hazard regression analyses.

	Patients	Univariate analysis			Multivariate analysis	
Variables	**n= 999**	**HR**	**95% CI**	**P-value**	**HR**	**95% CI**
Sex						
Male	763 (76)	Ref	-	0.548		
Female	236 (24)	0.94	0.78-1.14			
Age at HCC diagnosis						
≤62 years	466 (47)	Ref	-	0.005	Ref	-
>62 years	533 (53)	1.26	1.07-1.48		1.26	1.04-1.53
Etiology						
Hepatitis B	152 (15)	Ref	-		Ref	-
Hepatitis C	190 (19)	1.44	1.09-1.92		1.81	1.32-2.48
Co-infection	15 (2)	0.74	0.34-1.61		0.66	0.28-1.53
Hemochromatosis	20 (2)	1.21	0.66-2.22	0.037	1.09	0.58-2.05
Alcohol	292 (29)	1.56	1.20-2.03		1.18	0.88-1.59
NAFLD	168 (17)	1.48	1.10-1.98		1.21	0.86-1.69
Other	30 (3)	1.23	0.72-2.11		1.41	0.79-2.51
No risk factors known	132 (13)	1.40	1.03-1.90		1.04	0.72-1.49
Presence of cirrhosis						
Yes	709 (71)	Ref	-	0.033	Ref	-
No	279 (28)	0.93	0.78-1.11		0.76	0.59-0.97
Unknown	11 (1)	2.05	1.13-3.74		1.31	0.66-2.61
Surveillance						
Interval ≤9 months	167 (17)	0.64	0.51-0.81		0.51	0.39-0.67
Interval >9 months	112 (11)	0.55	0.42-0.73	<0.001	0.50	0.37-0.69
No	720 (72)	Ref	-		Ref	-
Performance status						
0	357 (36)	Ref	-		Ref	-
1	337 (34)	1.46	1.19-1.78	<0.001	1.25	1.00-1.57
2	106 (10)	2.65	2.02-3.46		1.99	1.45-2.71
>2	50 (5)	8.28	5.96-11.50		6.81	4.59-10.11
Unknown	149 (15)	1.84	1.45-2.33		1.61	1.22-2.12
Right upper quadrant abdominal pain						
Yes	284 (28)	1.69	1.43-1.99	<0.001	1.59	1.17-1.80
No	715 (72)	Ref	-		Ref	-
Weight loss						
Yes	195 (20)	1.91	1.59-2.30	<0.001	1.45	1.17-1.80
No	804 (80)	Ref	-		Ref	-
Year of HCC diagnosis						
2005	66 (7)	1.31	0.86-2.01			
2006	69 (7)	0.90	0.58-1.40			
2007	92 (9)	0.95	0.62-1.45			
2008	126 (13)	1.27	0.86-1.87	0.060		
2009	143 (14)	1.32	0.90-1.93			
2010	164 (16)	1.44	0.99-2.10			
2011	180 (18)	1.29	0.88-1.90			
2012	159 (16)	Ref	-			
MELD score	869 (87)	1.09	1.07-1.12	<0.001	1.09	1.06-1.12

Values in parentheses are percentages; HR=hazard ratio; 95% CI= 95 percent confidence interval; Co-infection=hepatitis B+C infection; NAFLD=non-alcoholic fatty liver disease.

et al. [23]. In three of these, survival advantage of screening disappeared with assumed tumor doubling times of ≥90 days [25,26,28]. In line with these data, in the current study survival benefit remained when tumor doubling times of 60 or 90 days was assumed, but disappeared with doubling times ≥120 days. We adjusted lead-time bias according to Duffy *et al.* [23], which may be the most correct method. When we used the formula of Schwartz *et al.* [30] survival benefit remained significant with assumed tumor doubling time of 60 days, but not with doubling times of 90 or 120 days (HR 0.82, 95% CI 0.68-0.99, HR 0.93, 95% CI 0.77-1.12 and HR 1.03, 95% CI 0.86-1.25 respectively).

Based on available data on HCC tumor doubling times, approximately 40% of HCCs could have a tumor doubling time of less than 90 days [29,31-35]. Nevertheless, this is controversial [36] and further research is needed to evaluate HCC tumor doubling time, to identify possible factors associated with slow- and fast-growing tumors and to define the impact of this information on screening strategies. Also, in a recently published study [25] survival benefit of HCC surveillance after correction for lead-time bias became evident after three years follow-up. In the current study, median follow-up time was 11 months.

Interestingly, in most earlier observational studies, patients in the non-surveillance group had more severe liver disease than patients in the surveillance group [11,12]. In contrast, in the current study, liver disease was worse in the surveillance group (cirrhosis in 97% vs. 60%; P<0.001 and MELD score higher: 10 vs. 9; P<0.001) thus excluding severity of liver disease as bias explaining observed better survival in our surveillance group. A potential explanation for our relatively low prevalence of cirrhosis in the non-surveillance group could be the relatively large contribution of HCC without risk factors for underlying liver disease (14% of total) in our cohort (especially in the non-surveillance group). This could be related to the low contribution of viral hepatitis to HCC in the Netherlands with low prevalence of HBV and HCV (both estimated 0.2-0.4% of the general population [37-39].

In previous observational studies 60-100% of patients in the surveillance group and 20-56% of patients in the non-surveillance group exhibited HCC within Milan criteria [11]. In contrast, 61% of our surveillance group and 21% of our non-surveillance group had HCC within Milan criteria. Resection was performed in 10% of our surveillance group vs. 20% in our non-surveillance group (3-24% in previously published surveillance groups [11]) and transplantation in 23% of our surveillance group vs. 5% of our non-surveillance group (1-30% in previously published surveillance groups [11]). Differences in treatment modalities applied in the current vs. previous studies could be due, at least in part, to the high percentage (97%) of cirrhotics in our surveillance group and the relatively large contribution of patients without underlying liver disease (favoring resection) in our non-surveillance group.

In the current report, observed 1-, 3- and 5-year survival rates were 68%, 47% and 39% vs. 55%, 29% and 22% in the surveillance vs. non-surveillance group, respectively. Other studies also reported better survival in the surveillance group than in the non-surveillance group. For comparison, previously reported pooled 3-year survival rate of 36 studies was 51% in surveillance groups vs. 28% in non-surveillance groups [12]. However, there was no significant survival benefit in our patients >75 years old who underwent surveillance. Probably, older patients are less likely candidates for curative surgical treatment. Therefore the cost benefit for surveillance in elderly patients needs further investigations.

Recall strategy and adherence to follow-up are important factors for success of a surveillance program [40,41]. A large retrospective cohort study in HCC patients with a prior diagnosis of cirrhosis demonstrated that only 17% received regular and 38% inconsistent surveillance [40]. Utilization of surveillance declined with time. In the current study median time interval between previous negative surveillance imaging and HCC diagnosis (7.5 months) was significantly longer than the surveillance interval of 6 months that is advised in the current guidelines [4-6], indicating that surveillance programs can be improved. Furthermore, 62% of patients in the non-surveillance group had an indication for HCC surveillance based on AASLD guideline criteria [4,19]. However, in the majority of these patients presence of liver disease was unknown. In the current study, virtually all patients received surveillance by ultrasound, with or without concomitant AFP. Surveillance was associated with survival benefit in subgroups with surveillance interval ≤9 months as well as surveillance interval >9-18 months, despite significantly larger tumor sizes in the latter group. Potential explanations could be that many tumors in the latter group exceeded limit of ultrasonographic detection only after prolonged time periods after last negative radiological surveillance imaging and/or relatively slow tumor growth. Similar findings were reported in a study from Taiwan comparing 4- and 12-month US screening intervals [17]. We cannot exclude that strict surveillance based on the recommended interval of 6 months could lead to better outcomes, although this is difficult to achieve in clinical practice [40,42].

In the current study, survival was not only determined by tumor features and treatment. Older age, HCV as cause of underlying disease and higher MELD scores were independent predictors for higher overall mortality. One previous study also reported a positive correlation between age and mortality [15], but this was not confirmed in other studies [16,43]. Although Trevisani *et al.* [43] suggested that prognosis is independent of etiology of liver disease, several other studies indicated that cause of underlying liver disease is an independent risk factor for higher mortality in HCC patients in line with our results [44-46]

The strength of our study is that a large cohort of Western HCC patients was included to examine the effect of surveillance in "real life" clinical practice. Also, there appears to be no bias from overrepresentation of cirrhosis in the group without surveillance (in

our study, cirrhosis prevalence was higher in the surveillance group). However, findings of this study are inherently limited by the retrospective study design. Despite correction for lead-time bias, surveillance might still preferentially detect slowly growing indolent tumors (length-time bias). Only patients who were diagnosed or referred to one of the five Dutch academic centers were included. HCC patients with a very poor prognosis could not have been referred.

In conclusion, in this "real life" study, HCC surveillance was associated with a smaller tumor size, earlier BCLC tumor stage, with impact on therapeutic strategy and was an independent predictor of increased survival.

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Supplementary table 1: Relation between patient/tumor characteristics and mortality in HCC patients in the Netherlands: univariate and multivariate Cox proportional hazard regression analyses. Uni- and multivariate analyses include BCLC stage and applied treatment.

	Patients	Univariate analysis			Multivariate analysis	
Variables	**n= 999**	**HR**	**95% CI**	**P-value**	**HR**	**95% CI**
Sex						
Male	763 (76)	Ref	-	0.548		
Female	236 (24)	0.94	0.78-1.14			
Age at HCC diagnosis						
≤62 years	466 (47)	Ref	-	0.005	Ref	-
>62 years	533 (53)	1.26	1.07-1.48		1.19	0.98-1.44
Etiology						
Hepatitis B	152 (15)	Ref	-		Ref	-
Hepatitis C	190 (19)	1.44	1.09-1.92		2.18	1.57-3.02
Co-infection	15 (2)	0.74	0.34-1.61		1.11	0.47-2.66
Hemochromatosis	20 (2)	1.21	0.66-2.22	0.037	1.28	0.68-2.42
Alcohol	292 (29)	1.56	1.20-2.03		1.19	0.87-1.61
NAFLD	168 (17)	1.48	1.10-1.98		1.16	0.82-1.65
Other	30 (3)	1.23	0.72-2.11		1.99	1.10-3.62
No risk factors known	132 (13)	1.40	1.03-1.90		1.11	0.47-2.66
Presence of cirrhosis						
Yes	709 (71)	Ref	-	0.033	Ref	-
No	279 (28)	0.93	0.78-1.11		0.78	0.61-0.99
Unknown	11 (1)	2.05	1.13-3.74		0.61	0.28-1.22
Surveillance						
Interval ≤9 months	167 (17)	0.64	0.51-0.81		0.80	0.60-1.06
Interval >9 months	112 (11)	0.55	0.42-0.73	<0.001	0.78	0.56-1.08
No	720 (72)	Ref	-		Ref	-
Performance status						
0	357 (36)	Ref	-		Ref	-
1	337 (34)	1.46	1.19-1.78	<0.001	1.07	0.84-1.35
2	106 (10)	2.65	2.02-3.46		1.49	1.08-2.05
>2	50 (5)	8.28	5.96-11.50		3.43	1.95-6.02
Unknown	149 (15)	1.84	1.45-2.33		1.40	1.06-1.86
Right upper quadrant abdominal pain						
Yes	284 (28)	1.69	1.43-1.99	<0.001	1.39	1.13-1.72
No	715 (72)	Ref	-		Ref	-
Weight loss						
Yes	195 (20)	1.91	1.59-2.30	<0.001	0.95	0.76-1.20
No	804 (80)	Ref	-		Ref	-
Year of HCC diagnosis						
2005	66 (7)	1.31	0.86-2.01			
2006	69 (7)	0.90	0.58-1.40			
2007	92 (9)	0.95	0.62-1.45			
2008	126 (13)	1.27	0.86-1.87	0.060		
2009	143 (14)	1.32	0.90-1.93			
2010	164 (16)	1.44	0.99-2.10			
2011	180 (18)	1.29	0.88-1.90			
2012	159 (16)	Ref	-			
MELD score	869 (87)	1.09	1.07-1.12	<0.001	1.04	1.01-1.08
BCLC stage						
0	63 (6)	Ref	-		Ref	-
A	266 (27)	1.35	0.83-2.21	<0.001	1.61	0.94-2.74
B	354 (35)	3.07	1.92-4.91		2.71	1.58-4.65
C	245 (25)	8.82	5.49-14.16		3.68	2.06-6.58
D	71 (7)	16.69	9.97-27.93		2.87	1.43-5.78

Treatment						
Surgical therapy	291 (29)	Ref	-		Ref	-
RFA	115 (12)	1.96	1.39-2.77		2.17	1.48-3.18
TACE/TARE	169 (17)	3.74	2.79-5.01	<0.001	3.65	2.60-5.14
Sorafeinb	104 (10)	9.19	6.69-12.62		6.06	4.00-9.20
Best supportive care	278 (28)	14.97	11.44-19.59		8.50	5.89-12.27
Unknown	42 (4)	7.41	4.89-11.22		5.44	3.28-9.00

Values in parentheses are percentages; HR=hazard ratio; 95% CI= 95 percent confidence interval; Co-infection=hepatitis B+C infection; NAFLD=non-alcoholic fatty liver disease; RFA=radiofrequency ablation; TACE=transarterial chemoembolization; TARE=transarterial radioembolization.

CHAPTER 6

PATIENTS' MISCONCEPTIONS ABOUT SURVEILLANCE FOR HEPATOCELLULAR CARCINOMA: EDUCATION IS NEEDED

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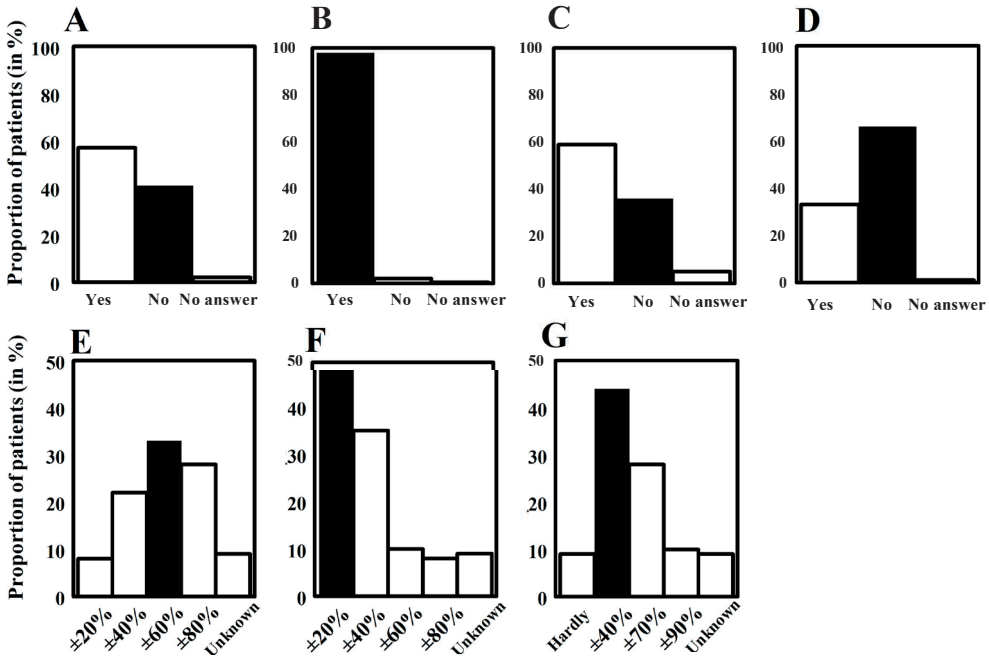
In follow up of recent publications in Journal of Hepatology [1,2], we would like to discuss patients' expectations of hepatocellular carcinoma (HCC) surveillance. Regular surveillance of high-risk patients is recommended by international guidelines [3]. The goal of surveillance is to improve the dismal prognosis by detecting HCC at earlier stages with potential curative treatment options. Unfortunately, sensitivities and specificities of ultrasound and alpha-fetoprotein (AFP) (the most widely used surveillance modalities) to detect HCC are far from perfect and high-level evidence supporting efficiency of surveillance is limited. As a result, HCC surveillance is highly controversial [4].

In clinical practice, there is widespread underuse of HCC surveillance: In the United States only 17% of 1873 cirrhotic patients who developed HCC received regular surveillance [5]. Also in the Netherlands, only 27% of patients underwent surveillance before HCC diagnosis [1]. Possible explanations for this underuse are a lack of knowledge, low detection rate of at-risk individuals, logistic factors and non-adherence of patients [5].

Patient knowledge and involvement in decision-making are associated with higher HCC surveillance rates [6]. We recently noticed a profound discrepancy between patients' expectations of HCC surveillance and real benefits in clinical practice, similar to the overestimation by women of the benefits of mammography for breast cancer surveillance [7]. We therefore asked 120 consecutive patients who visited our outpatient clinic and underwent regular HCC surveillance (ultrasound with or without AFP) at an approximately 6-month interval to fill in a 7-item questionnaire regarding their perceptions on HCC surveillance (response rate 92%, period: 2015). Furthermore, expectations of 7 Dutch experts in the field of hepatology were evaluated based on a similar questionnaire. The Figure shows expectations in the 111 responding high-risk patients with underlying chronic liver disease (i.e. 91 cirrhotic patients due to alcohol (n=14), hepatitis B (n=11), hepatitis C (n=32), NASH (n=11) or other risk factors (n=23), and 20 non-cirrhotic patients with increased HCC risk due to hepatitis B (n=18) or F3 fibrosis in hepatitis C (n=2)). In contrast to current knowledge, most patients expect that: 1. performing regular surveillance can prevent HCC development (Fig 1A), 2. HCC is always detected by ultrasound (Fig 1C), and 3. surveillance is only indicated if patients have complaints (Fig 1D). In multivariate logistic regression analyses (included variables: education level, gender, age, social-economic status, etiology of underlying liver disease, presence of cirrhosis, number of performed surveillance tests), only lower education level was an independent predictor of misconception.

Second, patients largely underestimate HCC-related mortality rates. Nearly 50% of patients think that curative treatment is still possible in ≥40% of HCC cases detected without surveillance (Fig 1F). Furthermore, many patients believed that surveillance reduces the risk of HCC deaths by ≥70% (Fig 1G). Unfortunately, based on current literature, numbers are less positive: curative treatment is possible in approximately 20% of HCC patients diagnosed without surveillance in Western countries [1,8]. The

Figure 1: Patients' perceptions of HCC surveillance. A: The goal of surveillance is to prevent liver cancer development; B: The goal of surveillance is to detect liver cancer in early stage when curative treatment is still possible; C: Abdominal ultrasound always detects liver cancer if present; D: Surveillance for liver cancer is only necessary if patients have complaints; E: When HCC is detected by surveillance, curative treatment is still possible in ±20%, 40%, 60% or 80%; F: When HCC is detected in patients without surveillance, curative treatment is still possible in ±20%, 40%, 60% or 80%; G: By performing surveillance with abdominal ultrasound at a 6-month interval, the 5-year mortality of HCC patients is reduced: hardly, with ±40%, 70% or 90%. Correct answers are marked with a solid black bar.



only randomized controlled trial on efficacy of HCC surveillance with ultrasound in chronic hepatitis B patients, demonstrated that surveillance lowered mortality rates by 37% [9]. However, several aspects of this study have been criticized [4]. In contrast to the first part of the questionnaire (Fig A-D), lower education level was no predictor for misconception about HCC-related mortality in multivariate analyses (Fig E-G). Expectations of Dutch experts in the field of hepatology were markedly lower than patients' expectations. For example, most experts thought that HCC surveillance in the current form does not lower HCC-related mortality at all.

Overestimation by patients of benefits of cancer surveillance programs is common [7]. Misconceptions impair the ability to make informed decisions. For example, clearing the misconception that surveillance is only indicated in case of complaints may result in higher rates of adequate surveillance. In the current era of increased awareness and doctors' obligations to provide adequate information about benefits and risk, better

education on HCC surveillance is needed in high-risk patients with underlying chronic liver disease. Finally, focus should be on healthcare delivery, for example by the implementation of quality improvement measures including recall policies, which will increase surveillance rates [10].

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CHAPTER 7

HEPATOCELLULAR CARCINOMA IN CIRRHOTIC VERSUS NON-CIRRHOTIC LIVERS: RESULTS FROM A LARGE COHORT IN THE NETHERLANDS

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ABSTRACT

Objectives: Hepatocellular carcinoma (HCC) usually occurs in patients with cirrhosis, but can also develop in non-cirrhotic livers. We here explore associated risk factors for HCC without cirrhosis and compare patient and tumor characteristics and outcomes in HCC patients with and without underlying cirrhosis.

Methods: Patients with HCC diagnosed in the period 2005-2012 in five Dutch academic centers were evaluated. Patients were categorized according to the presence of cirrhosis based on histology or combined radiological and laboratory features.

Results: In total, 19% of the 1221 HCC patients had no underlying cirrhosis. Non-cirrhotic HCC patients were more likely to be female and to have non-alcoholic fatty liver disease (NAFLD) or no risk factors for underlying liver disease, and less likely to have hepatitis C (HCV) or alcohol-related liver disease than cirrhotic HCC patients. HCCs in non-cirrhotic livers were more often unifocal (67% vs. 48%), but tumor size was significantly larger (8 vs. 4 cm). Despite the larger tumors, more patients underwent resection (50% vs. 10%) and overall survival was significantly better than in cirrhotics. In multivariate analyses, absence of cirrhosis (HR 0.49, 95% CI 0.38-0.63) and presence of hepatitis B (HR 0.68, 95% CI 0.51-0.91) were independent predictors for lower mortality, whereas HCV was associated with higher mortality (HR 1.32, 95% CI 1.01-1.65).

Conclusion: HCC without cirrhosis was strongly associated with female gender and presence of NAFLD or no risk factors for underlying liver disease. In absence of cirrhosis, resections were more often performed, with better survival despite larger tumor size.

Introduction

Primary liver cancer is the sixth most common cancer in the world and the second cause of cancer-related death [1]. Hepatocellular carcinoma (HCC) represents more than 90% of all primary liver cancers and typically occurs in patients with underlying cirrhosis. Nevertheless, HCC can also develop in non-cirrhotic livers. Based on previous studies, the proportion of HCC in the absence of cirrhosis varied widely (from 2% to 54%) between various geographical regions [2-7]. This may be the consequence of different patterns of underlying risk factors, such as viral hepatitis, alcohol abuse and non-alcoholic fatty liver disease (NAFLD).

Currently, NAFLD is a leading cause of chronic liver disease in Western countries and its prevalence is expected to increase further [8,9]. There is emerging evidence that presence of NAFLD and features of metabolic syndrome are associated with HCC [10-15]. Increased HCC risk seems to affect especially those with cirrhosis. Nevertheless, it has recently been demonstrated that also in non-cirrhotic livers, presence of NAFLD, and especially non-alcoholic steatohepatitis (NASH), is strongly associated with HCC [13,16-18]. Furthermore, steatosis is often present as co-factor in patients with other risk factors for underlying liver disease [19].

The severity of the underlying liver disease has a great impact on treatment decisions and prognosis in HCC patients: presence of cirrhosis and resulting impaired liver function may limit surgical and non-surgical options. In contrast, absence of cirrhosis could favor use of surgical treatment with curative intent [6,20,21]. Available data also suggest that etiology of underlying liver disease in HCC patients may influence outcomes [22].

In the present study we explore the prevalence of HCC in absence of cirrhosis and investigate associated risk factors in a large cohort of HCC patients. Furthermore, we compare patient and tumor characteristics as well as outcomes in HCC patients with and without underlying cirrhosis and with various causes of underlying liver disease.

Methods

All patients with an HCC diagnosis in the period 2005-2012 in the five major Dutch academic centers were evaluated. Diagnosis of HCC was based on AASLD 2005 and 2011 guideline criteria [23,24]. Data on the influence of HCC surveillance in the same cohort have recently been reported [25]. Collected data were obtained from (electronic) medical records. Extensive efforts were done to clarify all missing data, e.g. by contacting patients, referring hospitals or general practitioners.

Patients were categorized according to the presence or absence of cirrhosis. Patients were included in the “no cirrhosis” group based on the following criteria as essentially proposed by El-Sherag et al [26]: (A) histology without cirrhosis either in biopsy within one year of HCC diagnosis or in a resection specimen in combination with absence of radiological features of cirrhosis, or (B) (in absence of liver histology) all three of the following criteria: (1) an aspartate aminotransferase to platelet ratio index (APRI) ≤1,

(2) two of the three following laboratory tests within normal range: a) albumin >35g/L, b) platelet counts >200x10^9/L, c) INR <1.1, and (3) absence of radiological features of cirrhosis. The APRI score was calculated using the following formula: (AST/upper limit of normal)/platelet count × 100) [27]. Patients who had histology demonstrating cirrhosis or (in absence of histology) clear radiological features of cirrhosis and/or did not fulfill the above mentioned criteria for the "no cirrhosis" group were included in the "cirrhosis" group.

Information was collected on the cause of underlying liver disease: (A) alcohol-related liver disease (defined as a history of average alcohol use ≥3 alcoholic drinks/day [28]), (B) hepatitis B virus (HBV), (C) hepatitis C virus (HCV), (D) hemochromatosis, , (E) NAFLD (defined as steatosis >5%, steatohepatitis on liver biopsy or presence of metabolic syndrome in the absence of other risk factors for chronic liver disease such as alcohol abuse), (F) others or (G) absence of any risk factors for underlying liver disease.

Finally, data on tumor characteristics (e.g. number of tumor lesions, maximum diameter of the largest tumor lesion and tumor stage according to Barcelona Clinic Liver Cancer (BCLC) staging system), laboratory values at time of diagnosis, applied treatment(s) and survival data were obtained for each patient. Patients were categorized into treatment groups based on application of surgical therapy (resection or transplantation), radiofrequency ablation (RFA), transcatheter arterial chemoembolization (TACE) or transarterial radioembolization (TARE), systemic therapy (sorafenib) or best supportive care. Patients undergoing sequential therapy appertaining to two or more treatment groups were included in the treatment group that was presumed to have most impact on outcome. In case of RFA and subsequently TACE with at least a one month interval, patients were included in the RFA group. When a combination of RFA and TACE was performed within a one month interval, patients were included in the TACE group.

This study was in agreement with the ethical guidelines of the Declaration of Helsinki and analyses were performed with institutional medical ethical consent, in an anonymized database.

Statistical analysis

Continuous data were expressed as means and standard deviations (SD) or, in case of a nonparametric distribution, as medians and ranges, and discrete variables as absolute and relative frequencies. Independent samples t-test, one-way ANOVA, Mann-Whitney U or Kruskal-Wallis tests were applied to compare continuous data in the cirrhosis" vs. "no cirrhosis" groups. Post-hoc analyses using ANOVA with Bonferroni correction were performed to compare the five major etiology groups. Categorical variables were compared with Pearson's Chi-square or Fisher's exact tests.

Uni- and multivariate logistic regression analysis was performed to evaluate patient characteristics (i.e. sex, age and etiology of underlying liver disease) associated with risk of HCC in absence of cirrhosis.

Survival time was calculated from date of diagnosis to date of death or end of follow-up (latest: end of study 1-1-2013). Kaplan-Meier survival curves and log-rank tests were used to compare survival rates between the cirrhotic and non-cirrhotic patients in the total group and between the five major etiology groups: HBV, HCV, alcohol-related liver disease, NAFLD and absence of risk factors for underlying liver disease. Survival between cirrhotic and non-cirrhotic patients was also evaluated separately in the different treatment groups, except for RFA since this treatment was almost exclusively performed in cirrhotic patients. Possible predictors for overall mortality were tested by uni- and multivariate Cox proportional hazard regression. Besides the presence of cirrhosis and etiology of underlying liver disease, sex, age, tumor size, number of tumor lesions, BCLC stage and year of HCC diagnosis were included. Factors with a p-value <0.1 in univariate analyses were included in subsequent multivariate analyses. A two-sided p-value <0.05 was considered statistically significant. Statistical analysis was performed using SPSS (version 20; IBM Corp., Armonk, New York, USA).

Results

In the period January 2005- December 2012, 1290 HCC patients were under care in the five academic hospitals (i.e. approximately 60% of all Dutch HCC patients in this period [29]). After exclusion of 69 patients because of missing data, 1221 patients (95%) were included in this study.

Patient and clinical characteristics in the "cirrhosis" vs. "no cirrhosis" groups

In total, 983 of the 1221 included patients (81%) had underlying cirrhosis, without change during the study period. In 238 patients (19%) no cirrhosis was present: fibrosis grade was ≤F2 in 148 patients (62% of all non-cirrhotic patients), F3 in 19 (8%) and unknown in 71 (30%). Patient characteristics of all HCC patients, and separately for the "cirrhosis" and "no cirrhosis" groups, are given in Table 1. Median age at time of HCC diagnosis was 63 years (range 8-91 years) and did not differ between both groups. Proportion of males was significantly lower in non-cirrhotic patients than in cirrhotics (66% vs. 79%; P<0.001). Body mass index (BMI) was also significantly lower in non-cirrhotic than in cirrhotic patients (25.4 kg/m^2 vs. 27.1 kg/m^2, P<0.001). Furthermore, presence of NAFLD (12% vs. 28%) and absence of risk factors for underlying liver disease (idiopathic HCC; 7% vs. 30%) were more common in patients without cirrhosis than in those with cirrhosis. On the other hand, cirrhotic HCC patients were more likely to have alcohol-related liver disease (32% vs. 16%) and HCV (24% vs. 6%). The contribution of HBV-related HCC was similar in the "cirrhosis" and "no cirrhosis" groups (16% vs. 15%) (Table 1). Of the 37 patients with hemochromatosis (respectively 29 and 8 in the cirrhosis and no cirrhosis groups), diagnosis was based on positive genetic testing in 41%, whereas in 59% of patients diagnosis was based on a laboratory features

Table 1: Patient characteristics of 1221 cases with hepatocellular carcinoma subdivided according to presence or absence of cirrhosis

	Total group	Cirrhosis	No cirrhosis	P-value[a]
Patient nr	1221 (100)	983 (81)	238 (19)	
Male gender	936 (77)	779 (79)	157 (66)	<0.001
Age at HCC diagnosis	63 (8-91)	63 (8-91)	65 (11-88)	0.514
BMI (mean, SD)	26.7 (5.0)	27.1 (5.0)	25.4 (4.7)	<0.001
Etiology				<0.001
Alcohol	349 (29)	312 (32)	37 (16)	
Chronic viral hepatitis				
HBV	197 (16)	162 (16)	35 (15)	
HCV	249 (20)	236 (24)	13 (6)	
Co-infection	19 (2)	18 (2)	1 (<1)	
Hemochromatosis	37 (3)	29 (3)	8 (3)	
NAFLD	181 (15)	114 (12)	67 (28)	
Others	43 (3)	39 (4)	4 (2)	
No risk factors known	146 (12)	73 (7)	73 (30)	
ALT (U/L)	47 (4-1193)	49 (4-1193)	39 (8-712)	<0.001
AST (U/L)	66 (14-8678)	71 (15-8678)	46 (14-1344)	<0.001
Albumin	38 (13-62)	37 (13-58)	43 (16-62)	<0.001
Platelets	146 (8-985)	125 (8-985)	259 (62-724)	<0.001
INR	1.1 (0.8-2.9)	1.2 (0.8-2.9)	1.0 (0.8-1.8)	<0.001
PT	13.9 (9.7-36.7)	14.3 (10.0-36.2)	12.3 (9.7-36.7)	<0.001
APRI	1.6 (0.1-304)	2.0 (0.1-304)	0.6 (0.1-32)	<0.001
MELD score	9 (6-33)	10 (6-33)	7 (6-29)	<0.001

Results indicate nrs and, between brackets, percentages. Continuous variables reported as medians and, between brackets, ranges unless otherwise indicated.
ALT, alanine transaminase; APRI, aspartate aminotransferase to platelet ratio index; AST, aspartate aminotransaminase; coinfection, HBV +HCV infection; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; INR, international normalized ratio; MELD, Model For End-Stage Liver Disease; NAFLD, nonalcoholic fatty liver disease; PT, prothrombin time.
[a]P-value applies to the "cirrhosis" versus "no cirrhosis" groups.

(i.e. highly increased ferritin and high iron saturation) and typical histopathological characteristics of hemochromatosis.

Risk factors for HCC in absence of cirrhosis

In multivariate analysis, absence of risk factors for underlying chronic liver disease (odds ratio (OR): 4.28, 95% confidence interval (CI) 2.58-7.10), presence of NAFLD (OR: 2.59, 95% CI 1.58-4.26) and female gender (OR: 1.44, 95% CI 1.01-2.05) were independent risk factors for HCC in absence of cirrhosis. In contrast, presence of alcohol-related liver disease (OR: 0.56, 95% CI 0.33-0.93) and HCV (OR: 0.25, 95% CI 0.13-0.48) were less often present in HCC patients with non-cirrhotic livers (Table 2).

Table 2: Association between patient characteristics and the occurrence of hepatocellular carcinoma in absence of cirrhosis: uni- and multivariate logistic regression analysis

	Patients	Univariate analysis			Multivariate analysis	
Variables	**n= 1221**	**OR**	**95% CI**	**P-value**	**OR**	**95% CI**
Sex						
Male	936 (77)	Ref	-	<0.001	Ref	-
Female	285 (23)	1.97	1.45-2.68		1.44	1.01-2.05
Age at HCC diagnosis						
≤62 years	585 (48)	0.81	0.61-1.08	0.137	1.05	0.75-1.45
>62 years	636 (52)	Ref	-		Ref	-
Etiology						
Alcohol	349 (29)	0.55	0.33-0.91		0.56	0.33-0.93
Hepatitis B	197 (16)	Ref	-		Ref	-
Hepatitis C	249 (20)	0.26	0.13-0.50		0.25	0.13-0.48
Co-infection	19 (2)	0.26	0.03-1.99	<0.001	0.26	0.03-2.03
Hemochromatosis	37 (3)	1.28	0.54-3.03		1.34	0.56-3.24
NAFLD	181 (15)	2.72	1.69-4.37		2.59	1.58-4.26
Other	43 (3)	0.48	0.16-1.42		0.41	0.14-1.24
No risk factors known	146 (12)	4.63	2.84-7.55		4.28	2.58-7.10

Values in parentheses are percentages; CI, confidence interval; coinfection, hepatitis B +hepatitis C infection; HCC, hepatocellular carcinoma; NAFLD, nonalcoholic fatty liver disease; OR, odds ratio.

Tumor characteristics in the "cirrhosis" vs. "no cirrhosis" groups

Number of tumor lesions differed significantly between the cirrhotic and non-cirrhotic patients: unifocal HCC was significantly more common in patients without cirrhosis (48% vs. 67%), whereas proportion of multifocal or diffuse HCC was higher in patients with cirrhosis (32% vs. 23%) (P<0.001) (Table 3). Nevertheless, tumor size was significantly larger in non-cirrhotic patients than in cirrhotic patients (median tumor size: 8 cm versus 4 cm; P<0.001). In patients with cirrhosis, HCC was detected at an earlier tumor stage (BCLC 0 and A combined: 38% vs. 19%) than in patients without cirrhosis. Alpha-fetoprotein (AFP) level was significantly higher in patients with than in those without cirrhosis (35 µg/L vs. 10 µg/L; P<0.001) (Table 3).

Treatment in the "cirrhosis" vs. "no cirrhosis" groups

Despite the larger tumor size in patients without cirrhosis, overall proportion of patients who received surgical therapy was higher than in cirrhotic patients (53% vs. 22%): resection was more often performed in patients without cirrhosis (50% vs. 10%), whereas more patients with cirrhosis underwent transplantation (12% vs. 2%) (Table 3). RFA (15% vs. 2%) and TACE/TARE (18% vs. 13%) were also more often performed in cirrhotic patients. Proportion of patients who received sorafenib was higher in patients without cirrhosis (14% vs. 8%). Almost one-third of the cirrhotic patients received only best supportive care, whereas this was only 15% in non-cirrhotic patients (Table 3).

Table 3: Tumor characteristics of 1221 patients with hepatocellular carcinoma subdivided according to presence or absence of cirrhosis

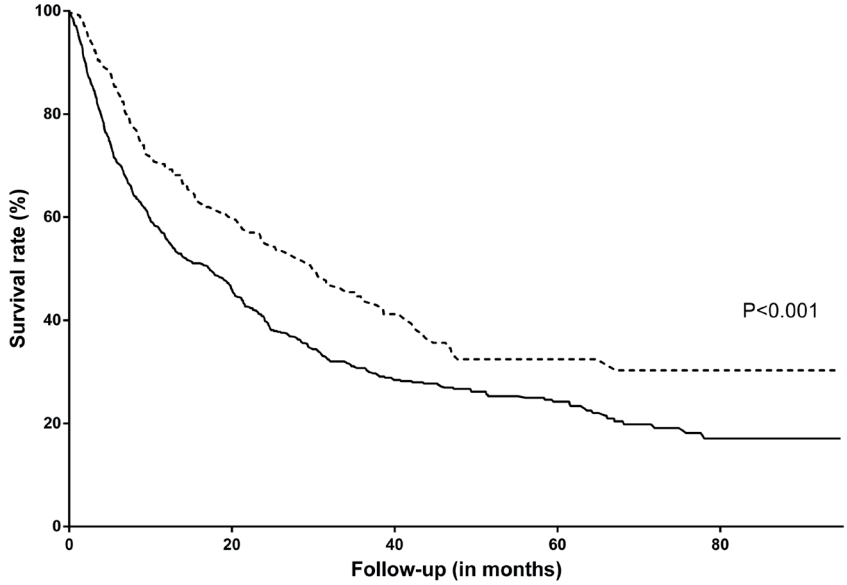
	Total group	Cirrhosis	No cirrhosis	P-value[a]
Patient nr	1221 (100)	983 (81)	238 (19)	
No. of lesions				<0.001
1	632 (52)	473 (48)	159 (67)	
2	152 (12)	132 (14)	20 (8)	
3	68 (6)	63 (6)	5 (2)	
Multifocal/diffuse	369 (30)	315 (32)	54 (23)	
Tumor size (cm)	5 (1-26)	4 (1-26)	8 (1-26)	<0.001
BCLC stage				<0.001
0	75 (6)	72 (7)	3 (1)	
A	345 (28)	301 (31)	44 (18)	
B	406 (33)	274 (28)	132 (56)	
C	299 (25)	247 (25)	52 (22)	
D	96 (8)	89 (9)	7 (3)	
Alpha-fetoprotein (μg/L)	29 (1-2.7x10^6)	35 (1-1.8x10^6)	10 (1-2.7x10^6)	<0.001
Treatments				<0.001
Surgical therapy	341 (28)	215 (22)	126 (53)	
Resection	214 (18)	95 (10)	119 (50)	
Transplantation	120 (10)	116 (12)	4 (2)	
Both	6 (<1)	4 (<1)	2 (1)	
RFA[b]	149 (12)	145 (15)	4 (2)	
TACE/TARE[c]	207 (17)	176 (18)	31 (13)	
Systemic therapy	118 (10)	85 (8)	33 (14)	
Best supportive care	351 (29)	314 (32)	36 (15)	
Unknown	55 (4)	48 (5)	8 (3)	

Results indicate nrs and, between brackets, percentages. Continuous variables reported as medians and, between brackets, ranges.
[a]P-value applies to "cirrhosis" versus "no cirrhosis" groups. [b]Thirteen patients received RFA and subsequently TACE with more than 1 month interval; [c]In 31 patients a combination of TACE and RFA within a one month interval was performed as initial therapy.
BCLC stage, tumor stage according Barcelona Clinic Liver Cancer staging system; RFA, radiofrequency ablation; TACE, transcatheter arterial chemoembolization; TARE, transarterial radioembolization.

Survival in "cirrhosis" vs. "no cirrhosis" groups

Data on vital status at the end of the study were available in 1148 patients (94% of all included patients). Median follow-up of the total group was 12 months (range 0.1–95 months). Follow-up time was significantly longer in non-cirrhotics than in cirrhotics (15 months vs. 11 months; P=0.001). The 1-, 3- and 5-year survival rates were significantly higher in non-cirrhotic than in cirrhotic patients (72%, 49% and 43% vs. 56%, 33%, 29%, respectively) (Fig. 1, log rank test P<0.001). In multivariate analysis, absence of cirrhosis was an independent predictor for lower overall mortality (hazard ratio (HR) 0.49, 95% CI 0.38-0.63), after adjusting for age, etiology of underlying liver disease

Figure 1: Observed survival of patients with hepatocellular carcinoma in the "cirrhosis" group (solid line) and "no cirrhosis" group (dotted line)(Kaplan Meier survival curve; log rank P<0.001)



Number at risk					
Cirrhosis	919	310	129	63	15
No-cirrhosis	229	99	47	24	6

tumor size, number of tumor lesions and BCLC stage (Table 4). An age ≤62 years (HR 0.76, 95% CI 0.63-0.91) and presence of HBV (HR 0.68, 95% CI 0.51-0.91) were also associated with lower mortality. In contrast, presence of HCV (HR 1.32, 95% CI 1.01-1.65), larger tumor size (HR 1.07, 95% CI 1.04-1.09), multifocal or diffuse HCC (HR 1.64, 95% CI 1.31-2.05) and a more advanced BCLC stage were independent predictors for higher overall mortality (Table 4).

In patients who underwent surgical treatment, absence of cirrhosis (HR 0.38, 95%CI 0.21-0.71) and treatment with transplantation instead of resection (HR 0.24, 95%CI 0.13-0.42) were both independently associated with lower overall mortality, after adjustment for etiology of underlying liver disease, tumor size, number of tumor lesions and BCLC stage. In patients who received TACE or sorafenib treatment, survival was not different between cirrhotic and non-cirrhotic patients (results not shown). RFA was almost exclusively performed in cirrhotic patients and therefore impact of cirrhosis could not be evaluated.

Table 4: Relation between patient/tumor characteristics and mortality in 1148 patients with hepatocellular carcinoma: univariate and multivariate Cox proportional hazard regression analyses.

	Patients	Univariate analysis			Multivariate analysis	
Variables	**n= 1148**	**HR**	**95% CI**	**P-value**	**HR**	**95% CI**
Presence of cirrhosis						
Yes	919 (80)	Ref	-	<0.001	Ref	-
No	229 (20)	0.67	0.55-0.82		0.49	0.38-0.63
Sex						
Male	879 (77)	Ref	-	0.46		
Female	269 (23)	0.94	0.79-1.12			
Age at HCC diagnosis						
≤62 years	543 (47)	0.77	0.67-0.90	0.001	0.76	0.63-0.91
>62 years	605 (53)	Ref	-		Ref	-
Etiology						
Alcohol	334 (29)	Ref	-		Ref	-
Hepatitis B	179 (16)	0.59	0.46-0.75		0.68	0.51-0.91
Hepatitis C	232 (20)	0.84	0.68-1.03		1.32	1.01-1.65
Co-infection	18 (2)	0.44	0.22-0.89	0.001	0.71	0.33-1.52
Hemochromatosis	36 (3)	0.96	0.63-1.47		0.94	0.57-1.56
NAFLD	175 (15)	0.90	0.71-1.13		0.92	0.70-1.21
Other	40 (3)	0.81	0.53-1.25		1.30	0.79-2.12
No risk factors known	134 (12)	0.44	0.22-0.89		0.70	0.32-1.49
Tumor size[a]	1008 (88)	1.08	1.06-1.09	<0.001	1.07	1.04-1.09
Number of tumor lesions						
1	602 (53)	Ref	-		Ref	-
2	142 (12)	1.24	0.97-1.59		1.27	0.98-1.65
3	66 (6)	1.55	1.12-2.15	<0.001	1.29	0.92-1.83
Multifocal (>3)/diffuse	338 (29)	3.93	3.31-4.66		1.64	1.31-2.05
BCLC stage						
0	73 (7)	Ref	-		Ref	-
A	334 (29)	1.18	0.77-1.82	<0.001	1.09	0.70-1.71
B	389 (34)	2.79	1.85-4.22		1.98	1.24-3.17
C	268 (23)	8.07	5.32-12.24		4.46	2.71-7.34
D	84 (7)	10.08	6.38-15.94		7.87	4.75-13.06
Year of HCC diagnosis						
2005	78 (7)	1.06	0.72-1.57			
2006	81 (7)	0.81	0.54-1.21			
2007	107 (9)	0.83	0.56-1.21			
2008	155 (14)	1.08	0.76-1.54	0.15		
2009	170 (15)	1.10	0.77-1.55			
2010	187 (16)	1.21	0.86-1.71			
2011	197 (17)	1.11	0.78-1.59			
2012	173 (15)	Ref	-			

Values in parentheses are percentages; CI, confidence interval; BCLC stage, tumor stage according Barcelona Clinic Liver Cancer staging system; coinfection, hepatitis B +hepatitis C infection; HR, hazard ratio; NAFLD, nonalcoholic fatty liver disease.
[a]Missing values: n=140.

Patient and clinical characteristics and outcome in relation to etiology of underlying liver disease

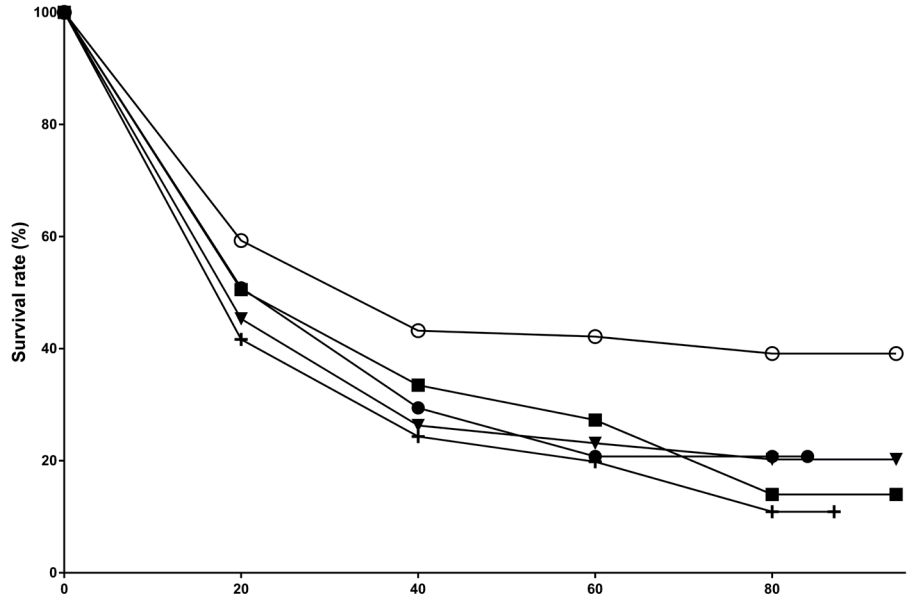
Of all included patients (n=1221), 29% had underlying alcohol-related liver disease, 20% HCV, 16% HBV, 15% NAFLD and 12% no risk factors. Patient characteristics were significantly different between the five major etiology groups (in total 1122 patients, included in the following analyses) (Supplementary table 1). Patients with HBV or HCV who developed HCC were significantly younger than patients with alcohol- related liver disease, NAFLD or absence of risk factors (median age: 57 and 59 years vs. 65, 67 and 68 years, respectively). Furthermore, female gender was significantly more frequent in patients with NAFLD (34%) or with no risk factors (42%) than in patients with HBV (15%), HCV (21%) or alcohol-related liver disease (15%). BMI was significantly higher in patients with alcohol- or NAFLD-related HCC (mean BMI 27.8 kg/m^2 and 28.9 kg/m^2, respectively) than in patients with HBV (25.8 kg/m^2), HCV (26.4 kg/m^2) or absence of risk factors (24.2 kg/m^2) (Supplementary table 1).

Alcohol-related HCCs were significantly more often multifocal or diffuse (38%) than HCCs in patients with HCV, HBV, NAFLD or no risk factors (range: 24-30%). Furthermore, tumor size was significantly larger in absence of risk factors (9 cm) and smaller in HCV patients (3 cm) than in patients with HBV (4 cm), alcohol-related liver disease (5 cm) or NAFLD (6 cm). BCLC tumor stage was also significantly more advanced in absence of risk factors (BCLC 0 and A combined: 12%) than in HCC patients with other disease etiology (for HBV-, HCV-, alcohol or NAFLD-related HCC: BCLC 0 and A combined in 42%, 50%, 31% and 25%, respectively). Median AFP levels were not significantly different in the various etiology groups (Supplementary table 2).

Applied treatments differed significantly between the various etiology groups (P<0.001) (Supplementary table 2). Proportion of patients who received (potentially curative) surgical therapy in general was significantly lower in HCC patients with alcohol-related liver disease (18%) than in patients with HBV, HCV, NAFLD or absence or risk factors who were treated with surgical therapy in 34%, 30%, 31% and 33% of cases, respectively. Proportion of patients who underwent resection was significantly higher in case of HBV (22%), NAFLD (25%) or absence of risk factors (27%) than in patients with HCV (14%) or alcohol-related liver disease (8%). Also, patients with HBV, HCV or alcohol-related liver disease received significantly more often a liver transplant (11%, 15% and 10%, respectively) than patients with NAFLD or no risk factors (6% and 5%, respectively). RFA was most frequently applied in HCV-related HCC (20%), whereas only 3% of patients without risk factors and 8% of patients with NAFLD received RFA. Proportion of patients who received TACE/TARE ranged between 12% and 20% in the different etiology groups. Furthermore, HCV patients were significantly less often treated with sorafenib (4%) than patients with HBV, NAFLD, alcohol-related liver disease or absence of risk factors (9%, 11%, 12% and 16%, respectively) (Supplementary table 2).

Survival rates differed significantly between the five major etiology groups (Fig. 2, log rank test P<0.001). In univariate analysis, survival was significantly longer in HCC patients with HBV (hazard ratio (HR) 0.59, 95% CI 0.46-0.75, reference group: patients with alcohol-related liver disease) than in the other four etiology groups. Survival rates between the other etiology groups did not differ. As mentioned earlier, in multivariate analyses, presence of HBV (HR 0.68, 95% CI 0.51-0.91) was associated with lower mortality. In contrast, presence of HCV (HR 1.32, 95% CI 1.01-1.65) was an independent predictor for higher overall mortality (Table 4).

Figure 2: Observed survival of patients with hepatocellular carcinoma with underlying hepatitis B (line with open circles), NAFLD (line with solid triangles), hepatitis C (line with solid blocks), absence of risk factors (line with solid circles), or alcohol-related liver disease (line with crosses) (Kaplan Meier survival curve; log rank P<0.001)



Number at risk					
Hepatitis B	179	81	45	28	7
Hepatitis C	232	85	42	22	6
Alcohol	334	99	37	13	4
NAFLD	175	55	19	10	3
No risk factors	134	52	25	11	2

Discussion

This study showed that absence of risk factors for underlying liver disease, presence of NAFLD and female gender were independently associated with HCC in non-cirrhotic livers. It has previously been reported that patients who develop HCC in a non-cirrhotic liver are younger and that there is a female predominance, although this was not confirmed in other studies [3,6,17]. Despite the larger tumor size and more

advanced BCLC tumor stage in HCC patients without cirrhosis, resections were more often performed and survival was significantly longer than in cirrhotic patients. Also, in multivariate analyses, absence of cirrhosis was an independent predictor of longer survival. To the best of our knowledge, the current work represent the largest study assessing the impact of cirrhosis on outcome in an unselected group of cirrhotic and non-cirrhotic HCC patients.

Recently, a large cohort study of HCC patients from Germany was published [30]. In line with the results of the current study, cirrhosis was present in 81% of cases and survival was significantly better in non-cirrhotic patients. Survival benefit in HCC patients with non-cirrhotic livers was also reported in two older studies with cohorts of unselected HCC patients [31,32]. Another study, published in 2005, reported only significant survival differences between patients with compensated liver disease and those with decompensated cirrhosis (Child Pugh B or C) [33]. Finally, two other recent studies did not find differences in survival rates between cirrhotic and non-cirrhotic HCC patients, despite the fact that presence of liver cirrhosis strongly affected HCC treatment choices [17,34]. Consistent with our findings, resection was more often applied in non-cirrhotic patients, whereas cirrhotic patients received more often other treatment modalities. In addition, several studies comparing survival between cirrhotics and non-cirrhotics in specific subgroups, e.g. after HCC resection, reported that overall and disease-free survivals were significantly better in non-cirrhotics [6]. Survival benefit in the total group of non-cirrhotic patients may be related to the fact that (potentially curative) surgical therapy was more frequently performed leading to a better outcome. Interestingly, in the current report, transplantation appeared to be associated with better outcome than resection, although patient numbers in the transplantation group were rather small and definite conclusions cannot be drawn.

In line with results of previous studies [4,6,17,30,35], causes for underlying liver disease in cirrhotic and non-cirrhotic HCC patients were different in our study. Alcohol abuse and viral hepatitis were the most important underlying causes in cirrhotic patients, whereas NAFLD was often present in HCC patients with a non-cirrhotic liver. Furthermore, a significant number of non-cirrhotic HCC patients proved to have no underlying risk factors despite extensive analyses. The hepatocarcinogenetic pathway of HCC might differ between cirrhotic and non-cirrhotic livers, but exact mechanisms are still unclear. In patients with HCV or alcohol-related liver disease, cirrhosis appears to be prerequisite for HCC development. HBV, on the other hand, could also exert a direct oncogenic effect regardless of presence of cirrhosis. HBV genome integration can lead to host DNA microdeletions and aberrant function of growth regulatory genes in the hepatocyte. In patients with NAFLD, it is thought that oxidative stress (as a results of increased intrahepatic fatty acid levels) may play an important role in the development of hepatocellular damage and HCC development [36].

One interesting finding of the current study is that there are significant differences in applied treatments modalities between patients with various etiologies of underlying liver disease with potential impact on survival. Some previous studies have suggested that cause of underlying liver disease could affect survival, but other studies were not able to confirm this [22,37-39]. In our study, resection was more often applied in patients with HBV, NAFLD or absence of underlying risk factors for chronic liver disease. In contrast, RFA was most often performed in case of HCV. Moreover, presence of HBV was an independent predictor for lower mortality, whereas presence of HCV was associated with higher mortality. A potential explanation for these findings could be that patients with HBV more often developed HCC before progression to cirrhosis, with a large proportion of solitary lesions. Also differences in efficacy of prior antiviral treatment for HBV and HCV could have contributed to these results obtained in a period before introduction of direct-acting anti-HCV agents.

An earlier cross-sectional study in USA veterans showed that NAFLD and features of metabolic syndrome (hypertension and diabetes) were strongly associated with HCC development in absence of cirrhosis, whereas non-cirrhotic HCC patients were less likely to have HCV or alcohol related liver disease [26]. These results are in line with our findings, although the proportion of non-cirrhotic patients was lower in that study (between 3%-16%) than in our study. A potential explanation for this difference could be the fact that almost exclusively males were included in the USA veteran study. Unfortunately, details of metabolic syndrome, except BMI values, were not available in the current study. Furthermore, BMI values in our study were not corrected for presence of ascites [40].

Findings of our study are inherently limited by the retrospective study design. In addition, only patients who were diagnosed or referred to one of the five major Dutch academic centers were included. HCC patients with a very poor prognosis were possibly not referred and thus not included in the current cohort. Furthermore, the term "non-cirrhotic" involves a heterogeneous group of conditions ranging from chronic hepatitis with stage III fibrosis to a morphologically healthy liver. Moreover, it may also be difficult to differentiate F3 and F4 based on liver biopsy, especially in case of macronodular cirrhosis.

In conclusion, in this large cohort of HCC patients in the Netherlands, presence of HCC without cirrhosis was strongly associated with absence of risk factors for underlying liver disease, presence of NAFLD and female gender. In absence of cirrhosis, resections were more often performed, despite larger tumor size. Survival in non-cirrhotic patients was significantly longer compared to those with liver cirrhosis.

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Supplementary table 1: Patient characteristics of 1122 cases with hepatocellular carcinoma subdivided into the five major etiology groups: alcohol-related liver disease, hepatitis C, hepatitis B, absence of risk factors and non-alcoholic fatty liver disease.

	Alcohol	Hepatitis C	Hepatitis B	NAFLD	No risk factors	P-value[a]
Patient nr (%)#	349 (29)	249 (20)	197 (16)	181 (15)	146 (12)	
Male gender	298 (85)	196 (79)	167 (85)	120 (66)	85 (58)	<0.001
Age at HCC diagnosis	65 (34-87)	59 (41-89)	57 (19-83)	67 (22-90)	68 (11-91)	<0.001
BMI (mean, SD)	27.8 (4.9)	26.4 (5.1)	25.8 (5.1)	28.9 (5.1)	24.2 (3.3)	<0.001
Cirrhosis	312 (89)	236 (95)	162 (82)	114 (63)	73 (50)	<0.001
ALT (U/L)	38 (7-1193)	68 (4-341)	46 (13-1158)	47 (12-767)	44 (8-908)	<0.001
AST (U/L)	62 (14-4782)	99 (17-415)	59 (18-8678)	58 (17-1610)	56 (16-1002)	<0.001
Albumin	36 (15-58)	37 (16-49)	41 (17-53)	40 (13-57)	40 (20-62)	<0.001
Platelets	134 (14-985)	111 (23-462)	147 (32-724)	179 (8-634)	236 (36-897)	<0.001
INR	1.2 (0.8-2.1)	1.1 (0.9-2.8)	1.1 (0.8-2.9)	1.1 (0.0-2.6)	1.1 (0.9-2.2)	<0.001
PT	14.5 (9.9-25.8)	14.1 (10.2-36.2)	13.6 (10.0-36.7)	13.4 (9.7-30.6)	13.4 (10.0-25.8)	<0.001
APRI	1.6 (0.1-146)	3.3 (0.3-17.8)	1.4 (0.2-304)	1.1 (0.2-68.8)	0.8 (0.2-51.0)	<0.001
MELD score	10 (6-31)	9 (6-28)	8 (6-33)	8 (6-27)	8 (6-26)	<0.001

Results indicate nrs and, between brackets, percentages. Continuous variables reported as medians and, between brackets, ranges unless otherwise indicated.
ALT, alanine transaminase; APRI, aspartate aminotransferase to platelet ratio index; AST, aspartate aminotransaminase; INR, international normalized ratio; NAFLD, non-alcoholic fatty liver disease; PT, prothrombin time.
#Percentages applies to all included patients (n=1221); [a]P-value applies to significant differences between various etiology groups. Clinically most relevant significant differences between individual groups by post-hoc analysis are mentioned in the text.

Supplementary table 2: Tumor characteristics of 1122 patients with hepatocellular carcinoma subdivided into the five major etiology groups: alcohol-related liver disease, hepatitis C, hepatitis B, absence of risk factors and non-alcoholic fatty liver disease.

	Alcohol	Hepatitis C	Hepatitis B	NAFLD	No risk factors	P-value[a]
Patient nr#	349 (29)	249 (20)	197 (16)	181 (15)	146 (12)	
No. of lesions						0.001
1	146 (42)	136 (55)	112 (57)	100 (55)	77 (53)	
2	44 (13)	38 (15)	23 (12)	12 (7)	23 (16)	
3	25 (7)	14 (6)	7 (3)	15 (8)	6 (4)	
Multifocal/diffuse	134 (38)	61 (24)	55 (28)	54 (30)	40 (27)	
Tumor size (cm)	5 (1-24)	3 (1-16)	4 (1-22)	6 (1-22)	9 (1-26)	<0.001
BCLC stage						<0.001
0	18 (5)	32 (13)	13 (7)	2 (1)	3 (2)	
A	90 (26)	92 (37)	68 (35)	44 (24)	15 (10)	
B	118 (34)	68 (27)	50 (25)	73 (40)	74 (51)	
C	90 (26)	47 (19)	56 (28)	50 (28)	40 (27)	
D	33 (9)	10 (4)	10 (5)	12 (7)	14 (10)	
Alpha-fetoprotein (µg/L)	20 (1-1.8x10^6)	26 (1-2.9x10^5)	35 (1-6.5x$10^{5)}$	25 (1-2.7x10^6)	33 (1-5.1x10^5)	0.832
Treatments						<0.001
Surgical therapy	64 (18)	75 (30)	67 (34)	57 (31)	47 (33)	
Resection	28 (8)	36 (14)	44 (22)	46 (25)	40 (27)	
Transplantation	34 (10)	37 (15)	21 (11)	11 (6)	7 (5)	
Both	2 (<1)	2 (1)	2 (1)	0 (0)	0 (0)	
RFA[b]	38 (11)	50 (20)	27 (14)	14 (8)	5 (3)	
TACE/TARE[c]	70 (20)	45 (18)	29 (15)	32 (18)	17 (12)	
Systemic therapy	41 (12)	11 (4)	18 (9)	20 (11)	24 (16)	
Best supportive care	121 (35)	57 (23)	46 (23)	49 (27)	47 (32)	
Unknown	15 (4)	11 (5)	10 (5)	9 (5)	6 (4)	

Results indicate nrs and, between brackets, percentages. Continuous variables reported as medians and, between brackets, ranges. #Percentages applies to all included patients (n=1221); [b] Thirteen patients received RFA and subsequently TACE with more than 1 month interval; [c]In 31 patients a combination of TACE and RFA within a one month interval was performed as initial therapy.

BCLC stage, tumor stage according Barcelona Clinic Liver Cancer staging system; TACE, transcatheter arterial chemoembolization; TARE, transarterial radioembolization; RFA, radiofrequency ablation. NAFLD, non-alcoholic fatty liver disease.

[a]P-value applies to significant differences between various etiology groups. Clinically most relevant significant differences between individual groups by post-hoc analysis are mentioned in the text.

CHAPTER 8

HEPATOCELLULAR CARCINOMA IN NON-CIRRHOTIC LIVERS IS ASSOCIATED WITH STEATOSIS RATHER THAN STEATOHEPATITIS: POTENTIAL IMPLICATIONS FOR PATHOGENESIS

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ABSTRACT

Objectives: Risk of hepatocellular carcinoma (HCC) is increased in patients with metabolic syndrome (MS), possibly related to non-alcoholic fatty liver disease (NAFLD). Since histological features of NAFLD may regress in cirrhosis, we compared steatosis vs steatohepatitis in the non-tumoral liver of non-cirrhotic HCC patients.

Methods: A retrospective clinicopathological analysis was performed in 91 non-cirrhotic HCC patients. Patients were divided into three subgroups, i.e. patients with: 1) MS without other risk factors for underlying liver disease, 2) no underlying risk factors or 3) other risk factors (with or without MS). NAFLD activity score (NAS) <3 was classified as no steatohepatitis, NAS 3-4 as borderline steatohepatitis and NAS≥5 as definite steatohepatitis.

Results: Eleven patients (12%) had MS without other risk factors (group 1). In the non-tumoral liver, significant steatosis (≥5% of hepatocytes) was generally present (in 10/11 patients), with mild lobular inflammation and absence of ballooning in most cases. Absence of steatohepatitis, borderline steatohepatitis and definite steatohepatitis were found in 55%, 45% and 0% of cases, respectively. In group 2 and 3, significant steatosis was frequently present (in 16/37 and 21/43 patients, respectively). Absence of steatohepatitis, borderline steatohepatitis and definite steatohepatitis were found in 84%, 16% and 0% of cases (group 2) respectively in 77%, 23% and 0% of cases (group 3).

Conclusions: In non-cirrhotic HCC patients, histological steatosis was frequently present, whereas overt steatohepatitis did not occur. These findings may be relevant for HCC pathogenesis in NAFLD.

Introduction

Primary liver cancer is the sixth most common cancer in the world and the third cause of cancer-related death [1]. Hepatocellular carcinoma (HCC) represents more than 90% of all primary liver cancers and often occurs in patients with cirrhosis due to viral hepatitis and/or alcohol abuse. Currently, non-alcoholic fatty liver disease (NAFLD) has become one of the leading causes of chronic liver disease and a common risk factor for HCC [2,3].

Non-alcoholic fatty liver disease (NAFLD) represents a wide histological spectrum ranging from non-alcoholic fatty liver (NAFL: simple steatosis without hepatocellular injury) to non-alcoholic steatohepatitis (NASH: steatosis with inflammation and ballooning of hepatocytes) and is strongly correlated with features of metabolic syndrome (MS) [4,5].

Increased HCC risk in NAFLD seems to affect especially those with cirrhosis and is generally attributed to NASH [5-9]. Nevertheless, simple steatosis may not be an entirely benign condition, and may exhibit a significant risk of progressive liver disease and even HCC development [10-12]. Furthermore, it has recently been suggested that also in non-cirrhotic livers, presence of NAFLD and/or MS is associated with HCC development [13,14]. It is noteworthy that in patients with MS as only risk factor, HCCs most often occur in a background liver without significant fibrosis and in absence of definite steatohepatitis [14].

Previous studies have described a steatohepatitic variant of HCC (SH-HCC), which is characterized by features resembling steatohepatitis in the tumor similar to the non-tumoral liver [15]. This phenotype has been reported to occur in the setting of underlying steatosis, steatohepatitis and MS [16,17].

As histological features of NAFLD may regress with the development of cirrhosis [18], we compared in the current study presence of steatosis versus steatohepatitis in the non-tumoral liver of non-cirrhotic HCC patients. Also, we explored histopathology of the tumor.

Materials and methods

Cases

All patients with HCC diagnosed in the period 2005-2012 in three Dutch academic centers (University Medical Center Utrecht, Erasmus Medical Center Rotterdam and Academic Medical Center Amsterdam) were evaluated. HCC diagnosis was based on AASLD 2005 and 2011 guideline criteria [19,20]. Data on the influence of HCC surveillance in this cohort have recently been reported [21]. Only those patients with available non-tumoral liver histology at a maximal distance from the tumor and less than 50% bridging fibrosis (i.e. few bridges, involving less than 50% of the portal tracts

and/or central veins) were included in the current study. In case of a fibrolamellar HCC, mixed tumors or progenitor-derived HCC, patients were excluded.

Clinical data

Clinical data were obtained from electronic medical records. Information on the cause of underlying liver disease was collected: (A) alcohol-related liver disease (defined as a history of average alcohol use ≥3 alcoholic drinks/day [22]), (B) hepatitis B (HBV), (C) hepatitis C (HCV), (D) hemochromatosis, (E) MS without other risk factors for underlying liver disease, (F) other causes or (G) absence of any risk factors for underlying liver disease. Patients were divided into three subgroups: 1) patients with a clinical diagnosis of MS without other risk factors for underlying liver disease, 2) patients with no risk factors for underlying liver disease or 3) patients with other risk factors for underlying liver disease (with or without coexistent MS).

MS was defined according to the National Cholesterol Education Program's Adult Treatment Panel III report [23]. Body mass index (BMI) >28.8 kg/m^2 rather than increased waist circumference was used as criterion, as previously validated [24]. Hypertension, diabetes and dyslipidemia were collected based on information in medical files [3].

Finally, data on tumor characteristics (e.g. number of tumor lesions, maximum diameter of the largest tumor lesion and tumor stage according to Barcelona Clinic Liver Cancer (BCLC) staging system) were obtained for each patient.

Histopathology

Paraffin-embedded formalin-fixed liver tissue slides were retrieved from the archives of the various departments of pathology. Hematoxylin and eosin (HE) stained slides were used, unless otherwise mentioned, and reviewed in detail by an experienced liver pathologist (JV), not aware of the clinical data. For each patient, microscopic features of non-tumoral liver tissue at maximal distance from the tumor were evaluated. Non-tumoral liver tissue was evaluated for grade of steatosis according to Kleiner *et al* [25]: grade 0 (<5% of the hepatocytes), grade 1 (5%–33% of the hepatocytes), grade 2 (34%–66% of the hepatocytes) or grade 3 (>66% of the hepatocytes). Furthermore, lobular inflammation (grade 0: no foci, grade 1: <2 foci per 200x field, grade 2: 2-4 foci per 200x field or grade 3: >4 foci per 200x field) and ballooning (none, few or many ballooning cells) were evaluated. Based on these three variables, the NAFLD activity score (NAS) was calculated [25]. NAS<3 was classified as no steatohepatitis, NAS of 3-4 as borderline steatohepatitis and NAS≥5 as definite steatohepatitis. Additionally, portal inflammation, interface activity (both classified as: none, mild, moderate or severe) and presence of siderosis (classified as: grade 0: none/not observed, grade 1: ≤25% of hepatocytes, grade 2: 26-50% of hepatocytes, grade 3: 51-75% of hepatocytes or grade 4: >75% of hepatocytes) in the non-tumoral tissue were reported. Finally, the

non-tumoral liver tissue was evaluated for fibrosis. Grade of fibrosis was descriptively reported, independent of underlying disease (if present): (A) none, (B) perisinusoidal or periportal, (C) perisinusoidal and portal/periportal or (D) bridging fibrosis, involving less than 50% of the portal tracts and/or central veins (patients with a more extensive fibrosis were excluded, see above). Collagen stainings (sirius red (SR) or Elastica van Giesson (EVG)) in addition to HE stains were used for evaluating fibrosis. Radiological imaging and/or Fibroscan® (Echosens, Paris, France) results were evaluated critically to confirm absence of cirrhosis.

Furthermore, morphologic characteristics of HCC lesions were evaluated: grade of steatosis in the tumor was classified as <5% or ≥5%, whereas ballooning and Mallory's hyaline were both classified as either present or absent [15]. Furthermore, tumor differentiation grade was determined (according to WHO: well, moderately or poorly differentiated) based on the areas of tumor showing the poorest differentiation grade. Intratumoral inflammation and pericellular fibrosis were not quantified in further detail, because of a large variability in morphology within different slides of single HCC lesions.

This study was in agreement with the ethical guidelines of the Declaration of Helsinki and analyses were performed with institutional medical ethical consent, in an anonymized database.

Statistical analysis

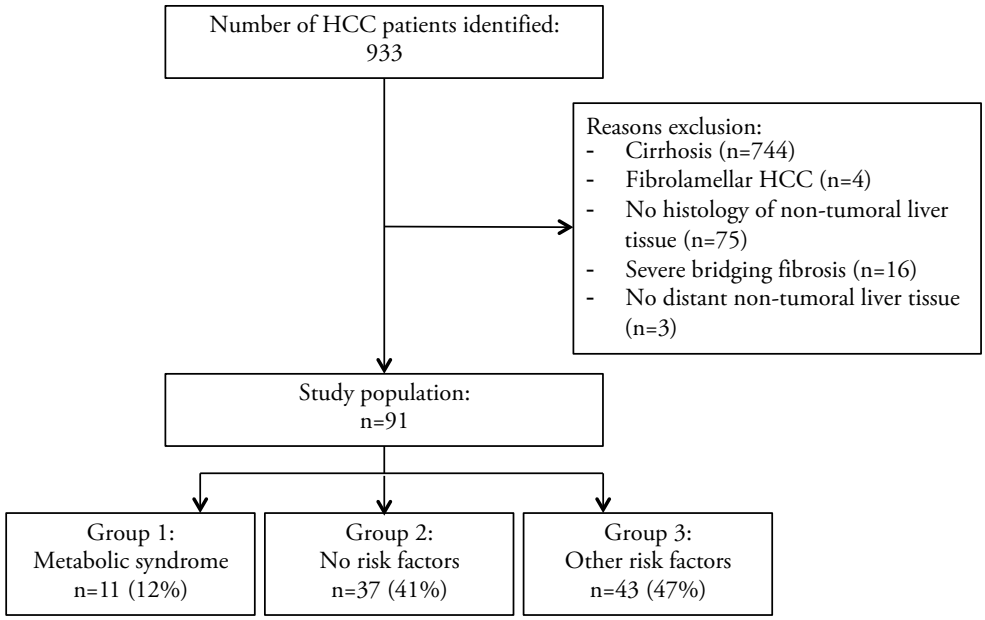
Continuous data are expressed as medians and ranges, and discrete variables as absolute and relative frequencies. Mann-Whitney U test was applied to compare continuous data in the three groups. Categorical variables were compared with Pearson's Chi-square or Fisher's exact tests. Odds ratios (OR) were calculated to estimate the strength of associations of binary variables with presence of significant steatosis in the non-tumoral liver tissue. A two-sided P-value <0.05 was considered statistically significant. Statistical analysis was performed using IBM SPSS statistics (version 20.0, IBM Corp., Armonk, New York, USA).

Results

Patient and tumor characteristics

In the period January 2005- December 2012, 933 HCC patients were under care in the three academic hospitals. After exclusion of 842 patients because of presence of cirrhosis based on histology or combined radiological and laboratory features (n=744), fibrolamellar HCC (n=4), absence of histology of non-tumoral liver tissue (n=75), severe bridging fibrosis (involving more than 50% of the portal tracts and/or central veins) at histopathological evaluation of non-tumoral liver tissue (n=16) and absence of distant non-tumoral liver tissue (n=3), a total of 91 HCC cases were identified who

Figure 1: Flow chart of patient inclusion and details of underlying liver disease



fulfilled the inclusion criteria (Figure 1). Patient characteristics are given in Table 1. Most patients were male (66%) and median age at time of HCC diagnosis was 64 years (range: 17-86). Of the total group, 11 patients (12%) had MS without other risk factors (group 1). In total, 37 patients had no risk factors for underlying liver disease (group 2). The remaining patients had other underlying risk factors for chronic liver disease (n=43, group 3): 16 alcohol, 11 HBV, 8 HCV, 4 hemochromatosis, 4 other risk factors (porphyria (2x), Von Gierke disease and Alagille syndrome (Table 1 and Supplementary table 1). Of note, none of the HCV patients had genotype 3. Coexistent MS was present in 9/43 patients (21%) of group 3 (Table 1).
Most patients had a single tumor (76%) and median tumor size was 7 cm (range: 2-22 cm). Furthermore, BCLC stage B was most common (66%). Median AFP level at time of diagnosis was 6.0 µg/L (range: 1.0-2.7x10^6 µg/L). Resection was performed in 89% of patients (Table 1).

Histopathology of the non-tumoral liver tissue

Histopathology of the non-tumoral liver tissue was evaluated in liver resection specimens of 81 patients, whereas in 10 patients liver biopsies were used (taken at distance from the tumor). Of the total group (n=91), 31 patients had no fibrosis, 18 perisinusoidal or periportal fibrosis, 22 perisinusoidal and portal/periportal fibrosis, and 20 bridging fibrosis <50%. Furthermore, hepatic steatosis was present in 47/91 patients (52%),

Table 1: Clinical characteristics of 91 patients with hepatocellular carcinoma in a non-cirrhotic liver (group 1: metabolic syndrome, group 2: no underlying risk factors, group 3: other risk factors).

No. of patients	Total group n=91	Group 1 n=11	Group 2 n=37	Group 3 n=43	P-value *	P-value **	P-value ***
Male gender	60 (66)	5 (46)	21 (57)	34 (79)	0.732	0.054	0.052
Age at HCC diagnosis (years)	64 (17-86)	70 (60-77)	63 (17-86)	64 (33-86)	0.086	0.216	0.318
Etiology of underlying liver disease					-	-	-
Alcohol	16 (18)	-	-	16 (37)			
Chronic viral hepatitis							
Hepatitis B	11 (12)	-	-	11 (26)			
Hepatitis C	8 (9)			8 (19)			
Hemochromatosis	4 (4)	-	-	4 (9)			
Metabolic syndrome without other underlying causes	11 (12)	11 (100)	-	-			
Others	4 (4)	-	-	4 (9)			
No risk factors known	37 (41)	-	37 (100)	-			
Features of metabolic syndrome							
BMI >28.8 kg/m^2^	20 (22)	6 (55)	6 (17)	8 (19)	0.034	0.050	0.958
Dyslipidemia	32 (35)	9 (82)	5 (14)	18 (42)	<0.001	0.039	0.006
Hypertension	48 (53)	11 (100)	14 (38)	23 (54)	<0.001	0.004	0.184
Diabetes	24 (26)	10 (91)	3 (8)	11 (26)	<0.001	<0.001	0.074
Definite metabolic syndrome	20 (22)	11 (100)	0 (0)	9 (21)	<0.001	<0.001	0.003
No. of tumor lesions					0.364	0.633	0.239
1	69 (6)	10 (91)	24 (65)	35 (81)			
2	12 (13)	1 (9)	6 (16)	5 (12)			
3	2 (2)	0 (0)	2 (5)	0 (0)			
Multifocal/diffuse	8 (9)	0 (0)	5 (14)	3 (7)			
Tumor size (cm)	7.0 (2.1-22.0)	5.6 (2.6-14.0)	10.0(2.4-22.0)	6.0 (2.1-20.0)	0.045	0.547	0.012

BCLC tumor stage					0.145	0.998	0.013
0	0 (0)	0 (0)	0 (0)	0 (0)			
A	24 (26)	4 (36)	4 (11)	16 (37)			
B	60 (66)	6 (55)	31 (83)	23 (54)			
C	6 (7)	1 (9)	1 (3)	4 (9)			
D	1 (1)	0 (0)	1 (3)	0 (0)			
Alpha-fetoprotein (µg/L)	6.0 (1.0-2.7x10^6)	4.0 (2.0 -2.9x10^4)	5.0 (1.0-2.7x10^6)	7.0 (2.0-2.2x10^5)	0.824	0.547	0.786
Treatments					0.523	0.786	0.775
Resection	81 (89)	11 (100)	33 (89)	37 (87)			
Radiological therapy	2 (2)	0 (0)	1 (3)	1 (2)			
Systemic therapy	1 (1)	0 (0)	0 (0)	1 (2)			
Best supportive care	6 (7)	0 (0)	3 (8)	3 (7)			
Unknown	1 (1)	0 (0)	0 (0)	1 (2)			

Results indicate numbers and, between brackets, percentages or in case of continuous variables, medians and ranges; *P-value applies to differences between group 1 versus group 2; **P-value applies to differences between group 1 versus group 3; *** P-value applies to differences between group 2 versus group 3; ^Data of 2 patients is missing. No statistical testing was performed on etiology of underlying liver disease, since patients were categorized in groups based on this variable.

moderate or severe interface activity (in total 8% of cases) was rare and moderate or severe portal inflammation (in total 38% of cases) was found in a significant proportion of patients. Presence of siderosis was rare (Table 2).

In group 1, significant steatosis (≥5% of hepatocytes) was generally present (in 10/11 patients: grade 0 in 10%, grade 1 in 45%, grade 2 in 45%), whereas lobular inflammation was often mild (in 8/11 patients) and ballooning absent (in 9/11 patients). As a result, no steatohepatitis was found in 6/11 patients and borderline steatohepatitis in 5/11 patients. None of the patients in this group exhibited definite steatohepatitis. In patients with no underlying risk factors (i.e. group 2: n=37), significant steatosis was also common: 16/37 patients had significant steatosis (grade 0 in 57%, grade 1 in 33%, grade 2 in 5%, grade 3 in 5%). No steatohepatitis was found in 31/37 patients (84%) and borderline steatohepatitis in 6/37 patients (16%). Again, definite steatohepatitis was not present. In patients with other underlying risk factors (i.e. group 3: n=43), significant steatosis was present in 21/43 patients (grade 0 in 51%, grade 1 in 33%, grade 2 in 14%, grade 3 in 2%). In total, 33/43 patients (77%) had no steatohepatitis in the background liver, whereas in 10/43 patients (23%) borderline steatohepatitis was present. Again, none of the patients in this group had definite steatohepatitis (Table 2 and Supplementary table 2).

Proportions of patients with significant steatosis (91% vs. 43% and 49%, P=0.006 and P=0.016, respectively) and NAS (median: 2 vs. 1 and 1, P=0.039 and P=0.017, respectively) were significantly higher in group 1 than in group 2 or 3 (Table 2 and Figure 2). Other histopathologic features of tumoral and non-tumoral liver tissue and clinical features were in general not significantly different between the three groups, except for presence of features of metabolic syndrome (Tables 1 and 2). Furthermore, severity of fibrosis did not correlate with grade of steatosis or NAS (data not shown).

Association between metabolic syndrome and histopathology of the non-tumoral liver tissue

In the total group, BMI >28.8 kg/m^2 (OR 27.8, 95% CI 3.52-219.93) and diabetes (OR 5.29, 95% CI 1.77-15.87) were significantly associated with presence of significant steatosis in the non-tumoral liver tissue, whereas age and gender were not (Table 3). Dyslipidemia and hypertension were also more frequently present in patients with significant steatosis in the non-tumoral liver tissue, but these associations did not reach statistical significance. Overall, presence of MS was significantly higher in HCC patients with ≥5% steatosis in non-tumoral liver tissue (OR 7.74, 95% CI 2.08-28.83) (Table 3).

Histopathology of HCC lesions

Histopathology of HCC tissue was available in 86 patients and characteristics are given in Table 2. A large variability in morphology was seen within HCC lesions. In

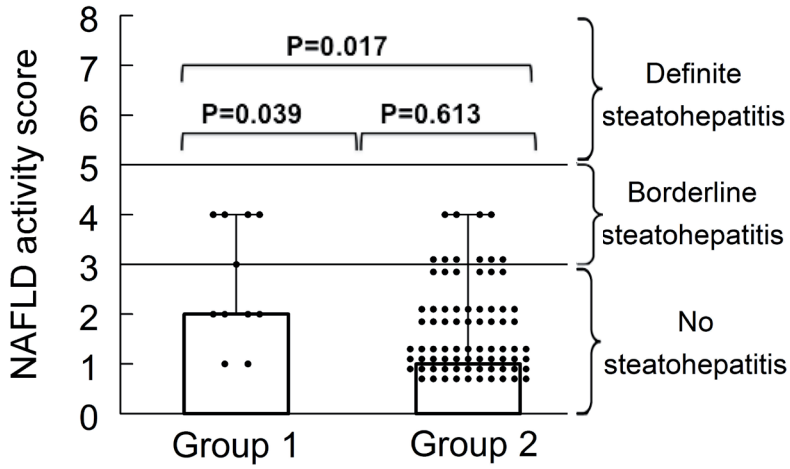
Table 2: Histopathologic features of non-tumoral liver tissue (at distance from the tumor) and tumor lesions in 91 patients with hepatocellular carcinoma in a non-cirrhotic liver (group 1: metabolic syndrome, group 2: no underlying risk factors, group 3: other risk factors).

Non-tumoral liver	Total group n=91	Group 1 n=11	Group 2 n=37	Group 3 n=43	P-value *	P-value **	P-value ***
Significant steatosis (≥5%)	47 (52)	10 (91)	16 (43)	21 (49)	0.006	0.016	0.658
Lobular inflammation							
Grade 0: No foci	6 (7)	1 (9)	3 (8)	2 (5)			
Grade 1: <2 foci per 200x field	79 (86)	8 (73)	32 (87)	39 (90)	0.394	0.247	0.801
Grade 2: 2-4 foci per 200x field	6 (7)	2 (18)	5 (4)	2 (5)			
Grade 3: <4 foci per 200x field	0 (0)	0 (0)	0 (0)	0 (0)			
Ballooning							
None ballooning cells	86 (94)	9 (82)	36 (97)	41 (95)	0.127	0.181	1.00
Few ballooning cells	5 (6)	2 (18)	1 (3)	2 (5)			
Many ballooning cells	0 (0)	0 (0)	0 (0)	0 (0)			
NAFLD activity score							
<3 (= no steatohepatitis)	70 (77)	6 (55)	31 (84)	33 (77)	0.043	0.256	0.577
3-4 (= borderline steatohepatitis)	21 (23)	5 (45)	6 (16)	10 (23)			
≥5 (= steatohepatitis)	0 (0)	0 (0)	0 (0)	0 (0)			
Interface activity							
None	56 (61)	6 (55)	25 (68)	25 (58)			
Mild	28 (31)	4 (36)	12 (32)	12 (28)	0.163	0.911	0.134
Moderate	6 (7)	1 (9)	(0)	5 (12)			
Severe	1 (1)	0 (0)	0 (0)	1 (2)			
Portal inflammation							
None	5 (6)	2 (8)	(0)	3 (7)			
Mild	51 (56)	5 (46)	24 (65)	22 (51)	0.038	0.334	0.230
Moderate	25 (27)	4 (36)	10 (27)	11 (26)			
Severe	10 (11)	0 (0)	3 (8)	7 (16)			
Fibrosis							
None	31 (34)	3 (27)	16 (44)	12 (28)			
Perisinusoidal or periportal	18 (20)	4 (37)	5 (13)	9 (21)	0.383	0.403	0.110
Perisinusoidal and portal/periportal	22 (24)	3 (27)	11 (30)	8 (19)			
Bridging fibrosis <50%	20 (22)	1 (9)	5 (13)	14 (32)			

Presence of siderosis							
Grade 0: none	83 (91)	11(100)	33 (89)	39 (90)			
Grade 1: ≤25 of hepatocytes	4 (5)	0 (0)	4 (11)	0 (0)	0.561	0.575	0.044
Grade 2: 26-50% of hepatocytes	2 (2)	0 (0)	0 (0)	2 (5)			
Grade 3: 51-75% of hepatocytes	2 (2)	0 (0)	0 (0)	2 (5)			
Tumor	n=86^						
Differentiation grade							
Good	8 (9)	0 (0)	2 (6)	6 (15)	0.597	0.332	0.429
Moderate	58 (68)	9 (82)	24 (68)	25 (63)			
Poor	20 (23)	2 (18)	9 (26)	9 (22)			
Steatosis							
<5%	56 (65)	6 (54)	22 (63)	28 (70)	0.728	0.472	0.625
≥5%	30 (35)	5 (46)	13 (37)	12 (30)			
Ballooning							
Absent	65 (76)	7 (64)	28 (80)	30 (75)	0.418	0.467	0.783
Present	21 (24)	4 (36)	7 (20)	10 (25)			
Mallory hyaline							
Absent	70 (81)	10 (91)	28 (80)	32 (80)	0.658	0.663	1.00
Present	16 (19)	1 (9)	7 (20)	8 (20)			

Results indicate numbers and, between brackets, percentages. *P-value applies to differences between group 1 versus group 2; **P-value applies to differences between group 1 versus group 3; *** P-value applies to differences between group 2 versus group 3; ^In 5 patients histopathology of HCC tissue was not available.

Figure 2: NAFLD activity score in 91 non-cirrhotic HCC patients (group 1: metabolic syndrome, group 2: no underlying risk factors, group 3: other risk factors)



most patients, HCC lesions were moderately differentiated (67%) and ≥5% steatosis within the tumor was present in 65% of cases. All HCCs had some inflammation and fibrosis (variable within the tumor). Furthermore, 24% of HCCs contained ballooning cells and 19% Mallory hyaline. In 11/86 patients (13%) a combination of steatosis, ballooning and Mallory hyaline was seen within the tumor, indicating a possible SH-HCC morphology (Table 2). No significant associations were found between SH-HCC morphology on the one hand and features of MS or histopathology of the non-tumoral liver tissue on the other hand (data not shown).

Table 3: Comparison of clinical characteristics of all 91 HCC patients with and without significant steatosis in the non-tumoral liver tissue

Characteristics	Non-tumoral liver without significant steatosis (<5%) n (%)	Non-tumoral liver with significant steatosis (≥5%) n (%)	P value	Odds ratio with 95% confidence interval
No. of patients	44 (100)	47 (100)		
Age at HCC diagnosis (years)	63 (17-86)	65 (33-86)	0.226	1.03 (0.99-1.06)
Male gender	29 (66)	31 (66)	0.966	1.00 (0.42-2.39)
BMI > 28.8 kg/m^2*	1 (2)	19 (40)	<0.001	27.8 (3.52-219.93)
Dyslipidemia	11 (34)	21 (66)	0.052	2.42 (1.00-5.91)
Hypertension	19 (43)	29 (62)	0.079	2.12 (0.92-4.90)
Diabetes	5 (11)	19 (40)	0.002	5.29 (1.77-15.87)
Definite metabolic syndrome	3 (7)	16 (34)	0.002	7.74 (2.08-28.83)

Results indicate numbers and, between brackets, percentages or in case of continuous variables, medians and ranges. *data of 2 patients is missing.

Discussion

In the current study, we compared morphologic characteristics of NAFLD in the non-tumoral liver in a large cohort of non-cirrhotic HCC patients. The main finding is that histological steatosis was frequently present in non-cirrhotic HCC patients, both in patients with and without known causes of underlying liver disease. On the other hand, overt steatohepatitis was surprisingly absent.

In line with our findings, the estimated prevalence of MS in the general adult European population is approximately 25% [26]. Moreover, available data suggest an overall prevalence rate of NAFLD between 20-40%, whereas in specific subgroups, such as obese adults or patients with diabetes, prevalence is even higher [27-30]. These rates are mainly based on studies using ultrasonography with a relativity low sensitivity for detection low-grade steatosis. NASH is significantly less common and affects approximately 2-3% of the general population [27]. In a recently published large Dutch population-based study including 3041 patients ≥45 years, steatosis on abdominal ultrasound was present in 35% of participants and 44% fulfilled the criteria for MS [31]. After exclusion of patients with secondary causes of steatosis, 33% of participants had NAFLD, which is comparable to findings in the current cohort. Currently, most experts consider NAFL a relatively benign condition. In contrast, two recently published studies suggested that also NAFL could progress to NASH and clinically significant fibrosis in a significant number of cases [10-12].

Previous studies have suggested that both NAFLD and MS could directly and indirectly promote HCC development [6,7,32]. Increased HCC risk is mainly reported in patients with NASH-related cirrhosis, but recent studies reported that also in non-cirrhotic livers presence of NAFLD and/or MS is associated with HCC development [13,14]. Based on preliminary data of the SEER-Medicare database, non-cirrhotic NAFLD was found to be the only identifiable risk factor in more than 5% of HCC cases [33]. The high prevalence of steatosis in the non-tumoral liver in the current study supports the hypothesis that hepatic steatosis may play a causal role in hepatocarcinogenesis, even in absence of steatohepatitis. Several possible underlying mechanisms have been suggested: for example, it is thought that oxidative stress, as a result of an increased level of intrahepatic fatty acids, is increased and causes hepatocyte injury and cancer-promoting mutations, which can lead to HCC development [34]. As hepatic steatosis was also often present in our patients with other well-known risk factors for underlying liver disease (n=21/43), it could possibly play an additional role as co-factor in the hepatocarcinogenesis in these patients as well.

In this study, steatosis in the non-tumoral liver was significantly associated with presence of diabetes, high BMI and MS. Previous studies also described associations between features of MS and presence of NAFLD [35]. Nevertheless, these findings were established in absence of HCC. In contrast, Alexander *et al.* [13] recently reported that only high BMI was significantly associated with steatosis in the non-tumoral liver of

non-cirrhotic HCC patients, whereas diabetes, dyslipidemia and hypertension were not. In general, the use of BMI instead of waist circumference in the definition of MS can be criticized, although there was a significant association between hepatic steatosis and high BMI in the current study. It is thought that body fat distribution plays an important role for NAFLD development and may also influence HCC risk [36]: i.e. excess of intra-abdominal fat may be a key determinant in the pathogenesis of NAFLD as visceral fat is strongly associated with insulin resistance and an important source of free fatty acids leading to steatosis [37].

Based on the currently accepted definition (which has been endorsed by experts in the field of NAFLD), the combination of three histological features: steatosis, ballooning and lobular inflammation is necessary to diagnose NASH [38]. This combination is incorporated in the NAS scoring system used in the current study. Nevertheless, a new histological algorithm for NASH has recently been developed with an alternative scoring system. In contrast to the NAS, the steatosis, activity, fibrosis (SAF) score separately assesses grade of steatosis, activity (the sum of hepatocyte ballooning and lobular inflammation) and fibrosis [39,40]. When this algorithm was applied to our cohort, occurrence of NASH remained rare: 5/91 (5%) patients exhibited NASH (2/11 patients in group 1, 1/37 in group 2 and 2/43 in group 3). The same patients with NASH based on the SAF score had borderline steatohepatitis according to NAS classification. A possible explanation for the absence of steatohepatitis in this cohort is the exclusion of patients with extensive fibrosis or cirrhosis.

As previously mentioned, a distinctive steatohepatitic histological variant of HCC has been described: SH-HCC [15]. This variant is characterized by features in the tumor resembling steatohepatitis in a non-tumoral liver. Recently, two studies reported that this morphologic variant is associated with patient's metabolic condition and presence of steatosis or steatohepatitis in non-tumoral liver [16,17]. In contrast, in a large cohort of non-cirrhotic HCC patients from the USA, only steatosis in the non-tumoral liver was associated with SH-HCC morphology, whereas NASH and features of MS were not [13]. Similarly, in the current study no significant associations were found between SH-HCC morphology on the one hand and features of MS or histopathology of the non-tumoral liver tissue on the other hand. Nevertheless, there was a large variability in morphology within single HCC lesions and numbers were relatively small compared to previous studies.

Findings of our study are inherently limited by the retrospective study design. Also, we were obviously restricted by availability of histology specimens, which were mostly resection specimens. Selection bias may have occurred by including HCC patients with a favorable, less-aggressive natural tumor behavior, who were still eligible for curative treatment at time of diagnosis. Furthermore, no control group was included due to the absence of histology specimens in the general population. Another point of notice is that the morphological characteristics of NAFLD are often indistinguishable from alcoholic

fatty liver disease. Definition of alcoholic liver disease was based on patient-reported mean alcohol consumption, which may introduce bias. Finally, although our study does not provide evidence of causality, findings suggest that hepatic steatosis may be involved in hepatocarcinogenesis.

In conclusion, histological steatosis is frequently present in non-cirrhotic HCC patients. Since overt steatohepatitis was rare in our HCC patients, current paradigmas on the role of NAFLD in HCC development may be reconsidered. Further studies are needed to elucidate the exact role of hepatic steatosis in HCC development in non-cirrhotic livers and to determine exact HCC risk in patients with steatosis versus steatohepatitis.

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Supplementary table 1: Comparison of presence of features of metabolic syndrome in 91 patients with hepatocellular carcinoma in a non-cirrhotic liver according to etiology of underlying liver disease

Characteristics	Metabolic syndrome	Alcohol	HBV	HCV	Hemochromatosis	Other	No risk factors
	n=11	n=16	n=11	n=8	n=4	n=4	n=37
Age at HCC diagnosis*	70 (60-77)	73 (41-86)	53 (33-78)	58 (45-63)	68 (64-77)	53 (44-67)	63 (17-86)
Male gender	5 (45)	13 (81)	8 (73)	7 (88)	4 (100)	2 (50)	21 (57)
BMI > 28.8 kg/m^2	6 (55)	3 (19)	2 (18)	1 (13)	1 (25)	1 (25)	6 (16)
Dyslipidemia	9 (82)	9 (56)	3 (27)	3 (38)	2 (50)	1 (25)	5 (14)
Hypertension	11 (100)	11 (69)	6 (54)	4 (50)	2 (50)	0 (0)	14 (38)
Diabetes	10 (91)	5 (31)	3 (27)	1 (13)	2 (50)	0 (0)	3 (8)
Metabolic syndrome	11 (100)	3 (19)	3 (27)	1 (13)	2 (50)	0 (0)	0 (0)

Results indicate numbers and, between brackets, percentages or in case of continuous variables, medians and ranges; HBV=hepatitis B virus; HCV= hepatitis C virus; *in years. Numbers of patients in various etiology groups were too low to compare all these groups separately.

Supplementary table 2: Comparison of histopathologic features of non-tumoral liver tissue in 91 patients with hepatocellular carcinoma in a non-cirrhotic liver according to etiology of underlying liver disease

Characteristics	Metabolic syndrome	Alcohol	HBV	HCV	Hemochromatosis	Other	No risk factors
	n=11	n=16	n=11	n=8	n=4	n=4	n=37
Significant steatosis (≥5%)	10 (91)	8 (50)	4 (36)	4 (50)	4 (100)	1 (25)	16 (43)
Lobular inflammation							
Grade 0: No foci	1 (9)	0 (0)	1 (9)	0 (0)	0 (0)	1 (25)	3 (8)
Grade 1: <2 foci per 200x field	8 (73)	16 (100)	10 (91)	6 (75)	4 (100)	3 (75)	32 (87)
Grade 2: 2-4 foci per 200x field	2 (18)	0 (0)	0 (0)	2 (25)	0 (0)	0 (0)	2 (5)
Grade 3: <4 foci per 200x field	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Ballooning							
None balloon cells	9 (82)	15 (94)	10 (91)	8 (100)	4 (100)	4 (100)	36 (97)
Few balloon cells	2 (18)	1 (6)	1 (9)	0 (0)	0 (0)	0 (0)	1 (3)
Many balloon cells	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
NAFLD activity score	2 (1-4)	1 (1-3)	1 (0-4)	1 (1-3)	2 (1-3)	1 (0-2)	1 (0-4)
NAFLD activity score							
<3 (= no steatohepatitis)	6 (55)	13 (81)	9 (82)	5 (63)	2 (50)	4 (100)	31 (84)
3-4 (= borderline steatohepatitis)	5 (45)	3 (19)	2 (18)	3 (37)	2 (50)	0 (0)	6 (16)
≥5 (= steatohepatitis)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Interface activity							
None	6 (55)	15 (94)	4 (36)	1 (13)	2 (50)	3 (75)	25 (68)
Mild	4 (36)	1 (6)	7 (64)	1 (13)	2 (50)	1 (25)	12 (32)
Moderate	1 (9)	0 (0)	0 (0)	5 (61)	0 (0)	0 (0)	0 (0)
Severe	0 (0)	0 (0)	0 (0)	1 (13)	0 (0)	0 (0)	0 (0)
Portal inflammation							
None	2 (8)	1 (6)	2 (18)	0 (0)	0 (0)	0 (0)	0 (0)
Mild	5 (46)	11 (69)	4 (36)	1 (13)	3 (75)	3 (75)	24 (65)
Moderate	4 (36)	4 (25)	5 (46)	0 (0)	1 (25)	1 (25)	10 (27)
Severe	0 (0)	0 (0)	0 (0)	7 (87)	0 (0)	0 (0)	3 (8)
Fibrosis							
None	3 (27)	8 (50)	3 (27)	0 (0)	0 (0)	1 (25)	16 (44)
Perisinusoidal or periportal	4 (37)	4 (25)	3 (27)	1 (13)	1 (25)	0 (0)	5 (13)
Perisinusoidal and portal/periportal	3 (27)	3 (19)	2 (19)	1 (13)	1 (25)	1 (25)	11 (30)
Bridging fibrosis <50%	1 (9)	1 (6)	3 (27)	6 (74)	2 (50)	2 (50)	5 (13)

Presence of siderosis							
Grade 0: none	11 (100)	15 (94)	11 (100)	8 (100)	3 (75)	2 (50)	33 (89)
Grade 1: ≤25 of hepatocytes	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	4 (11)
Grade 2: 26-50% of hepatocytes	0 (0)	1 (6)	0 (0)	0 (0)	0 (0)	1 (25)	0 (0)
Grade 3: 51-75 of hepatocytes	0 (0)	0 (0)	0 (0)	0 (0)	1 (25)	1 (25)	0 (0)

Results indicate numbers and, between brackets, percentages or in case of continuous variables, medians and ranges; HBV=hepatitis B virus; HCV= hepatitis C virus; NAFLD=non-alcoholic fatty liver disease. Numbers of patients in various etiology groups were too low to compare all these groups separately.

CHAPTER 9

DIAGNOSTICS AND TREATMENT OF HEPATOCELLULAR CARCINOMA: TRENDS IN THE NETHERLANDS IN THE PERIOD 2003-2011

(Translation from article in Dutch: Hepatocellulair carcinoom: diagnostiek en behandeling. Trends in Nederland in de periode 2003-2011. Ned Tijdschr Geneeskd. 2014;158:A7074.)

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Ned Tijdschr Geneeskd. 2014;158:A7074

ABSTRACT

Aim: To evaluate trends in distribution of diagnostic testing and treatment of hepatocellular carcinoma (HCC) in the past decade.

Design: Retrospective study

Method: Data on diagnostic testing and initial treatment of patients with HCC diagnosis in the period 2003-2011 was collected from the Netherlands Cancer Registration.

Results: In the period 2003-2011, 2915 patients were diagnosed with HCC. Proportion of patients who received palliative treatment increased significantly, whereas proportion of patients treated with surgical resection remained stable (approximately 10%). Tumour biopsies were performed in virtually all hospitals. Despite a significant decrease during the investigated period, tumour biopsy was performed in more than half of all cases. Furthermore, there was a significant increase in proportion of hospitals that performed HCC treatment (from 33% to 62% of all hospitals) and the contribution of university hospitals decreased significantly (from 83% to 75% of all treatments). Transarterial chemoembolization (TACE), radiofrequency ablation (RFA) and resection were mainly performed in university hospitals (respectively 99%, 95% and 79%), whereas half of initial sorafenib treatments were given in non-university hospitals. Only in a few university hospitals at least five resections were performed annually or - only during the last three years - at least five patients were initially treated with sorafenib. There were significant differences between regions in the use of RFA or TACE (both $p<0.001$, after casemix correction).

Conclusion: In the past decade there was no trend towards centralization of diagnostic testing and treatments for HCC.

Introduction

Primary liver cancer is the sixth most common cancer in the world and the third cause of cancer-related death. Hepatocellular carcinoma (HCC) represents more than 90% of primary liver cancers and occurs mostly in patients with underlying cirrhosis. HCC is relatively rare in the Netherlands; however, incidence has increased [1]. In tumours ≥1 cm HCC can be diagnosed based on 3-phase CT or MR imaging, if the lesion is hypervascular in the arterial phase and shows washout in the delayed venous phase [2-4]. Therefore, diagnostic biopsy is unnecessary in most cases.

Liver transplantation and resection are the most important curative treatment options. In the last few years, new treatment strategies for HCC have been introduced, most in palliative setting. With radiofrequency ablation (RFA), a thin needle electrode is inserted (mostly percutaneous) in the tumour using ultrasound guidance and tumour destruction is obtained by tissue heating up to 60-100°C [5]. During transarterial chemoembolization (TACE), a catheter is placed through the hepatic artery directly in the supplying artery of the tumour. Subsequently, chemotherapy is administered in combination with embolization agents [6]. In some cases, RFA and TACE can be curative. Finally, systemic treatment with multikinase inhibitor sorafenib leads to improved survival in patients with compensated cirrhosis (Child-Pugh class A) and advanced HCC stage (tumour invasion of the portal vein and/or distant metastases); median survival benefit ranges from two to seven months compared to placebo [7].

Not only tumour characteristics, but also severity of liver disease is decisive for treatment choice. The recently published first Dutch guideline "Hepatocellular carcinoma" recommends centralization of diagnostics and treatment of HCC in expert centres [4]. In this article we describe trends in diagnostics and treatment of HCC in the Netherlands in the past decade.

Patients and methods

Trends in diagnostic testing and initial treatment of patients with HCC diagnosis in the period 2003-2011 were evaluated based on data of the Netherlands Cancer Registry (NCR). For this study, all patients with an invasive malignancy primary located in the liver (C22.0) were selected from the NCR [8]. Exclusion criteria were: age ≤18 years, residing abroad, HCC diagnosed at autopsy and proven primary hepatic malignancy other than HCC (e.g. cholangiocarcinoma, hepatoblastoma, lymphoma). The NCR is a nationwide cancer registry mainly based on notification of all newly diagnosed malignancies in the Netherlands by the automated pathological archive (PALGA). An additional source of notification is the national registry of hospital discharges. Completeness of the NCR is estimated to be at least ***95***% [9,10].

Data on mortality was obtained by annual linkage to ***municipal*** population registries. Survival time was calculated from date of diagnosis till date of death, or end of follow-up on 1-1-2013**.** Tumour stage of pathologically confirmed HCCs is registered in the

NCR according to the Tumour-Node-Metastasis (TNM) classification and summarized according to the seventh edition [11]. For tumours without pathological confirmation, tumour stage was registered according to the 'Extent of Disease' (EoD) staging system.
The NCR exclusively records initial tumour-directed treatments after primary diagnostics until approximately nine months after diagnosis. In this report, the following treatments were evaluated: resection, loco-regional therapy (RFA and TACE), targeted systemic therapy and other systemic chemotherapy (for example, doxorubicin, potentially in combination with thalidomide). Targeted systemic therapy included sorafenib, which was registered in the Netherlands for treatment of renal cell carcinoma in July 2006 and for treatment of HCC in October 2007, and sporadically linifanib. Traditionally, HCC patients are often treated in university hospitals, because of the possibility of liver transplantation. Therefore, hospitals were classified as university and non-university hospitals (reference year 2013: 9 respectively 85). Since liver transplantations are exclusively performed in university hospitals in the Netherlands, liver transplantations were not addressed in the current study.
Hospital volume was calculated based on number of patients who underwent surgical resection respectively received initial sorafenib treatment (low volume: <5 per year; or high volume: ≥5 per year). The former regions of the Comprehensive Cancer Centre were classified based on zip codes of patients.

Statistical analyses

The study period was divided into three periods: 2003-2005, 2006-2008 and 2009-2011. Chi-square tests were used to evaluate trends. To compare regions, standardized percentages ([observed/expected] x national percentage) were calculated with 'case mix'-correction based on sex, age, socioeconomic status and tumour stage. Standardized percentages are shown in a radar chart, in which each radius represents a region. Statistical analysis of variation between regions was performed with likelihood-ratio tests. A p-value <0.05 was considered statistically significant. All analyses were performed using STATA version 12.0.

Results

The total study period 2003-2011 was divided into three periods: 2003-2005, 2006-2008 and 2009-2011. In total, 2915 patients were diagnosed with HCC. Patients and tumour characteristics are given in Table 1. Three-quarters of all patients were male and 44% were between 60-74 years of age. At time of diagnosis, 7% of all patients had regional metastases in lymph nodes (TNM-and EoD classifications) and 19% had distant metastases. In 45% of all patients with distant metastases, metastases were located in the lungs, in 23% in extra-regional lymph nodes, in 20% in bones and in 14% in the peritoneum or retroperitoneum. Moreover, additional data on distant metastases

Table 1: Patient and tumour characteristics of 2915 patients with hepatocellular carcinoma in the Netherlands in the period: 2005-2011

	n	%
Total	2915	100
Period		
2003-2005	780	27
2006-2008	956	33
2009-2011	1179	40
Sex		
Male	2184	75
Female	731	25
Age		
< 60 years	802	28
60-74 years	1290	44
≥75 years	823	28
Socioeconomic status		
High (1st-3rd decile)	875	30
Moderate (4th-7th decile)	1166	40
Low (8th-10th decile)	874	30
Diagnosis based on		
Clinical examination/imaging	1006	34
Cytology	77	3
Histology	1832	63
TNM-stage for pathologically confirmed HCCs[1]		
I (T1N0M0)	390	20
II (T2N0M0)	393	21
III (T3ab-4N0M0)	392	21
IV-A (N1)	145	8
IV-B (M1)	401	21
X (unknown)	169	9
EoD-stage for HCC when TNM was not available[2]		
EOD 1-2: local	573	56
EOD 3-4-5: regional	163	16
EOD 6: distant metastases	141	14
EOD 9: unknown	148	14
Distant metastases		
No	1900	65
Yes	542	19
Unknown	473	16
Differentiation grade		
Good/moderate	368	13
Poor	133	4
Unknown	2414	83

[1] Total 1890 patients. Based on 7th edition of Tumour-Node-Metastasis (TNM) classification: pTNM, in case of X or no resection based on cTNM. NX and MX are classified as N0 and M0.
[2] Total 1025 patients. Tumour stage according to the Extent of Disease (EoD) classification when TNM was not available.

were obtained during the study period: in the first period presence of metastases was unknown in 24% of patients, whereas in the last period this was the case in 7%.
In the periods 2003-2005, 2006-2008 and 2009-2011, 1-year survival rates of the total group of patients were 31, 37 and 38% respectively (p=0.004), 3-years survival rates 15, 19 and 18% respectively (p=0.006) and 5-years survival rates (only calculated for the first two periods) 10 and 14% respectively (p=0.009).

Trends in diagnostics and treatment

Despite a significant decrease in the number of diagnostic biopsies in the period 2003-2011, this procedure was still performed in more than half of all patients (61, 56 and 52% of all patients in the three periods, respectively) (Table 2). In 54% of all patients of whom pathology material was examined after resection tumour differentiation grade was unknown. There was a significant increase of treatment in general (25, 36 and 47% of all patients received treatment in the three periods, respectively). However, proportion of patients who underwent surgical resection remained stable (11, 11 and 13% respectively). Proportion of patients treated with RFA, TACE or sorafenib increased significantly, whereas there was a significant decrease of proportion of patients treated with other systemic chemotherapy (Table 2).
Of all patients without pathological confirmation of HCC diagnosis, 17, 26 and 34% received treatment for HCC, such as RFA, TACE and sorafenib. For example, in the last period 15% of patients without pathological confirmation of their HCC diagnosis received RFA, 12% TACE and 7% sorafenib.

Table 2: Location of diagnostics and initial treatment of hepatocellular carcinoma (HCC) in the Netherlands, period 2003-2011.

	Patients (%) of total			**Involved hospitals (%) of total [3,4]**			**Patients in university hospitals (%) of total [4]**		
Period	03-05	06-08	09-11	03-05	06-08	09-11	03-05	06-08	09-11
	n=780	n=956	n=1179				n=780	n=956	n=1179
Diagnoses [1]	100	100	100	94	96	97	26	29	25
Biopsies [2]	61	56	52*	-	94	97	23	24	27
Treatments	25	36	47*	33	43	62*	83	83	75*
Resection	11	11	13	18	22	27	82	82	75
RFA	4	10	10*	9	11	10	89	96	96
TACE	1	3	10*	1	4	10*	100	100	99
Sorafenib	<1	6	14*	1	28	53*	100	49	47
Chemo, overig	3	2	1*	11	10	9	60	67	60

[1]Hospital of diagnosis in defined as the hospital of the clinical HCC diagnosis; [2]Biopsy: pathological diagnosis without surgical treatment or pathological diagnosis on another date than surgical treatment. Date of surgery before 2005 not available; [3]Compared to the total number of hospitals in the Netherlands (n=93); [4]Exclusion of 17 patients due to resection performed abroad or hospital unknown. *Chi^2 test for trend p<0,05. RFA= radiofrequency ablation; TACE= transarterial chemoembolization

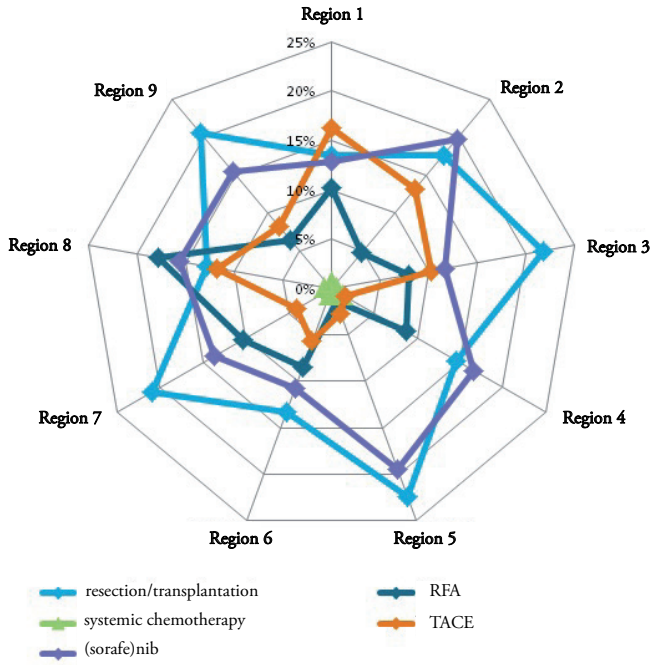
Trends in number of hospitals

In the period 2003-2011 diagnostic biopsies were performed in virtually all hospitals. There was a slight increase in proportion of hospitals that performed resections (18, 22 and 27% of all hospitals in the three periods, respectively). Moreover, there was a significant increase of proportion of hospitals in which initial sorafenib treatment was given (1, 28 and 53% of all hospitals in the three periods, respectively) and in which TACE was performed (1, 4 and 10%, respectively). Besides, only a few patients were treated with TACE or sorafenib in the first period. Proportion of hospitals in which RFA was performed or other systemic chemotherapy was given, did not change (Table 2).

Variation between regions

Figure 1 shows the variation of investigated treatments between regions in the last period (2009-2011). There were significant differences in the use of RFA and TACE between regions (both $p<0.001$ after case mix correction).

Figure 1: Radar chart with standardized percentages of patients per region in the period 2009-2011 who received one of the following treatments: resection of transplantation, radiofrequency ablation (RFA), transarterial chemoembolization (TACE), targeted therapy (sorafenib). Percentages of patients are standardized per treatment for casemix differences between regions (sex, age, socio-economic status (SES, based on zip codes of patients) and tumorstage). Each radius of the radar charts represents a region. Regions are based on zip codes of patients.



Trends in hospital type and volume

Although majority of patients was treated in university hospitals, there was a significant decrease of this proportion in the period 2003-2011. RFA, TACE and to a lesser extent resection were mainly performed in university hospitals, whereas in the last two periods half of initial sorafenib treatments were given in non-university hospitals (Table 2).

In Figure 2 the number of hospitals that performed an average of less than 1, 1 to 2, 3 to 4, or 5 or more resections per year is shown. Only a few university hospitals performed 5 or more resections per year. Of the 321 resections performed in the period 2003-2011, 60% was performed in university high volume hospitals (≥5 resections per year), 18% in university low volume hospitals (<5 resections per year) and 22% in non-university hospitals. There were no significant differences between university and non-university hospitals in proportion of laparoscopic procedures or extensiveness of resections. Median survival after resection was 47 months. In the periods 2003-2005, 2006-2008 and 2009-2011, 1-year survival rates after resection were 75, 86 and 83% respectively (p=0.11) and 3-year survival rates 55, 61 and 58% respectively (p=0.52).

Figure 3 shows the number of hospitals in which an average of less than 1, 1 to 2, 3 to 4, or 5 or more initial sorafenib treatment per year was given. Only in a few academic hospitals, in the last period, at least 5 patients per year started with sorafenib treatment shortly after diagnosis. Of the 227 sorafenib treatments in the period 2003-2011, 25% was given in university high volume hospitals (≥5 initial sorafenib treatments per year), 23% in university low volume hospitals (<5 initial sorafenib treatments per year) and

Figure 2: Number of hospitals that performed resection as initial therapy for HCC based on the average performed resections per hospital in the periods: 2003-2005, 2006-2008, 2009-2011. In light blue less than 1 per year, in dark blue 1 to 2 per year, in green 3 to 4 per year and in orange 5 or more resections per year.

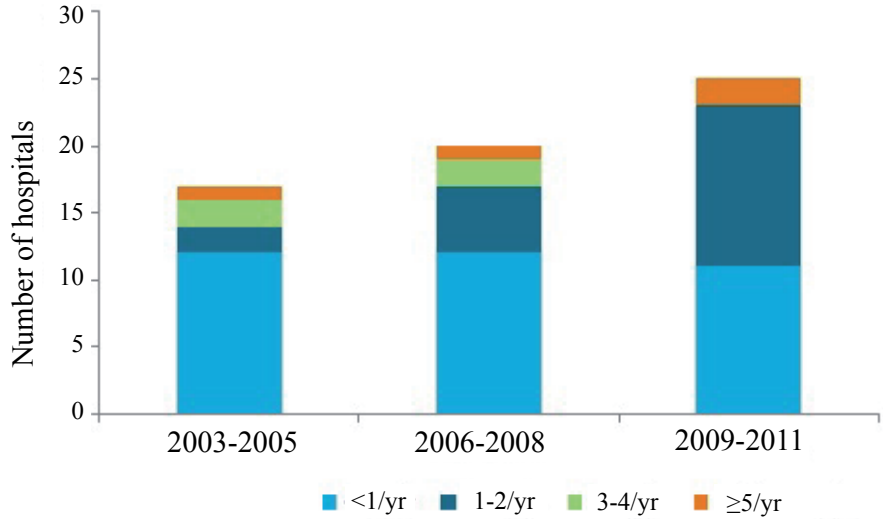
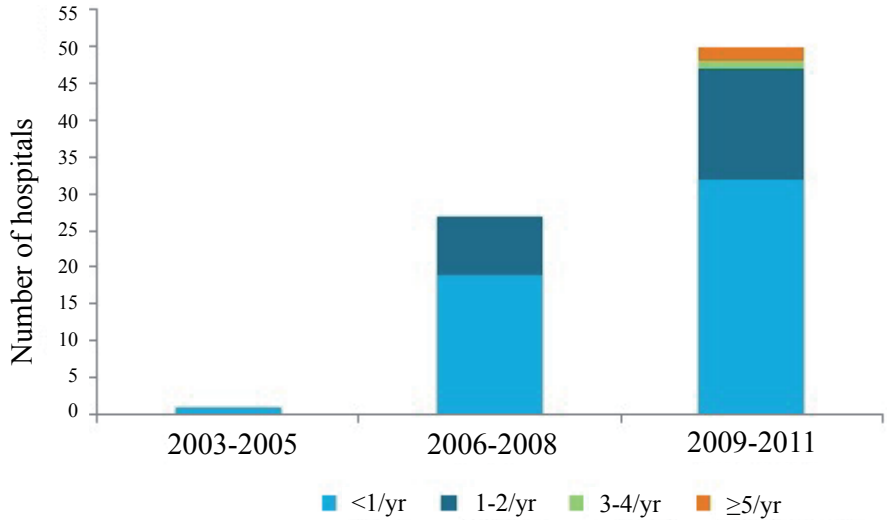


Figure 3: Number of hospitals that has given sorafenib as initial therapy for HCC based on the average sorafenib treatments per hospital in the periods: 2003-2005, 2006-2008, 2009-2011. In light blue less than 1 per year, in dark blue 1 to 2 per year, in green 3 to 4 per year and in orange 5 or more sorafenib treatments per year.



52% in non-university low volume hospitals. Median survival of patients who were treated with sorafenib was 7 months. In the periods 2006-2008 and 2009-2011, 1-year survival rates after sorafenib treatment were 42 and 25% respectively (p=0.05) and 3-year survival rates 5 and 4% respectively (p=0.03). However, patients who were treated with sorafenib in the last period were significantly older.

Discussion

In the current report, we evaluated trends in diagnostics and treatment of patients with HCC in the Netherlands in the past decade. Since 2005 international guidelines recommend ultrasonographic surveillance in patients with liver cirrhosis and a high HCC risk [2,3,12]. The goal of surveillance is to detect HCC at earlier stages, when tumour size is smaller and chance on curative treatment is higher, which could possibly lead to better survival [13]. Despite the lack of data about HCC surveillance in the Netherlands, our data show no increase in the number of resections with curative intention. However, number of patients who underwent transplantation increased significantly (10, 49 and 67 liver transplantations in the 3 periods, respectively) (source: Eurotransplant-database).

A Dutch study published in 2012 reported that proportion of patients who received surgical treatment (defined as tumour resection, transplantation and/or RFA) increased in the period 1989-2009 [1], but since then this proportion has not further increased (respectively 18, 24 and 24% in the three periods). On the other hand, proportion

of patients treated with TACE, RFA and mainly sorafenib increased significantly. Interestingly, there was no trend towards centralization. Although resection and loco-regional treatments were still mainly performed in university hospitals, only half of sorafenib treatments were given in university hospitals. Moreover, significant differences in the use of loco-regional therapy existed between the former regions of the Comprehensive Cancer Centre. A possible explanation is that these new treatments are not introduced in the clinical practice in all regions at the same time ('early' vs 'late adopters').

Remarkably, in more than half of patients a diagnostic biopsy was performed, despite a slight decrease in the period 2003-2011. Also, tumour differentiation grade after resection was only known in half of patients. The Dutch guideline 'Hepatocellular carcinoma' recommends to perform diagnostic biopsy only as indicated and to report the tumour differentiation grade in the pathology report of the resection specimens [4]. For several tumours it is demonstrated that hospital type and patient volume have a clear influence on treatment results [14,15]. Proponents of centralization point out the possibility of knowledge development. On the other hand, outcome-based centralization may be better for quality of care than volume-based centralization [16]. Furthermore, as a result of centralization knowledge and experience in the remaining hospitals will decrease and travel distance for patients will increase.

In multivariate analysis, hospital type was an independent predictor of postoperative mortality (within 30 days after resection) and long-term mortality after resection respectively sorafenib treatment (results not shown). Despite the correction for available casemix variables in this multivariate analysis, we cannot rule out that differences in patient populations are responsible for these findings.

The main shortcoming of our database is the absence of important prognostic factors, such as etiology and severity of underlying liver disease and performance status of the patient. The 'Barcelona Clinic of Liver Cancer'-staging combines HCC extensiveness, Child-Pugh-classification of the liver disease and performance status, and is nowadays the preferred staging system instead of the TNM-classification [2-4]. Furthermore, HCC patients could be missing in the NCR database when there is no pathologically confirmed diagnosis and no clinical admissions have occurred. Also, only initial treatments are recorded in the NCR database.

Conclusion

In the Dutch guideline "Hepatocellular carcinoma" centralization of diagnostics and treatment in expert centers is recommended, however, there was no trend towards centralization in the past decade. Current clinical practice has to change in order to achieve this recommendation.

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CHAPTER 10

SURVIVAL IN RELATION TO HOSPITAL TYPE AFTER RESECTION OR SORAFENIB TREATMENT FOR HEPATOCELLULAR CARCINOMA IN THE NETHERLANDS

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Summary

Background and objective: Despite an increase in recent years, hepatocellular carcinoma remains uncommon in the Netherlands. The aim of the current study is to explore potential effects of hospital type and volume on outcomes after resection or sorafenib in patients with hepatocellular carcinoma.

Methods: Initial treatment and survival of patients with hepatocellular carcinoma diagnosed in the period 2005-2011 were based on data of the Netherlands Cancer Registration. Potential risk factors (including hospital type and volume) for 30-days postoperative and long-term mortality in patients who underwent resection and in patients treated with sorafenib were evaluated by uni- and multivariate analyses.

Results: In the period 2005-2011, 2402 patients were diagnosed with hepatocellular carcinoma: 12% received resection and 9% sorafenib. Postoperative mortality was higher in non-university hospitals (13% versus 4%; P=0.01). Resection in non-university hospitals was associated with higher postoperative mortality (odds ratio 3.38, 95% confidence interval 1.37-10.68) and long-term mortality (hazard ratio 1.21, 95% confidence interval 1.04-1.40). Sorafenib treatment in non-university hospitals was also associated with higher long-term mortality (hazard ratio 1.39, 95% confidence interval 1.06-1.82). Hospital volume was no independent predictor for outcome.

Conclusion: In low incidence countries, outcome after resection or sorafenib for hepatocellular carcinoma may differ between various hospital types.

Introduction

Primary liver cancer is the sixth most common cancer in the world and the second cause of cancer-related death [1]. Hepatocellular carcinoma (HCC) represents more than 90% of primary liver cancers. In the USA, HCC incidence rates have tripled over the last two decades, whereas in Europe there is a mixed pattern, with increasing or decreasing incidence rates in various countries [1,2]. HCC generally occurs in patients with underlying chronic liver disease. Geographical differences in trends of incidence rates with time are thought to be the consequence of changing patterns of underlying risk factors for HCC, such as viral hepatitis, alcohol abuse and non-alcoholic steatohepatitis (NASH) [2-4].

Liver transplantation and resection are the most important curative treatment options for HCC. In the last few years, new treatment strategies for HCC –most in palliative setting- have been introduced: radiofrequency ablation (RFA) and transarterial chemoembolization (TACE). However, especially in small HCCs, RFA could also be curative [5]. Finally, multikinase inhibitor sorafenib leads to improved survival in patients with compensated liver disease (mainly Child-Pugh class A) and advanced HCC. Median survival benefit ranges from two to seven months compared to placebo [6,7].

As HCC is relatively rare in the Netherlands (HCC incidence in 2009: 3.4 respectively 1.2 per 100,000 person-years in men and women) [8], centralization of diagnostics and treatment of this disease to optimize outcome is subject to discussion. Indeed, for several other tumours, an association has been shown between hospital type and patient volume on the one hand and outcomes of treatment on the other hand [9-22]. In this nationwide cohort study, we evaluate the influence of hospital type and volume on outcomes of HCC treatment in the Netherlands.

Methods

Initial treatment and survival of patients with HCC diagnosis in the period January 2005-December 2011 were evaluated based on data of the Netherlands Cancer Registry (NCR). The NCR is a nationwide cancer registry mainly based on notification of all newly diagnosed malignancies in the Netherlands by the automated pathological archive (PALGA). An additional source of notification is the national registry of hospital discharges. Information on patient, tumour and treatment characteristics is extracted retrospectively from the medical records in all Dutch hospitals. Completeness of the NCR is estimated to be at least ***95***% [23,24]. The NCR prospective database is completely anonymous, without possibility to identify individual patients or hospitals (hospital type (i.e. university or non-university) is addressed in the NCR database). The international classification of diseases for oncology (ICD-O) was used as basis for coding of tumours [25]. For this study, all patients with a primary malignancy located in the liver (ICD-10 code C22.0) were selected from the NCR. Exclusion criteria were

age ≤18 years, residing abroad, proven primary hepatic malignancy other than HCC (e.g. cholangiocarcinoma, hepatoblastoma, lymphoma) and HCC diagnosed at autopsy. Information on mortality was obtained by annual linkage to municipal population registries. These registries provide complete coverage of all deceased Dutch residents.
No data on Barcelona Clinic of Liver Cancer staging (combining extension of HCC, Child-Pugh classification of liver disease and performance score) are available in the NCR database. Tumour stage of pathologically proven HCCs is registered in the NCR according to the Tumor-Node-Metastasis (TNM) classification and summarized according to the seventh edition [26]. 'Extent of Disease' (EoD) staging system was used for HCCs when TNM was not available [27,28]. The NCR records initial treatments until approximately nine months after diagnosis.
Descriptive data on trends in distribution of various diagnostic procedures and treatment modalities between all 85 general hospitals and 8 university hospitals in the Netherlands in this cohort have recently been reported [29]. The current report focuses on characteristics and outcomes of resection and sorafenib treatment for HCC in the Netherlands, as these important curative and palliative treatment modalities were provided in a relatively large number of hospitals (29 and 53 hospitals respectively) [29]. Traditionally, HCC treatment is often performed in university hospitals. Therefore, hospitals were classified as university or non-university hospitals. Since in the Netherlands liver transplantation is restricted to three university hospitals, outcomes of this procedure are not reported here. Hospital volumes were calculated for each year and each institution based on numbers of patients treated with liver resection or initial multikinase inhibitor sorafenib treatments respectively (<5 or ≥5 treatments per year). Since annual volumes could change with time, hospitals could switch from low volume to high volume institutions and vice versa.

Statistical analyses

Chi-square tests were used to compare groups of patients. A p-value <0.05 was considered to be significant. Logistic regression analysis was performed to evaluate the influence of patient and tumour characteristics on mortality within 30 days after resection (regardless of cause of death). Kaplan-Meier mortality curves and Cox proportional hazard regression analyses were used to evaluate the relation between patient and tumour characteristics on the one hand and risk of **1.** long-term mortality after resection (patients with mortality within 30 days after resection excluded) and **2.** long-term mortality of patients treated with sorafenib on the other hand. For the resection and sorafenib subgroups, survival time was calculated based on date of diagnosis and separately based on date of resection or for the patients in whom date of sorafenib prescription was known based on date of start of sorafenib treatment. In all groups, survival time ended at time of death or end of follow-up on 1-1-2013. The following variables were included in univariate regression analyses: sex, age, country of birth, socioeconomic status, tumour stage, hospital type,

hospital volume and additional treatment with chemotherapy (only in the evaluation of long-term mortality after resection). Factors with a p-value <0.1 in univariate analyses were included in subsequent multivariate analyses. All analyses were performed using STATA/SE (version 12.0; STATA Corp., College Station, Texas, USA).

Results

Patient and tumour characteristics

In the period 2005-2011, 2402 patients were diagnosed with HCC in the Netherlands. Most patients (75%) were males and 45% was between 60 and 74 years of age (Table 1). At time of diagnosis, 573 patients (24%) had locally advanced disease (TNM+EoD together) and 456 patients (19%) had distant metastases (Table 1). Overall, 279 patients (12%) underwent liver resection and 225 patients (9%) received sorafenib treatment. RFA and TACE were performed in 222 (9%) and 137 (6%) patients respectively. Transplantation was performed in 89 patients (4%). Systemic treatment other than sorafenib was used in 2% of all patients.

Resection group

In the period 2005-2011, liver resection was performed in 272 patients (after exclusion of 7 patients who had resection in a hospital outside the Netherlands). Patients who underwent resection were more often female, were younger and were more often born in Asian or sub-Sahara countries than in the total group of HCC patients (all $P<0.05$). Tumour stage was generally limited (TNM+EoD together) and distant metastases were rare in patients who underwent resection. Most resected patients had a solitary HCC without vascular invasion (TNM=I), whereas in 30% of cases HCC was multifocal with a maximum tumour diameter <5 cm or with vascular invasion (TNM=II). Seventy-eight percent of all liver resections was performed in university hospitals. Patients in non-university hospitals had a slightly more advanced stage of disease ($P=0.07$; Table 1). There were no other remarkable differences in patient or tumour characteristics between university and non-university resection groups (Table 1). As indicated in Table 1, there was no significant difference in proportion of laparoscopic procedures (data available since 2010), type of resection or proportion of radical resections. Fifty-nine percent of resections was performed in high-volume hospitals (≥5 resections/year). Postoperative mortality during the first 30 days after resection was higher in non-university hospitals (13% versus 4%; $P=0.01$). Resection in a non-university hospital was the only factor associated with higher postoperative mortality (odds ratio 3.38, 95% confidence interval (CI) 1.37-10.68) (Table 2). The 1-, 3- and 5-year survival rates based on date of diagnosis in patients who underwent resection were 84%, 59% and 44% respectively. Median survival was 47 months. In patients who survived the first 30 days after resection, long-term mortality based on date of diagnosis was significantly

Table 1: Patient and tumour characteristics of patients with hepatocellular carcinoma in the Netherlands in the period: 2005-2011 and separately a comparison of the subgroups receiving resection or sorafenib in university and non-university hospitals.

	Total	Resection			Sorafenib		
		UMC	Non-UMC	P-value	UMC	Non-UMC	P-value
	n=2402 (%)	n=211 (%)	n=61 (%)		n=107 (%)	n=118 (%)	
Sex							
Male	1799 (75)	144 (68)	43 (70)	0.74	84 (79)	89 (75)	0.58
Female	603 (25)	67 (32)	18 (30)		23 (21)	29 (25)	
Age							
< 60 years	642 (27)	76 (36)	14 (23)	0.16	38 (36)	27 (23)	0.007
60-74 years	1080 (45)	95 (45)	34 (56)		63 (59)	70 (59)	
≥75 years	680 (28)	40 (19)	13 (21)		6 (6)	21 (18)	
Country of birth							
The Netherlands	1957 (81)	165 (78)	50 (82)	0.66	91 (85)	83 (83)	0.83
Asian/sub-Sahara	135 (6)	22 (11)	4 (7)		3 (3)	5 (4)	
Other/unknown	311 (13)	24 (11)	7 (11)		13 (12)	15 (13)	
Socioeconomic status							
High (1st-3rd decile)	721 (30)	66 (29)	22 (33)	0.86	35 (33)	38 (32)	0.77
Moderate (4th-7th decile)	962 (40)	84 (42)	23 (41)		42 (39)	42 (36)	
Low (8th-10th decile)	719 (30)	61 (29)	16 (26)		30 (28)	38 (32)	
Diagnosis based on							
Clinical examination and imaging	856 (36)	0 (0)	0 (0)		27 (25)	22 (19)	0.08
Cytology	53 (2)	1 (<1)	1 (1)	0.35	1 (1)	7 (6)	
Histology	1493 (62)	210 (100)	60 (99)		79 (74)	89 (75)	
Time between histological diagnosis and resection	[a]						
Date of histology=resection	176 (63)	132 (63)	43 (71)	0.09			
1 day - 3 months	45 (16)	28 (13)	11(18)				
3 or more months	58 (21)	51 (24)	7 (11)				
Neoadjuvant treatment							
No		198 (94)	58 (95)	0.72	92 (86)	111 (94)	0.04
Yes		13 (6)	3 (5)		15 (14)	7 (6)	

Stage (TNM + EoD)							
Localized	1142 (47)	175 (83)	47 (77)		16 (15)	30 (25)	
Locally advanced	573 (24)	35 (17)	11 (18)	0.07	47 (44)	47 (40)	0.30
Distant metastasis	456 (19)	0 (0)	1 (2)		42 (39)	40 (34)	
Unknown	231 (10)	1 (<1)	2 (3)		2 (2)	1 (1)	
TNM-stage for pathologically confirmed HCCs[b]							
I (T1N0M0)	324 (21)	106 (51)	32 (53)		2 (2)	5 (5)	
II (T2N0M0)	322 (21)	67 (32)	14 (23)		7 (9)	12 (13)	
III (T3ab-4N0M0)	309 (20)	30 (14)	11 (18)	0.07	23 (29)	30 (31)	0.68
IV-A (N1)	120 (8)	5 (2)	0 (0)		12 (15)	12 (13)	
IV-B (M1)	338 (22)	0 (0)	1 (2)		35 (44)	37 (38)	
X (unknown)	123 (8)	1 (<1)	2 (4)		1 (1)	0 (0)	
EoD-stage for HCC when TNM was not available[c]							
EOD 1-2: local	496 (57)	2 (100)	1 (100)		7 (26)	13 (59)	
EOD 3-4-5: regional	144 (17)				12 (44)	5 (23)	0.01
EOD 6: distant metastases	118 (14)				7 (26)	3 (14)	
EOD 9: unknown	108 (12)				1 (4)	1 (4)	
Type of resection	[a]						
1-2 segments	193 (71)	147 (70)	46 (75)	0.39			
Lobectomy (≥3 segments)	47 (17)	40 (19)	7 (12)				
(Extended) hemihepatectomy	32 (12)	24 (11)	8 (13)				
Laparoscopic resection[d]	[a]						
No	85 (89)	62 (89)	23 (92)	0.63			
Yes	10 (11)	8 (11)	2 (8)				
Radical resection[e]	[a]						
R0	228 (87)	179 (89)	49 (80)	0.14			
R1-2	24 (9)	16 (8)	8 (13)				
Unknown	9 (4)	5 (3)	4 (7)				

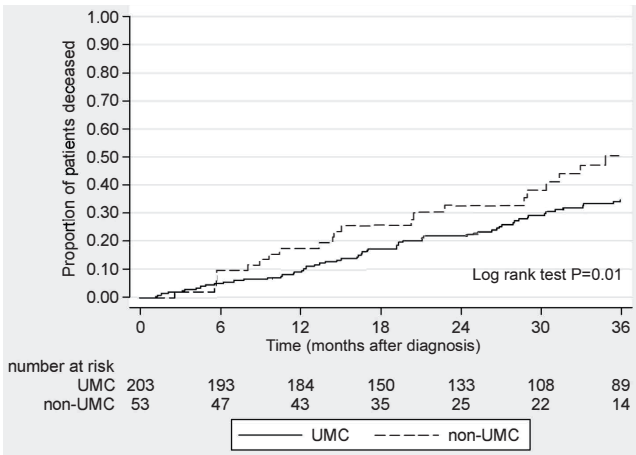
Values in parentheses are percentages; HCC= hepatocellular carcinoma; [a]Patients who underwent resection in the Netherlands; [b]Total 1536 patients. Based on 7th edition of Tumour-Node-Metastasis (TNM) classification: pTNM, in case of X or no resection based on cTNM. NX and MX are classified as N0 and M0; [c]Total 866 patients. Tumour stage according to the Extent of Disease (EoD) classification when TNM was not available; [d]Data available for patients who underwent resection in the period 2010-2011; [e]Exclusion of 11 patients with combined RFA treatment; *P-value applies to UMC versus non-UMC.

Table 2: Relation between patient and tumour characteristics and 30-days mortality after resection for hepatocellular carcinoma in the Netherlands in the period: 2005-2011 (univariate logistic regression analysis)

	Patients with resection	30-days mortality after resection (%)	Univariate analysis		
Variables	**n=272 (%)**	**5.9**	**OR**	**95 per cent CI**	**P-value**
Sex					
Male	187 (69)	6.4	1	-	0.58
Female	85 (31)	4.7	0.72	0.23-2.30	
Age					
< 75 years	219 (81)	5.0	1	-	0.23
≥ 75 years	53 (19)	9.4	1.97	0.65-5.93	
Country of birth					
The Netherlands	215 (79)	6.5	1	-	0.73
Asian/sub-Sahara	26 (10)	7.7	1.29	0.28-6.09	
Other/unknown	31 (11)	3.2	0.52	0.07-4.10	
Socioeconomic status					
High (1st-3rd decile)	82 (30)	4.9	1	-	0.45
Moderate (4th-7th decile)	113 (42)	8.0	1.69	0.50-5.68	
Low (8th-10th decile)	77 (28)	3.9	0.79	0.17-3.65	
Tumour stage					
TNM=I	138 (51)	4.4	1	-	0.55
TNM=II	81 (30)	7.4	1.76	0.55-5.65	
TNM=III/other	53 (19)	7.6	1.80	0.49-6.34	
Hospital type (resection)					
University	211 (78)	3.8	1	-	0.01
Non-university	61 (22)	13.1	3.83	1.37-10.68	
Hospital volume					
<5 resections / year	111 (41)	7.2	1	-	0.44
≥5 resections / year	161 (59)	5.0	0.67	0.24-1.85	

Values in parentheses are percentages; OR= odds ratio; 95 per cent CI= 95 per cent confidence interval; TNM= Tumour-Node-Metastasis

Figure 1: Long-term mortality based on date of diagnosis in patients in the Netherlands who underwent resection for hepatocellular carcinoma in university and non-university hospitals in the period: 2005-2011 and survived the first 30 days after resection (Kaplan-Meier curves, log rank test P=0.01). UMC= university medical centre; non-UMC= non-university medical centre.



higher in patients who underwent resection in a non-university hospital than in patients who underwent resection in an university hospital (Figure 1, log rank test *P*=0.01). In multivariate analysis, both hospital type and advanced tumour stage were independently associated with higher long-term mortality (based on date of diagnosis) after resection (hazard ratio (HR) non-university hospital: 1.21, 95% CI 1.04-1.40) (Table 3). Hospital volume was no independent predictor for short- or long-term outcomes after resection. Time between diagnosis and resection was longer in university hospitals, probably related in many cases to referral time from the hospital where HCC was initially diagnosed. The 1-, 3- and 5-year survival rates (based on date of resection) after resection were 84%, 57% and 44% respectively. Median survival after resection was 46 months. In patients who survived the first 30 days after resection, there was a trend towards higher long-term

Table 3: Relation between patient and tumour characteristics and long-term mortality (based on date of diagnosis) in patients who underwent resection for hepatocellular carcinoma in the Netherlands in the period: 2005-2011 (univariate and multivariate Cox proportional hazard analyses)

	Patients with resection	Univariate analysis			Multivariate analysis		
Variables	**n = 256[a] (%)**	**HR**	**95 per cent CI**	**P-value**	**HR**	**95 per cent CI**	**P-value**
Sex							
Male	175 (68)	1	-	0.83			
Female	81 (32)	0.96	0.64-1.44				
Age							
< 75 years	208 (81)	1	-	0.06	1	-	0.08
≥ 75 years	48 (19)	1.53	0.99-2.36		1.49	0.96-2.31	
Country of birth							
The Netherlands	202 (79)	1	-	0.59			
Asian/sub-Sahara	24 (9)	0.87	0.46-1.71				
Other/unknown	30 (12)	0.71	0.36-1.42				
Socioeconomic status							
High (1st-3rd decile)	78 (30)	1	-	0.92			
Moderate (4th-7th decile)	104 (41)	0.93	0.60-1.45				
Low (8th-10th decile)	74 (29)	0.91	0.56-1.49				
Tumour stage							
TNM=I	132 (52)	1	-	<0.001	1	-	
TNM=II	75 (29)	2.11	1.33-3.33		2.14	1.35-3.40	0.001
TNM=III/other	49 (19)	2.36	1.47-3.77		2.29	1.43-3.66	0.001
Chemotherapy							
No	246 (96)	1	-	0.89			
Yes (systemic-TACE-sorafenib)	10 (4)	0.92	0.29-2.91				
Hospital type (resection)							
University	203 (79)	1	-	0.02	1	-	0.01
Non-university	53 (21)	1.72	1.11-2.67		1.21	1.04-1.40	
Hospital volume							
<5 resections / year	103 (40)	1	-	0.88			
≥5 resections / year	153 (60)	0.97	0.66-1.42				

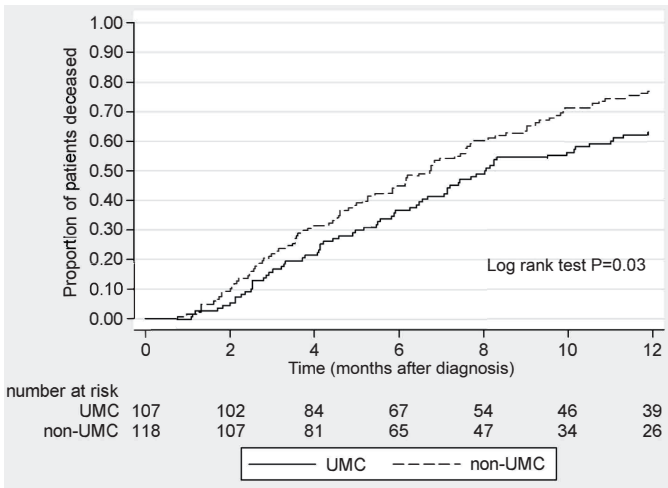
Values in parentheses are; [a]Exclusion of 16 deceased patients within 30 days after resection; HR= hazard ratio; 95 per cent CI= 95 per cent confidence interval; TNM= Tumour-Node-Metastasis

mortality (based on date of resection) after resection in a non-university hospital than after resection in an university hospital (Supplementary figure 1, log rank test *P*=0.06). Also, in multivariate analysis, the influence of hospital type on long-term mortality (based on date of resection) after resection was significant (hazard ratio (HR) 1.57, 95% CI 1.01-2.44). On the other hand, advanced TNM stage was independently associated with higher long-term mortality after resection (Supplementary table 1). Hospital volume was again no independent predictor for long-term outcomes after resection.

Sorafenib group

Initial sorafenib treatment was given to 225 patients. Tumour and patient characteristics of this subgroup are given in Table 1. Patients receiving sorafenib treatment were significantly younger (78% of patients younger than 75 years) than the total group of HCC patients (*P*<0.05). Also, patients who received sorafenib treatment had a more advanced tumour stage (TNM+EoD together) and frequently had metastatic disease. The majority of patients receiving initial sorafenib treatment had an advanced HCC tumour stage at time of diagnosis (80%; locally advanced and/or distant metastasis). Patients in non-university hospitals were significantly older than patients in university hospitals (18% and 6% of patients were 75 years or older respectively) (Table 1). Number of prescribed initial sorafenib treatments was low in the large majority of hospitals: only in a few university hospitals, at least five patients per year received sorafenib (only in the period 2009-2011). Forty-eight percent of sorafenib treatments was given in university

Figure 2: Long-term mortality based on date of date of diagnosis in patients in the Netherlands with sorafenib treatment for hepatocellular carcinoma in university and non-university hospitals in the period: 2005-2011 (Kaplan-Meier curves, log rank test P=0.03). UMC= university medical centre; non-UMC= non-university medical centre.



hospitals, and 25% in high-volume hospitals (≥5 sorafenib treatments/year). The 1- and 3-year survival rates based on date of diagnosis of patients treated with sorafenib were 29% and 3% respectively. Median survival from date of diagnosis was 7.3 months. Long-term mortality (based on date of diagnosis) after sorafenib treatment was higher in non-university hospitals (Figure 2, log rank test *P*=0.03). In multivariate analysis, advanced tumour stage (HR 1.75, 95% CI 1.23-2.47) and sorafenib treatment in non-university hospitals (HR 1.39, 95% CI 1.06-1.82) were independently associated

Table 4: Relation between patient and tumour characteristics and long-term mortality (based on date of diagnosis) in patients who received sorafenib treatment for hepatocellular carcinoma in the Netherlands in the period: 2005-2011 (univariate and multivariate Cox proportional hazard analyses)

	Patients with sorafenib	Univariate analysis			Multivariate analysis		
Variables	**n=225 (%)**	**HR**	**95 per cent CI**	**P-value**	**HR**	**95 per cent CI**	**P-value**
Sex							
Male	173 (77)	1	-	0.19			
Female	52 (23)	0.81	0.59-1.12				
Age							
< 75 years	198 (88)	1	-	0.97			
≥ 75 years	27 (12)	1.01	0.66-1.54				
Country of birth							
The Netherlands	189 (84)	1	-	0.57			
Asian/sub-Sahara	8 (4)	1.50	0.74-3.06				
Other/unknown	28 (12)	1.04	0.69-1.57				
Socioeconomic status							
High (1st-3rd decile)	73 (33)	1	-	0.15			
Moderate (4th-7th decile)	84 (37)	0.79	0.57-1.10				
Low (8th-10th decile)	68 (30)	1.08	0.77-1.52				
Tumour stage							
TNM=I-II; EoD=local	46 (20)	1	-	0.002	1	-	0.002
TNM=III-IV-X; EoD=other	179 (80)	1.70	1.20-2.40		1.75	1.23-2.47	
Hospital type (sorafenib)							
University	107 (48)	1	-	0.03	1	-	0.02
Non-university	118 (52)	1.34	1.02-1.76		1.39	1.06-1.82	
Hospital volume							
<5 sorafenib treatments / year	169 (75)	1	-	0.28			
≥5 sorafenib treatments / year	56 (25)	1.19	0.87-1.62				

Values in parentheses are percentages; HR= hazard ratio; 95 per cent CI= 95 per cent confidence interval; TNM= Tumour-Node-Metastasis; EoD= Extent of Disease

with higher long-term mortality (based on date of diagnosis) after sorafenib treatment (Table 4). Hospital volume was no independent predictor for long-term outcomes after sorafenib treatment.

In patients in whom date of sorafenib prescription was known (n=137), time between diagnosis and start of sorafenib treatment was significantly longer in patients treated in university hospitals (median: 58 days (IQR: 39-81) in university vs. 31 days (IQR: 16-45) in non-university hospitals; $P<0.05$; Supplementary table 2). The 1- and 3-year survival rates (based on date of start of sorafenib treatment) after sorafenib treatment were 25% and 2% respectively. Median survival from start of treatment was 5.6 months. Long-term mortality (based on date of start of sorafenib treatment) after sorafenib treatment was slightly higher in non-university hospitals (Supplementary figure 2, log rank test $P=0.05$). In multivariate analysis, advanced tumour stage (HR 1.89, 95% CI 1.21-2.96) and sorafenib treatment in non-university hospitals (HR 1.58, 95% CI 1.10-2.28) were again independently associated with higher long-term mortality (based on date of start of sorafenib treatment) after sorafenib treatment (Supplementary table 3). Hospital volume was again no independent predictor for long-term outcomes after sorafenib treatment.

Discussion

This national population-based study showed that postoperative mortality and (less pronounced) long-term mortality were significantly higher in patients who underwent HCC resection in non-university compared to university hospitals. Long-term mortality in patients who had initial sorafenib treatment in non-university hospitals was also significantly higher than in patients treated in university hospitals. Hospital volume (based on number of resections and sorafenib treatments respectively) was no independent predictor for these short- and long-term outcomes. Available patient and tumour characteristics, and surgical parameters like type of resection, could not explain these results. However, important prognostic factors such as aetiology and severity of underlying liver disease and performance score were not available. Furthermore, Barcelona Clinic of Liver Cancer staging (combining extension of HCC, Child-Pugh classification of liver disease and performance score) is currently the first choice for HCC staging rather than TNM classification which was used in the current report. Therefore, no definite conclusion can be drawn on quality of care in university vs. non-university hospitals and potential impact of hospital type on mortality.

We calculated long-term mortality based on date of diagnosis and separately based on date of resection respectively date of start of sorafenib treatment, since time between diagnosis and resection respectively start of sorafenib treatment was longer in university than in non-university hospitals. In fact, differences in long-term mortality between both hospital types were more pronounced when date of diagnosis was used. A potential explanation for this phenomenon could be selection bias: In some patients it may

have been decided not to perform resection or to start sorafenib treatment because of progression of tumour or liver disease during the longer waiting time in university hospitals.

Despite the relatively low incidence of HCC in the Netherlands, resection and sorafenib treatment were not concentrated in centres of expertise, as we reported previously [29]. In the current report, we therefore explore the potential impact of this phenomenon on patient survival. We focused in particular on resection and sorafenib, since these are quantitatively the main curative and palliative treatment options. An additional advantage of this choice is that a substantial number of these patients were treated in non-university hospitals, allowing to study the effect of hospital type on outcome. In contrast, RFA and TACE are performed almost exclusively, and liver transplant exclusively, in university hospitals [29].

In the current report, 1-, 3- and 5-year survival rates in patients who underwent resection are in line with previous reports from Japan and the USA [30,31]. In our study, postoperative mortality within 30 days after resection was 6%. In previous reports, early mortality after HCC resection varied between 1% and 24%, related to definitions of postoperative mortality [11-13,17-22,32]. According to previous literature, hepatic resection for HCC is associated with better survival in high volume centres than in low volume centres, mainly due to lower early postoperative mortality [11-13,17,18,22]. Also, the experience of the individual surgeon may be an important factor in this respect [16]. In the current study, hospital volume (based on number of resections performed in each centre) was no independent predictor for short- and long-term outcomes. One may speculate that a distinction between high or low volume hospitals based on a volume of less than five versus five or more resections per year, as used in the current report, is too low to have a significant impact on treatment outcome. However, only one hospital performed more than 10 resections per year. Furthermore, earlier studies regarding HCC outcomes after resection used all different definitions for high-volume centres, ranging from more than five resection per year to more than 299 resections in a period of three years [11,13,16]. In one study volume was reported as categorical variable [12]. In our study, multivariate analysis indicated that hospital type was a relevant factor for short-term outcome and (less pronounced) also for long-term outcome after HCC resection. No previous data are available on the impact of hospital type after resection for HCC. Nevertheless, for oesophageal and gastric cancer and some urological tumours, an association has been shown between hospital type (e.g. academic status) and outcome [9,14,15]. On the other hand, outcome of surgery for colorectal liver metastasis may not be influenced by hospital type [33].

In the current report, cumulative 1- and 3-year survival rates based on date of HCC diagnosis in patients treated with sorafenib were 29% and 3% respectively. Patients in our population-based study receiving sorafenib treatment were older, had a more advanced tumour stage and a worse overall survival compared to patients enrolled

in previous randomized or observational studies [6,34]. Furthermore, most patients who were included in previous studies had a Child-Pugh A status. Unfortunately, data on severity of liver disease are not available in our study. Sorafenib treatment in an university hospital was associated with slightly better survival rates according to multivariate analysis. In contrast, hospital volume was not associated with outcome. To our knowledge, there are no earlier data available about the influence of hospital type or volume on outcomes after sorafenib treatment. Nevertheless, for several other tumours an effect of hospital type and number of medical oncologists on outcome of chemotherapy has been demonstrated [22]. Another study demonstrated the impact of physician's level of experience on HCC outcome in general: HCC patients who were managed by a high-volume physician (a hepatologist who devoted more than 80% of daily practice to liver disease) had significantly better survival than patients allocated to low-volume physicians (general gastroenterologists who devoted less than 30% of daily practice to liver disease), after adjusting for other important prognostic factors [35]. Apart from a higher level of expertise in university hospitals, another possible explanation for differences in outcomes between university and non-university hospitals could in theory be a better multidisciplinary approach in the allocation process of treatment in university hospitals. Nevertheless, our data do not allow to confirm or refute these possibilities.

Our study has several limitations. Firstly, as mentioned above, important prognostic factors were not available. To obtain some additional information, we have availed of the complementary cooperative hepatocellular carcinoma database of the 5 major university hospitals in the Netherlands for the period: 2005-2012, overlapping with the NCR database. All patients in the cooperative university hospital database are also included in the NCR database. The cooperative university hospital database comprises approximately 60% of all HCC patients in the Netherlands during this period. Resection was performed in 221 patients (18% of all HCC cases) and sorafenib prescribed in 118 patients (10% of all HCC cases). For the resection and sorafenib groups, underlying causes were hepatitis B in 22% and 16%, hepatitis C in 17% and 9%, alcohol abuse in 14% and 35%, NAFLD in 17% and 14%, hemochromatosis in 5% and 1% and others in 3% and 2%, whereas no underlying risk factors were identified despite extensive investigations in 18% and 20% respectively. Majority of patients had a ECOG performance score of 0-1 (86% in both groups). In the resection and sorafenib groups, 3% and 0% of patients had a BCLC stage 0 at time of HCC diagnosis, 44% and 2% BCLC stage A, 52% and 27% BCLC stage B, 2% and 70% BCLC stage C and 0% and 1% BCLC stage D, respectively. For the resection group, presence of cirrhosis, advanced tumour size and number of nodules were all independent factors associated with mortality in multivariate analysis. For the sorafenib group, no independent predictors for mortality were found in multivariate analysis.

Another criticism could be that long-term outcomes may be influenced by many factors, including patient condition, disease status and (second-line) treatment during follow-up. Only initial treatments until approximately nine months after diagnosis are registered in the NCR database. Furthermore, in earlier years the availability of dates of (starting) initial sorafenib treatment was limited. Finally, HCC patients could be missed in the NCR database when HCC diagnosis was not based on histology and the patient was never admitted for HCC during the course of the disease. Death certificates are not available as a source of notification for registration in the NCR database. However, the NCR database contains unique data on diagnosis, treatment and survival of the vast majority of HCC patients in the Netherlands and therefore reflects current clinical practice.

In conclusion, postoperative and (less pronounced) long-term mortality was significantly higher in patients who underwent resection in non-university compared to university hospitals. Long-term mortality in patients who had initial sorafenib treatment in non-university hospitals was also significantly higher than in patients treated in university hospitals. Hospital volume was no independent predictor for mortality. Further prospective research in necessary to evaluate potential impact of hospital type on outcome in HCC patients.

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Supplementary table 1: Relation between patient and tumour characteristics and long-term mortality (based on date of resection) in patients who underwent resection for hepatocellular carcinoma in the Netherlands in the period: 2005-2011 (univariate and multivariate Cox proportional hazard analyses)

	Patients with resection	Univariate analysis			Multivariate analysis		
Variables	**n=256[a] (%)**	**HR**	**95 per cent CI**	**P-value**	**HR**	**95 per cent CI**	**P-value**
Sex							
Male	175 (68)	1	-	0.85			
Female	81 (32)	0.96	0.65-1.43				
Age							
< 75 years	208 (81)	1	-	0.07	1	-	0.09
≥ 75 years	48 (19)	1.51	0.99-2.32		1.45	0.94-2.23	
Country of birth							
The Netherlands	202 (79)	1	-	0.71			
Asian/sub-Sahara	24 (9)	0.85	0.44-1.62				
Other/unknown	30 (12)	0.80	0.43-1.50				
Socioeconomic status							
High (1st-3rd decile)	78 (30)	1	-	0.87			
Moderate (4th-7th decile)	104 (41)	0.95	0.62-1.47				
Low (8th-10th decile)	74 (29)	0.88	0.54-1.42				
Tumour stage							
TNM=I	132 (52)	1	-	<0.001	1	-	
TNM=II	75 (29)	2.31	1.48-3.58		2.33	1.49-3.63	<0.001
TNM=III/other	49 (19)	2.33	1.46-3.72		2.27	1.42-3.63	0.001
Chemotherapy							
No	246 (96)	1	-	0.95			
Yes (systemic-TACE-sorafenib)	10 (4)	1.04	0.33-3.27				
Hospital type (resection)							
University	203 (79)	1	-	0.07	1	-	0.045
Non-university	53 (21)	1.52	0.99-2.36		1.57	1.01-2.44	
Hospital volume							
<5 resections / year	103 (40)	1	-	0.79			
≥5 resections / year	153 (60)	1.05	0.72-1.54				

Values in parentheses are percentages; [a]Exclusion of 16 deceased patients within 30 days after resection; HR=hazard ratio; 95 per cent CI= 95 per cent confidence interval; TNM= Tumour-Node-Metastasis

Supplementary table 2: Comparison of patient and tumour characteristics of patients with hepatocellular carcinoma in university and non-university hospitals receiving sorafenib treatment in whom date of start of sorafenib treatment was known in the Netherlands in the period: 2005-2011

	Sorafenib		
	UMC n=67 (%)	Non-UMC n=70 (%)	P-value*
Sex			
Male	52 (78)	50 (71)	0.41
Female	15 (22)	20 (29)	
Age			
< 60 years	27 (40)	14 (20)	0.003
60-74 years	36 (54)	40 (57)	
≥75 years	4 (6)	16 (23)	
Country of birth			
The Netherlands	53 (79)	58 (83)	0.84
Asian/sub-Sahara	3 (5)	3 (4)	
Other/unknown	11 (16)	9 (13)	
Socioeconomic status			
High (1st-3rd decile)	20 (30)	21 (30)	0.03
Moderate (4th-7th decile)	32 (48)	20 (29)	
Low (8th-10th decile)	15 (22)	29 (41)	
Diagnosis based on			
Clinical examination/imaging	13 (19)	12 (17)	0.08
Cytology	0	5 (7)	
Histology	54 (81)	53 (76)	
Stage (TNM + EoD)			
Localized	9 (13)	22 (31)	
Locally advanced	28 (42)	26 (38)	0.06
Distant metastasis	29 (43)	22 (31)	
Unknown	1 (2)	0	
TNM-stage for pathologically confirmed HCCs[a]			
I (T1N0M0)	1 (2)	4 (7)	
II (T2N0M0)	4 (7)	8 (14)	
III (T3ab-4N0M0)	16 (30)	18 (31)	0.46
IV-A (N1)	7 (13)	7 (12)	
IV-B (M1)	26 (48)	21 (36)	
X (unknown)	0	0	
EoD-stage for HCC when TNM was not available[b]			
EOD 1-2: local	4 (31)	10 (84)	
EOD 3-4-5: regional	5 (38)	1 (8)	0.07
EOD 6: distant metastases	3 (23)	1 (8)	
EOD 9: unknown	1 (8)	0	
Interval date of (histological) diagnosis and (start of) treatment			
Interval 1 day - 3 months	53 (79)	66 (94)	0.009
Interval 3 or more months	14 (21)	4 (6)	
Unknown (missing dates)	-	-	

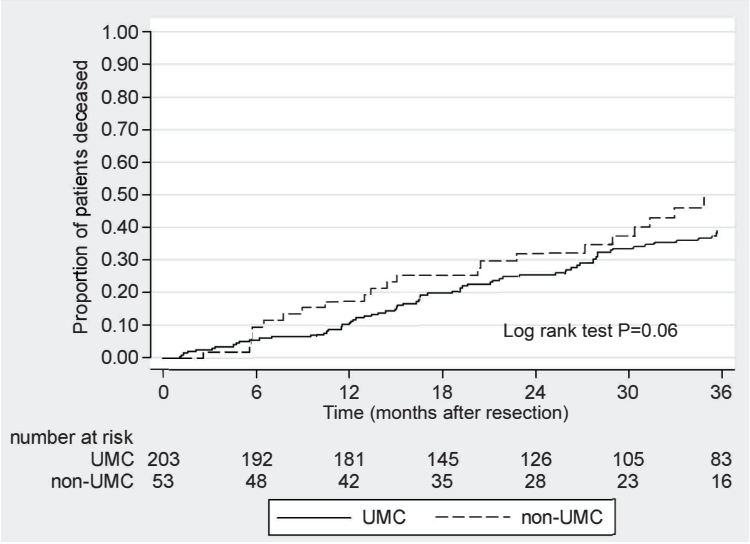
Values in parentheses are percentages; [a]Total 112 patients. Based on 7th edition of Tumour-Node-Metastasis (TNM) classification: pTNM, in case of X or no resection based on cTNM. NX and MX are classified as N0 and M0; [b]Total 25 patients. Tumour stage according to the Extent of Disease (EoD) classification when TNM was not available; **P*-value applies to UMC versus non-UMC.

Supplementary table 3: Relation between patient and tumour characteristics and long-term mortality (based on date of start of sorafenib treatment) in patients who received sorafenib treatment for hepatocellular carcinoma in the Netherlands in the period: 2005-2011 (univariate and multivariate Cox proportional hazard analyses)

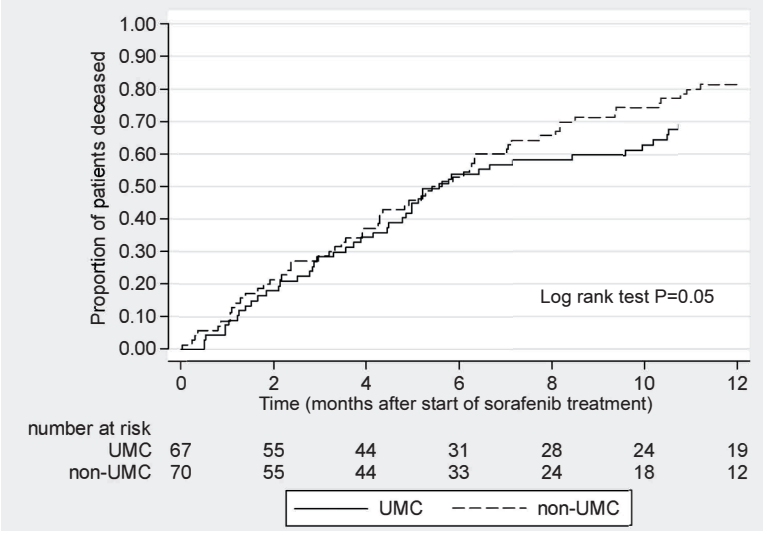
	Patients with sorafenib	Univariate analysis			Multivariate analysis		
Variables	**n=137[a] (%)**	**HR**	**95 per cent CI**	**P-value**	**HR**	**95 per cent CI**	**P-value**
Sex							
Male	102 (74)	1	-	0.21			
Female	35 (26)	0.78	0.52-1.16				
Age							
< 75 years	117 (85)	1	-	0.66			
≥ 75 years	20 (15)	1.12	0.68-1.86				
Country of birth							
The Netherlands	111 (81)	1	-	0.76			
Asian/sub-Sahara	6 (4)	1.35	0.59-3.08				
Other/unknown	20 (15)	0.94	0.58-1.56				
Socioeconomic status							
High (1st-3rd decile)	41 (30)	1	-	0.59			
Moderate (4th-7th decile)	52 (38)	0.83	0.54-1.28				
Low (8th-10th decile)	44 (32)	1.01	0.65-1.56				
Tumour stage							
TNM=I-II; EoD=local	31 (23)	1	-	0.01	1	-	0.005
TNM=III-IV-X; EoD=other	106 (77)	1.71	1.10-2.66		1.89	1.21-2.96	
Hospital type (sorafenib)							
University	67 (49)	1	-	0.05	1	-	0.013
Non-university	70 (51)	1.43	1.00-2.04		1.58	1.10-2.28	
Hospital volume							
<5 sorafenib treatments /yr	104 (76)	1	-	0.50			
≥5 sorafenib treatments / yr	33 (24)	1.15	0.77-1.72				

Values in parentheses are percentages; [a]Exclusion of 88 patients due to missing treatment dates; HR=hazard ratio; 95 per cent CI= 95 per cent confidence interval; TNM= Tumour-Node-Metastasis; EoD= Extent of Disease

Supplementary figure 1: Long-term mortality based on date of resection in patients in the Netherlands who underwent resection for hepatocellular carcinoma in university and non-university hospitals in the period: 2005-2011 and survived the first 30 days after resection (Kaplan-Meier curves, log rank test P=0.06). UMC= university medical centre; non-UMC= non-university medical centre.



Supplementary figure 2: Long-term mortality based on date of start of sorafenib treatment in patients in the Netherlands with sorafenib treatment for hepatocellular carcinoma in university and non-university hospitals in the period: 2005-2011 (Kaplan-Meier curves, log rank test P=0.05). UMC= university medical centre; non-UMC= non-university medical centre.



CHAPTER 11

GENERAL DISCUSSION AND SUMMARY

The burden of hepatocellular carcinoma (HCC) has changed in the past few decades. Although the majority of HCC cases develops in East Asia and Sub-Saharan Africa, HCC has become an increasing problem in Western countries, partly due to the increased prevalence of non-alcoholic fatty liver disease (NAFLD), one of the most common risk factors for underlying chronic liver disease [1-4]. HCC surveillance has been introduced (despite the current discussion concerning its value) in high-risk patients to detect HCC at earlier stages enabling curative therapies with a better outcome and decreased mortality [5-8]. At the same time, several new treatment options – most in palliative setting - have been introduced. In this chapter we discuss the main findings of this thesis in view of current clinical practice and future perspectives.

PART I IDENTIFICATION OF PATIENTS AT RISK AND SURVEILLANCE FOR HEPATOCELLULAR CARCINOMA

In the first chapters of this thesis we focused on high-risk patients for HCC development and HCC surveillance. Despite a large number of studies available, HCC surveillance remains controversial [9]. Nowadays, several international en national guidelines recommend HCC surveillance in patients at risk for HCC development [5-8]. In **Chapter 2** we summarized the existing evidence for surveillance including ultrasound (US), other radiological modalities and various serum biomarkers, and current international guideline recommendations for surveillance. US and alpha–fetoprotein (AFP) (alone or in combination) are most frequently used for surveillance, but their sensitivities and specificities are still far from perfect [10]. According to a meta-analysis by Singal *et al* [10] pooled sensitivities of US to detect early stage HCC is only 63%. The potential benefit of combining US with AFP results in an increase of sensitivity from 63% to 69%. Various other potential surveillance tools have been evaluated, including serum marker such as des-carboxyprothrombin and lectin-bound alpha-fetoprotein, and radiological investigations such as CT- or MRI-scan [11-13]. Although early results seem promising, these tools have generally been tested in a diagnostic rather than a surveillance setting, and in most cases, no detailed information is available on their cost-effectiveness. It remains to be seen, whether these new serum biomarkers and radiological investigations will provide satisfactory and cost-effective results in the setting of surveillance in daily clinical practice.

Identification of the target population for surveillance, i.e. especially high-risk patients who will benefit most, in combination with a well-defined screening project organization is important for a successful surveillance program. However, selection of patients remains a subject of debate. Cost-effectiveness studies have indicated that HCC surveillance becomes cost-effective when HCC incidence rates exceed 1.5%/year in patients with cirrhosis and 0.2%/year in non-cirrhotics [5,14,15]. Annual incidence rates have been investigated extensively in cirrhotic hepatitis B (HBV) and hepatitis C (HCV) patients. However, in patients with cirrhosis due to other causes, HCC incidence

rates are often not clear. Therefore, one of the main challenges for the near future is to define those high-risk subgroups that will benefit most from surveillance. In **Chapter 3** we demonstrated that, even in case of cirrhosis, HCC risk in patients with Wilson disease (WD) is far below the accepted threshold incidence for HCC surveillance to be cost-effective. Results of our meta-analysis confirmed the low HCC incidence as well as a low HCC-related mortality rate in WD patients. Our data do therefore not support regular HCC surveillance in patients with WD in absence of other risk factors. This position is in contrast to the EASL guideline in which HCC surveillance is recommended in all cirrhotic patients, regardless of underlying cause [6]. The reason for the low HCC incidence in WD patients compared to other chronic liver diseases remains unclear. It has previously been suggested that excessive copper accumulation might have a protective effect on hepatocarcinogenesis [16]. However, nowadays it is thought that chronic copper accumulation leads to chronic inflammation and cirrhosis resulting in an increased risk of HCC, which may be prevented by administration of D-penicillamine [17].

Worldwide, chronic infection with HBV is one of the most important risk factors for HCC development [18,19]. In low endemic countries, such as the Netherlands, prevalence of chronic HBV infection is mainly concentrated in migrants from intermediate and high endemic countries [20]. Therefore, it is debated whether first generation migrants should be screened for chronic HBV (and HCV), since these infections are often asymptomatic and antiviral treatment options have improved in recent years. In **Chapter 4** we studied the clinical impact of five large-scale Dutch screening projects for HBV (and partly also for HCV) in first generation Chinese migrants. The main finding was that detection of HBV infection in Chinese migrants can have considerable impact on patient care: in 25% of all positive HBsAg patients analyzed in referral centers antiviral treatment was indicated. Furthermore, in 50% of all positive HBsAg patients analyzed in referral centers, strict follow-up because of high HBV DNA levels and/or HCC surveillance was initiated. These results stress the need for future research to compare costs and benefits of various screening strategies for chronic HBV in migrants. In current clinical practice, indication for HCC surveillance in non-cirrhotic HBV patients is controversial and there are discrepancies between recommendations of several guidelines [5,6,21-23]. In non-cirrhotic HBV patients with increased HCC risk due to additional risk factors such as race (i.e. Asian or African/North American Blacks), family history of HCC, ALT elevation and/or a high HBV DNA level, HCC surveillance should be considered. Of note, prevalence of HCV infection in Chinese migrants was low and therefore no recommendation regarding HCV screening or surveillance could be made based on our data (**Chapter 4**). Nonetheless, one HCV infected patient was identified who had already developed HCC

Successful antiviral treatment leading to sustained virological response (SVR) in chronic HCV patients, and HBeAg seroconversion or sustained HBV-DNA suppression in

chronic HBV patients can reduce HCC risk remarkably, even in case of cirrhosis [24-26]. In Asian HBV patients, treatment with nucleos(t)ide analogs (NAs) results in an overall HCC risk reduction of approximately 30% in cirrhotic patients and approximately 80% in non-cirrhotic patients [26]. For patients of Caucasian origin, no appropriate comparative studies are available to date to evaluate the impact of NAs treatment on HCC risk. In HCV patients with advanced hepatic fibrosis who achieve SVR after interferon based therapy, HCC risk is reduced with approximately 75%, resulting in a 10-year cumulative HCC incidence rate of approximately 5% [25]. However, despite the reduced HCC risk in HBV and HCV patients with successful antiviral treatment, HCC development cannot be completely eliminated, probably due to risk factors that are not amenable to be changed by antiviral therapy, or events that may have taken place before treatment initiation. Therefore, current guidelines recommend to continue HCC surveillance in these patients [5,6]. Future studies are needed to identify accurate predictors for HCC development in patients receiving successful antiviral therapy and to develop valid HCC risk calculators [26].

High-level evidence for the efficacy of surveillance is limited. Two randomized controlled trials (RCTs) on HCC surveillance, investigating a surveillance strategy including combined ultrasound (US) and alpha–fetoprotein (AFP) or AFP alone, both at 6-month intervals, versus no surveillance, yielded contradictory results [27,28]. Therefore, lower level evidence from observational studies could contribute to advise in guidelines. In **Chapter 5** we investigated the contribution of surveillance in a large group of HCC patients in "real life" clinical practice in the Netherlands. Patients with HCC diagnosed in the period 2005-2012 in five Dutch academic centers were evaluated. The main findings were that HCC patients who underwent surveillance prior to diagnosis had a smaller tumor size, earlier BCLC tumor stage and lower AFP levels. Furthermore, they received more often surgical therapy and had a better overall survival than patients without surveillance. Survival benefit was also present in multivariate analysis. Interestingly, findings suggest that surveillance results in better survival through HCC detection at an earlier BCLC stage, enabling more effective therapy. These findings are in line with previously published retrospective cohort studies, meta-analyses and systematic reviews [29,30]. However, the main limitation of all observed cohort studies is the potential lead-time bias (i.e. detection of tumors at earlier stages by surveillance) and/or length-time bias (i.e. detection of indolent tumors by surveillance). Although several statistical techniques can be used to adjust for lead-time bias, adjustments remain controversial since HCC tumor doubling times are not clear [31-33]. In **Chapter 5**, survival benefit remained when tumor doubling times of 60 or 90 days were assumed, but disappeared with doubling times ≥120 days. Despite correction for lead-time bias, surveillance might still preferentially detect slowly growing indolent tumors (length-time bias). Based on available data on HCC tumor doubling times, approximately 40% of HCCs could have a tumor doubling time of less than 90 days. Nevertheless, this is

controversial and further research is needed to evaluate HCC tumor doubling times, to identify possible factors associated with slow- and fast-growing tumors and to define the impact of this information on surveillance strategies [34]. Unfortunately, the possibility of a new RCT investigating efficiency of HCC surveillance in a Western population seems unlikely due to ethical reasons [9].

The optimal interval for HCC surveillance should be based on rate of tumor growth up to the limit of its detectability, and may therefore differ from several surveillance modalities. In current guidelines a surveillance interval of 6 months in recommended [5-8]. This is partially based on a meta-analysis by Singal *et al* [10], showing that sensitivity for detecting early-HCC may be improved by US at 6-month intervals compared to surveillance intervals between six and twelve months. However, in **Chapter 5** a survival benefit was not only found in subgroups with surveillance interval ≤9 months but also with surveillance interval >9-18 months, despite significantly larger tumor sizes in the latter group. Potential explanations could be that many tumors in the latter group exceeded the limit of US detection only after prolonged time periods after the last negative radiological surveillance imaging and/or relatively slow tumor growth rates. Similar findings were reported in a study from Taiwan comparing 4- and 12-month US screening intervals [35]. It cannot be excluded that strict surveillance based on the recommended interval of 6 months could lead to better outcomes, although this is difficult to achieve in clinical practice.

Utilization of surveillance in clinical practice is low [36,37]. In the United States for example only 17% of in total 1873 cirrhotic patients who developed HCC received regular surveillance, which declined even further with time [37]. Also in the Netherlands, only 27% of patients underwent surveillance before HCC diagnosis. Furthermore, mean surveillance interval was significantly longer than the recommended surveillance interval of 6 months based on current guidelines, indicating there is room for improving surveillance programs (**Chapter 5**). Possible explanations for the low utilizations and longer surveillance intervals are a lack of knowledge, low detection rate of at-risk individuals, logistic factors, non-adherence of patients and/or low level of enthusiasm for evidence supporting the efficiency of surveillance or for the surveillance tests [37]. Patient knowledge and involvement in decision-making have been shown to be are associated with higher HCC surveillance rates [38]. In **Chapter 6** we noticed a profound discrepancy between patients' expectations of HCC surveillance and the real benefits in daily clinical practice, similar to the overestimation by patients of benefits of other cancer surveillance programs [39,40]. Misconceptions impair the ability to make informed decisions [39]. Therefore, in the current era of increased awareness and doctors' obligations to provide adequate information about benefits and risk, better education on HCC surveillance is needed in high-risk patients with underlying chronic liver disease.

Final conclusions

- Ultrasound and alpha–fetoprotein (alone or in combination) are most frequently used for surveillance, but their sensitivities and specificities are still far from perfect.
- Even in case of cirrhosis, HCC risk was low in patients with Wilson disease and therefore our data do not support regular HCC surveillance.
- Detection of chronic hepatitis B infection in Chinese migrants can have considerable impact on patient care.
- In "real life" clinical practice, HCC surveillance was associated with smaller tumor size, earlier tumor stage, with impact on therapeutic strategy. Participation in an HCC surveillance program was an independent predictor of survival.
- Survival benefit by surveillance disappeared after adjustment for lead-time bias based on assumed HCC doubling time of ≥120 days.
- There was a profound discrepancy between patients' expectations of HCC surveillance and the real benefits in daily clinical practice.

PART II PATIENTS WITH HEPATOCELLULAR CARCINOMA WITHOUT CIRRHOSIS

HCC usually occurs in patients with cirrhosis, but can also develop in non-cirrhotic livers. Proportion of HCC in absence of cirrhosis varied widely between various geographical regions. This may be the consequence of different patterns of underlying risk factors [41]. In **Chapter 7** we explored risk factors for HCC without cirrhosis and compared patient and tumor characteristics and outcomes in HCC patients with and without underlying cirrhosis. In total, 19% of the 1221 included HCC patients had no underlying cirrhosis. The main findings of this study were: 1) HCC without cirrhosis was strongly associated with female gender and presence of NAFLD or no risk factors for underlying liver disease, and 2) in absence of cirrhosis, resection of HCC was more frequently performed and overall survival was significantly better despite larger tumor size. Previous studies have reported contradictory results regarding differences in survival rates between cirrhotic and non-cirrhotic HCC patients [42-45]. Severity of underlying liver disease has a great impact on treatment decisions as the presence of cirrhosis and resulting impaired liver function may limit surgical and non-surgical options [41]. Therefore, a survival benefit in non-cirrhotic patients may be related to the fact that (potentially curative) surgical therapy can more frequently been performed leading to a better outcome, which is in line with results of **Chapter 7.** Interestingly, there were also significant differences in applied treatments modalities between patients with various etiologies of underlying liver disease with potential impact on survival. A potential explanation for these findings could be differences in tumor characteristics. For example, patients with HBV or NAFLD more often developed HCC before progression to cirrhosis enabling the possibility of extensive surgical resections [46].

Increased HCC risk in NAFLD seems to affect especially those with cirrhosis and is generally attributed to non-alcoholic steatohepatitis (NASH) [47,48]. Nevertheless, recent studies have demonstrated that also simple steatosis may exhibit a significantly increased risk of progressive liver disease and even HCC development [49-51]. Furthermore, it has been suggested that also in non-cirrhotic livers, presence of NAFLD and/or metabolic syndrome is associated with HCC development [52,53]. In **Chapter 8** presence of steatosis versus steatohepatitis was compared in non-tumoral liver of non-cirrhotic HCC patients. Only non-cirrhotic patients were included in this study since histological features of NAFLD may regress with the development of cirrhosis. The main finding was that histological steatosis was frequently present in non-cirrhotic HCC patients, both in patients with and without known causes of underlying liver disease. On the other hand, overt steatohepatitis was surprisingly absent. The high prevalence of steatosis in the non-tumoral liver supports the hypothesis that hepatic steatosis may play a causal role in hepatocarcinogenesis, even in absence of steatohepatitis and may be a cofactor in patients with other well-known risk factors for underlying liver disease. Several possible underlying mechanisms have been suggested. For example, oxidative stress, as a result of an increased level of intrahepatic fatty acids, may be increased and causing hepatocyte injury and cancer-promoting mutations, which can lead to HCC development [54]. Furthermore, alterations in the function of specific genes, such as Pten and Mir122, due to presence of hepatic steatosis are described [55,56]. Important pathophysiologic mechanisms in NALFD patients such as insulin resistance, hyperinsulinemia and dysregulation in cytokine/adipocytokine signaling pathways may also promote HCC development [57]. A possible explanation for the absence of steatohepatitis in this cohort is the exclusion of patients with extensive fibrosis or cirrhosis. However, since overt steatohepatitis was rare in our HCC patients, current paradigmas on the role of NAFLD in HCC development may be reconsidered. Further studies are needed to elucidate the exact role of hepatic steatosis in HCC development in non-cirrhotic livers and to determine the exact HCC risk in patients with steatosis versus steatohepatitis.

Final conclusions:

- In the Netherlands, approximately 20% of HCC patients had no underling cirrhosis.
- Absence of cirrhosis in HCC patients was strongly associated with female gender and presence of NAFLD or no risk factors for underlying liver disease.
- In non-cirrhotic HCC patients, resections were more often performed, with better overall survival despite larger tumor size compared to HCC patients with cirrhosis.
- There were significant differences in applied treatments modalities between patients with various etiologies of underlying liver disease with potential impact on survival.

- Histological steatosis was frequently present in the non-tumoral liver of non-cirrhotic HCC patients, both in patients with and without known causes of underlying liver disease.
- Since overt steatohepatitis was surprisingly rare in non-cirrhotic HCC patients, current paradigmas on HCC pathogenesis in NAFLD may be reconsidered.

PART III HEPATOCELLULAR CARCINOMA IN THE NETHERLANDS

Finally, we focused on presence and treatment of HCC in the Netherlands (**Chapter 9 and 10**). In the past decade, 2915 HCC patients were diagnosed with HCC in the Netherlands. Despite the introduction of HCC surveillance for high-risk patients, proportion of patients who underwent surgical resection did not increase, whereas proportion of patients who received palliative treatment (e.g. arterial chemoembolization (TACE), radiofrequency ablation (RFA) and sorafenib treatment) increased significantly. The main finding of **Chapter 9** was the absence of a trend toward centralization diagnostic testing and treatments for HCC, in contrast to the recommendation of the Dutch guideline [8]. TACE, RFA and resection were mainly performed in university hospitals, whereas half of initial sorafenib treatments was given in non-university hospitals. Furthermore, there were only a few high volume hospitals (based on number of patients who underwent surgical resection respectively received initial sorafenib treatment with low volume defined as <5 per year and high volume: ≥5 per year), which were all university hospitals. Interestingly, significant differences in the use of loco-regional therapy existed between regions. A possible explanation for this is that these new treatments are not introduced simultaneously in clinical practice in all regions ('early' vs 'late adopters').

Subsequently, in **Chapter 10** we demonstrated that in low incidence countries such as the Netherlands, outcome after resection or sorafenib for HCC may differ between various hospital types. Postoperative mortality and (less pronounced) long-term mortality were significantly higher in patients who underwent HCC resection in non-university compared to university hospitals. Furthermore, long-term mortality in patients who had initial sorafenib treatment in non-university hospitals was also significantly higher than in patients treated in university hospitals. Available patient and tumour characteristics, and surgical parameters could not explain these results. No previous data are available on the impact of hospital type after resection or sorafenib treatment for HCC. Nevertheless, for other types of cancer an association has been shown between hospital type (e.g. academic status) and outcome [58-61]. Apart from a higher level of expertise in university hospitals, another possible explanation for differences in outcomes between university and non-university hospitals could theoretically be a better multidisciplinary approach in the allocation process of treatment in university hospitals. Nevertheless, our data do not allow confirming or refuting these possibilities. Furthermore, an important limitation of these findings is that important prognostic factors such as etiology and severity of

underlying liver disease and performance score were not available. Therefore, no definite conclusion can be drawn on quality of care in university vs. non-university hospitals and potential impact of hospital type on mortality. Interestingly, hospital volume was no independent predictor for the short- and long-term outcomes in **Chapter 10**, in contrast to previous literature regarding hepatic resection for HCC [62-64]. One may speculate that a distinction between high or low volume hospitals based on a volume of <5 versus ≥5 resections per year, respectively, as used in the current report, is too low to have a significant impact on treatment outcome. Further prospective research in indicated to evaluate the potential impact of hospital type and volume on outcome in HCC patients.

Final conclusions:

- In the Netherlands, proportion of HCC patients who underwent surgical resection remained stable during the last decade, whereas proportion of patients who received palliative treatment increased significantly.
- Significant differences in the use of loco-regional therapy for HCC existed between regions.
- There was no trend towards centralization of diagnostic testing and treatments for HCC in the Netherlands.
- In low incidence countries such as the Netherlands, outcome after resection or sorafenib for hepatocellular carcinoma may differ between various hospital types (university versus non-university hospitals).

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CHAPTER 12

NEDERLANDSE SAMENVATTING
DANKWOORD
LIST OF PUBLICATIONS
CURRICULUM VITAE

Nederlandse samenvatting

Hepatocellulair carcinoom (HCC) is wereldwijd een veel voorkomende vorm van kanker. Meestal betreft het patiënten met onderliggend levercirrose. Het voorkomen van HCC kent wereldwijd een sterke variatie mede als gevolg van de verdeling van onderliggende risicofactoren. Hoewel het merendeel van de HCC's ontstaat in Oost-Azië en sub-Sahara Afrika, neemt in Westerse landen zoals Nederland de incidentie toe, mede als gevolg van de stijgende prevalentie van niet-alcoholische leververvetting (NAFLD) [1-4]. In het afgelopen decennium is surveillance voor HCC geïntroduceerd bij hoog-risico patiënten, ondanks de lopende discussie over de waarde hiervan. Het doel van surveillance is het detecteren van HCC in een vroeg stadium om curatieve therapie met een betere uitkomst mogelijk te maken [5-8]. Tegelijkertijd zijn er de laatste jaren enkele nieuwe therapieën voor HCC geïntroduceerd, nl. radiofrequente ablatie (RFA), transarteriële chemo-embolisatie (TACE) en radio-embolisatie (TARE), en systemische behandeling met multikinaseremmer sorafenib.

In dit proefschrift worden studies beschreven die zich richten op hoog-risico patiënten en HCC surveillance (deel 1), aanwezigheid van HCC in een niet-cirrotische lever (deel 2) en trends in diagnostiek en behandeling voor HCC in Nederland (deel 3). In deze samenvatting bespreken we de belangrijkste conclusies van dit proefschrift.

DEEL 1: IDENTIFICATIE VAN HOOG-RISICO PATIENTEN EN SURVEILLANCE VOOR HEPATOCELLULAIR CARCINOOM

HCC surveillance is controversieel, ondanks de vele beschikbare studies [9]. Toch adviseren huidige richtlijnen het uitvoeren voor HCC surveillance bij hoog-risico patiënten [5-8]. In **hoofdstuk 2** wordt een samenvatting gegeven van de belangrijkste literatuur omtrent HCC surveillance. Daarnaast worden adviezen van de huidige internationale richtlijnen besproken. Echografie en de serummarker alfa-foetoproteïne (AFP) zijn de meest gebruikte testen voor de uitvoering van HCC surveillance ondanks de suboptimale sensitiviteit voor het detecteren van HCC in een vroeg stadium: 63% voor echografie en 69% voor echografie en AFP gecombineerd [10]. Veel andere potentiële surveillance modaliteiten zijn geëvalueerd, inclusief serum biomarkers zoals des-carboxyprothrombine en lectine-gebonden AFP, en radiologische onderzoeken zoals CT en MRI [11-13]. Deze studies zijn voornamelijk verricht in een diagnostische setting. Hoewel de eerste resultaten veelbelovend zijn, is het nog maar de vraag wat de waarde van deze ontwikkelingen is in de setting van surveillance in de dagelijkse klinische praktijk. Bovendien is er geen informatie beschikbaar over de kosteneffectiviteit.

Identificatie van de juiste doelgroep die in aanmerking komt voor HCC surveillance (nl. hoog-risico patiënten) is belangrijk voor het succes van een surveillance programma. Het selecteren van deze patiënten blijkt in de praktijk echter lastig. Op basis van theoretische modellen wordt surveillance kosteneffectief geacht, indien de HCC incidentie >1,5%/jaar is bij patiënten met cirrose en >0.2%/jaar bij patiënten zonder cirrose [5,14,15].

Het voorkomen van HCC bij patiënten met een hepatitis B (HBV) of hepatitis C (HCV) infectie is uitgebreid onderzocht, maar de incidentie in andere patiëntengroepen is veelal onduidelijk. Eén van de belangrijkste uitdagingen voor de nabije toekomst is het verder definiëren van deze hoog-risico subgroepen. In **hoofdstuk 3** onderzochten we het risico op het ontwikkelen van HCC bij patiënten met de ziekte van Wilson. De ziekte van Wilson is een erfelijke stofwisselingsziekte waarbij als gevolg van een mutatie in het ATP7B gen koperstapeling optreedt in verschillende organen zoals de lever. De belangrijkste bevinding was dat bij patiënten met de ziekte van Wilson, ook in aanwezigheid van cirrose, het risico op HCC laag is. Op basis van deze gegevens is HCC surveillance bij patiënten met de ziekte van Wilson zonder andere risicofactoren dan ook niet geïndiceerd. Deze stelling is in strijd met de adviezen van de Europese richtlijn waarin wordt geadviseerd om HCC surveillance toe te passen bij alle patiënten met cirrose, ongeacht de onderliggende oorzaak [6].
Wereldwijd is een chronische HBV infectie één van de belangrijkste risicofactoren voor het ontstaan van HCC [16,17]. In Nederland is de prevalentie van chronische HBV infecties laag en komt met name voor bij migranten afkomstig van matig tot hoog endemische landen [18]. Screening van eerste generatie migranten is mogelijk effectief gezien het asymptomatische beloop van de ziekte en de verbeterde antivirale behandeling in de laatste jaren. In **hoofdstuk 4** hebben we gekeken naar de klinische impact van vijf grootschalige Nederlandse screeningsprojecten voor HBV (en gedeeltelijk voor HCV) bij eerste generatie Chinese migranten. De conclusie van deze studie was dat detectie van een HBV infectie bij Chinese migranten een aanzienlijke impact kan hebben op de patiëntenzorg: van alle nieuw gediagnosticeerde HBsAg positieve patiënten die waren doorverwezen had 25% een indicatie voor antivirale therapie en 50% een indicatie voor HCC surveillance en/of strikte follow-up vanwege een hoge virale load. Mogelijk geldt deze bevinding ook voor migranten afkomstig uit andere hoog endemische HBV-regio's. In de toekomst zijn nieuwe studies nodig om de kosten en baten te vergelijken van verschillende screeningsstrategieën voor HBV infecties in migranten. Noemenswaardig is dat de prevalentie van HCV infecties in deze studie laag was. Er kunnen dan ook er geen adviezen worden gegeven ten aanzien van screening voor HCV. Opvallend was wel dat één van de nieuw gediagnosticeerde HCV geïnfecteerde patiënten reeds een HCC had ontwikkeld.
Hoog niveau bewijs voor de effectiviteit van surveillance is beperkt. Er zijn slechts twee gerandomiseerde studies uitgevoerd naar het effect van HCC surveillance op de mortaliteit met tegenstrijdige resultaten [19,20]. Daarom zal lager niveau bewijs afkomstig van observationele studies een bijdrage moeten leveren aan de verdere onderbouwing van de richtlijnen. In **hoofdstuk 5** onderzochten we de waarde van surveillance bij een grote groep HCC patiënten in de dagelijkse klinische praktijk in Nederland. Patiënten met een diagnose HCC in de periode 2005-2012 in vijf academische ziekenhuizen werden geëvalueerd (in totaal 1074 patiënten). Patiënten die surveillance hadden ondergaan

voorafgaande aan de diagnose HCC hadden significant kleinere tumoren, een vroeger Barcelona Clinic Liver Cancer (BCLC) tumorstadium en lagere AFP waarden. Bovendien werd er bij deze patiënten vaker chirurgie toegepast en was de totale overleving beter dan die van HCC patiënten zonder surveillance voorafgaande aan de diagnose. Ook in een multivariate analyse was surveillance een onafhankelijke voorspeller voor een betere overleving. Interessant is dat op basis van deze bevindingen gesuggereerd kan worden dat surveillance tot een betere overleving leidt door het detecteren van HCC in een vroeg stadium met als gevolg dat effectievere behandeling mogelijk is. Deze resultaten komen overeen met eerder gepubliceerde retrospectieve cohortstudies, meta-analyses en systematische reviews [21,22]. De belangrijkste beperking van observationele cohortstudies is de mogelijke invloed van lead-time bias (het eerder detecteren van een ziekte door een gericht surveillance programma) en/of length-time bias (het detecteren van indolente tumoren). Ondanks het feit dat er verschillende statistische technieken beschikbaar zijn om te corrigeren voor lead-time bias, blijft deze correctie controversieel aangezien de HCC tumor verdubbelingstijd niet geheel duidelijk is [23-25]. In **hoofdstuk 5** bleef het overlevingsvoordeel voor patiënten die surveillance hebben ondergaan bestaan na correctie voor lead-time bias als men uitging van een tumor verdubbelingstijd van 90 dagen, maar verdween als men uitging van verdubbelingstijden van 120 dagen of langer. Op basis van de beschikbare literatuur heeft ongeveer 40% van de HCC's een tumor verdubbelingstijd kleiner dan 90 dagen [25]. In de toekomst is verder onderzoek nodig om factoren te identificeren die geassocieerd zijn met langzaam en snel groeiende tumoren en de impact van deze informatie te bepalen voor surveillance strategieën [26]. De komst van een nieuwe gerandomiseerde studie naar de effectiviteit van HCC surveillance in een Westerse populatie is op basis van ethische redenen niet aannemelijk [9].

Het optimale interval voor HCC surveillance is afhankelijk van de snelheid van tumorgroei maar ook van de ondergrens voor de detectie van een tumor en kan hierdoor per surveillance modaliteit wisselen. In de huidige richtlijnen wordt een surveillance interval van 6 maanden geadviseerd [5-8]. Dit is gedeeltelijk gebaseerd op de meta-analyse van Singel *et al* [10] die laat zien dat de sensitiviteit voor het detecteren van een vroeg stadium HCC verbetert door het uitvoeren van 6-maandelijkse echografie in vergelijking met intervallen tussen de 6 en 12 maanden. In **hoofdstuk 5** werd echter niet alleen een overlevingsvoordeel gezien in de subgroep met een surveillance interval ≤9 maanden, maar ook in de groep met een interval tussen de 9 en 18 maanden, ondanks significant grotere tumoren in de laatste groep. Mogelijke verklaringen kunnen zijn dat veel tumoren in de laatste groep echografisch pas detecteerbaar waren na een langere tijdsperiode en/of een relatief langzame tumorgroei. Het is niet uit te sluiten dat strikte surveillance uitgaande van het in de internationale richtlijnen geadviseerde interval van 6 maanden kan leiden tot een betere uitkomst.

De toepassing van surveillance in de klinische praktijk is laag [27,28]. In Nederland had slechts 27% van de HCC patiënten surveillance ondergaan voorafgaande aan de diagnose (**hoofdstuk 5**). Bovendien was het gemiddelde surveillance interval significant langer dan de geadviseerde 6 maanden. Hieruit blijkt dat er mogelijkheden zijn tot verbetering van de huidige surveillance programma's. Mogelijke verklaringen voor het weinig toepassen van surveillance en/of de lange surveillance intervallen zijn een gebrek aan kennis, een lage detectiegraad voor patiënten met een verhoogd risico, logistieke factoren, niet-naleving door patiënten en/of een gebrek aan enthousiasme voor het bewijs ter ondersteuning van de effectiviteit van surveillance [28]. Kennis en betrokkenheid van patiënten met cirrose bij het nemen van beslissingen zijn geassocieerd met hogere HCC surveillance percentages [29]. In **hoofdstuk 6** constateerden we echter dat er sprake was van een sterke discrepantie tussen de verwachtingen van patiënten ten aanzien van HCC surveillance en de te verwachten effecten in de dagelijkse klinische praktijk, overeenkomstig de overschatting van voordelen van andere kanker surveillance programma's door patiënten [30,31]. Misconcepties bij patiënten maken het moeilijker om te komen tot het maken van geïnformeerde keuzes [30]. In de huidige periode met toegenomen bewustwording en verplichtingen van artsen om patiënten van adequate informatie over voordelen en risico's te voorzien, is betere educatie ten aanzien van HCC surveillance nodig bij hoog-risico patiënten met een onderliggende chronische leveraandoening. Tenslotte moet er aandacht zijn voor de uitvoering van surveillance, bijvoorbeeld door de implementatie van kwaliteit verbeterende maatregelen zoals een duidelijk oproepbeleid, wat het gebruik van surveillance zal vergroten [32].

Belangrijkste conclusies

- Echografie en alfa-foetoproteïne zijn de meest gebruikte testen voor de uitvoering van surveillance ondanks de suboptimale sensitiviteit voor het detecteren van HCC in een vroeg stadium.
- Ook in aanwezigheid van cirrose was het HCC risico bij patiënten met de ziekte van Wilson laag; dus op basis van deze gegevens is HCC surveillance dan ook niet geïndiceerd.
- Detectie van een chronische hepatitis B-infectie bij Chinese migranten kan veel impact hebben op de patiëntenzorg.
- HCC surveillance was geassocieerd met het detecteren van kleinere tumoren en een vroeger tumorstadium met impact op de behandelstrategie. Deelname aan een HCC surveillance programma was een onafhankelijke voorspeller voor overleving.
- Het overlevingsvoordeel als gevolg van surveillance verdween na correctie voor lead-time bias als men uitging van een HCC verdubbelingstijd van 120 dagen of langer.
- Er was een sterke discrepantie tussen de verwachtingen van patiënten ten aanzien van HCC surveillance en de te verwachten effecten in de dagelijkse klinische praktijk.

DEEL 2: PATIENTEN MET EEN HEPATOCELLULAIR CARCINOOM ZONDER CIRROSE

Hoewel een HCC meestal voorkomt bij patiënten met cirrose, kunnen ook patiënten met een niet-cirrotische lever een HCC krijgen [33]. In **hoofdstuk 7** onderzochten we risicofactoren voor het voorkomen van HCC in afwezigheid van cirrose en vergeleken we patiënt- en tumorkarakteristieken en uitkomsten van HCC patiënten met en zonder cirrose. In Nederland had 19% van alle HCC patiënten een niet-cirrotische lever, waarbij er een sterke associatie was met het vrouwelijke geslacht, de aanwezigheid van NAFLD of de afwezigheid van risicofactoren voor een onderliggende leverziekte. In niet-cirrotische HCC patiënten werd ondanks de aanwezigheid van grotere tumoren vaker een resectie uitgevoerd en was de overleving significant beter dan in cirrotische HCC patiënten. Eerdere studies laten tegenstijdige resultaten zien ten aanzien van mogelijke verschillen in overleving tussen cirrotische en niet-cirrotische HCC patiënten [34-37]. De ernst van een onderliggende leverziekte heeft grote invloed op de therapiekeuze, aangezien de aanwezigheid van cirrose en de daarmee gepaard gaande verminderde leverfunctie en portale hypertensie de chirurgische en niet-chirurgische opties kan beperken [33]. Een betere overleving bij niet-cirrotische patiënten is daarom mogelijk gerelateerd aan het feit dat (potentieel curatieve) chirurgische therapie frequenter toegepast kan worden met als resultaat een betere uitkomst, overeenkomstig de resultaten in **hoofdstuk 7**. Interessant is ook dat er significante verschillen waren in toegepaste behandelmodaliteiten tussen patiënten met verschillende oorzaken van onderliggend leverlijden met een potentieel gevolg voor de overleving. Een mogelijke verklaring voor deze bevinding zijn de patiënt- en tumorkarakteristieken in de desbetreffende etiologie-groepen. Patiënten met een HBV infectie of NAFLD ontwikkelen bijvoorbeeld vaker een HCC voorafgaand aan progressie naar cirrose waardoor een uitgebreide chirurgische resectie een mogelijkheid is [38].

NALFD is tegenwoordig één van de belangrijkste oorzaken van een chronische leverziekte en een veelvoorkomende risicofactor voor HCC. Het verhoogde HCC risico bij patiënten met NAFLD werd met name toegeschreven aan de aanwezigheid van cirrose als gevolg van niet-alcoholische steatohepatitis (NASH) [39,40]. Echter recente studies laten zien dat ook simpele steatose kan leiden tot progressieve leverziekte en ontwikkeling van HCC [41-43]. Bovendien wordt gesuggereerd dat ook in niet-cirrotische levers, de aanwezigheid van NAFLD en/of een metabool syndroom is geassocieerd met de ontwikkeling van HCC [44,45]. In **hoofdstuk 8** hebben we de aanwezigheid van steatose versus steatohepatitis vergeleken in het leverparenchym dat geen tumor bevat bij HCC patiënten. Omdat de typische histologische kenmerken van NAFLD kunnen verdwijnen met het ontstaan van cirrose werden alleen niet-cirrotische patiënten geïncludeerd. De belangrijkste bevinding was dat steatose vaak aanwezig was zowel bij patiënten met en zonder een andere bekende oorzaak voor onderliggend leverlijden. Opvallend was bovendien dat steatohepatitis afwezig was. De hoge prevalentie

van steatose in het niet-tumorale leverparenchym van HCC patiënten ondersteunt de hypothese dat steatose mogelijk een rol speelt bij de hepatocarcinogenese, ook in afwezigheid van steatohepatitis, en een cofactor kan zijn voor het ontstaan van HCC bij patiënten met andere risicofactoren voor onderliggend leverlijden. Een potentiële verklaring voor de afwezigheid van steatohepatitis in dit cohort is de exclusie van patiënten met gevorderde fibrose en cirrose. Niettemin dienen de huidige paradigma's over de rol van NAFLD bij de ontwikkeling van HCC te worden heroverwogen gezien de afwezigheid van steatohepatitis in dit cohort. Toekomstige studies zijn nodig om de precieze rol van hepatische steatose bij de ontwikkeling van een HCC in een niet-cirrotische lever duidelijk te maken en het HCC risico bij patiënten met steatose versus steatohepatitis te bepalen.

Belangrijkste conclusies

- In Nederland had circa 20% van de HCC patiënten geen onderliggende cirrose.
- Afwezigheid van cirrose bij HCC patiënten was sterk geassocieerd met het vrouwelijke geslacht en aanwezigheid van NAFLD of geen risicofactoren voor onderliggend leverlijden.
- In niet-cirrotische HCC patiënten werden resecties vaker uitgevoerd met een betere algehele overleving ondanks grote tumoren in vergelijking met HCC patiënten met cirrose.
- Er waren significante verschillen in toegepaste behandelmodaliteiten tussen patiënten met verschillende oorzaken van onderliggend leverlijden met potentiele impact op de overleving.
- Histologische steatose was in tegenstelling tot steatohepatitis vaak aanwezig in het niet-tumorale leverparenchym van niet-cirrotische HCC patiënten, zowel bij patiënten met als zonder andere bekende oorzaak van onderliggende leverziekte, wat de hypothese ondersteunt dat steatose mogelijk een rol speelt bij de hepatocarcinogenese.

DEEL 3: HEPATOCELLULAIR CARCINOOM IN NEDERLAND

In de periode 2003-2011 werden 2915 HCC patiënten gediagnosticeerd in Nederland (**hoofdstuk 9**). Ondanks de introductie van HCC surveillance voor hoog-risico patiënten, steeg het percentage patiënten dat een resectie onderging gedurende deze tijdsperiode niet, terwijl het percentage patiënten dat een TACE, RFA of sorafenib behandeling kreeg significant toenam. De belangrijkste bevinding in **hoofdstuk 9** was dat er geen trend tot centralisatie zichtbaar was ten aanzien van diagnostiek en behandeling van HCC ondanks het advies van de Nederlandse richtlijn voor HCC om centralisatie te bewerkstellingen [8]. TACE, RFA en resecties werden voornamelijk in academische ziekenhuizen uitgevoerd, terwijl de helft van alle sorafenib behandelingen werd gegeven in niet-academische ziekenhuizen. Er waren slechts een paar hoog-volume ziekenhuizen

(gebaseerd op het aantal uitgevoerde resecties/sorafenib behandelingen met laag-volume gedefinieerd als <5 per jaar en hoog-volume als ≥5 per jaar), die allemaal academische ziekenhuizen bleken te zijn. Interessant is dat er significante regionale verschillen bestonden in het gebruik van locoregionale therapie. Een mogelijke verklaring hiervoor is dat deze nieuwe behandelingen niet in alle regio's tegelijk werden geïntroduceerd in de dagelijkse klinische praktijk (vroege versus late 'adopters').
Vervolgens hebben we in **hoofdstuk 10** laten zien dat in landen met een lage HCC incidentie zoals Nederland, de uitkomst na resectie of sorafenib behandeling voor HCC kan verschillen tussen diverse ziekenhuistypen. De postoperatieve mortaliteit en (minder duidelijk) de lange-termijn mortaliteit waren significant hoger bij patiënten die resectie van een HCC ondergingen in een niet-academische ziekenhuis dan in een academisch ziekenhuis. Daarnaast was de lange-termijn mortaliteit van patiënten die initiële behandeling met sorafenib in een niet-academische ziekenhuis hadden gekregen ook significant hoger dan van patiënten die behandeld waren in academische ziekenhuizen. Beschikbare patiënt- en tumorkarakteristieken en chirurgische parameters gaven geen goede verklaringen voor deze resultaten. Naast een hoger niveau van expertise in academische ziekenhuizen kan theoretisch een andere mogelijke verklaring zijn dat er in academische ziekenhuizen een betere multidisciplinaire aanpak wordt toegepast bij het bepalen van de optimale behandeling. Aan de hand van onze data is het echter niet mogelijk om deze verklaringen te bevestigen dan wel te verwerpen. Bovendien was een belangrijke beperking voor de interpretatie van deze resultaten de onbekendheid van belangrijke prognostische factoren zoals de etiologie, de ernst van het onderliggend leverlijden en de mate van functioneren van de patiënt ("performance score"). Daarom kunnen er geen definitieve conclusies worden getrokken over de kwaliteit van zorg in academische en niet-academische ziekenhuizen en de potentiële invloed van ziekenhuistype op de mortaliteit. Opvallend was dat ziekenhuisvolume geen onafhankelijke voorspeller was voor korte- en lange-termijn uitkomsten in **hoofdstuk 10,** in tegenstelling tot eerdere literatuur betreffende HCC resecties [46-48]. Men kan speculeren dat een onderscheid tussen hoog- en laag-volume ziekenhuizen gebaseerd op respectievelijk ≥5 of <5 resecties per jaar, zoals gebruikt in de huidige studie, te laag is om een significante impact te hebben op de behandeluitkomst. Verder prospectief onderzoek is nodig om de potentiële invloed van ziekenhuistype en -volume op de uitkomst van HCC patiënten te evalueren.

Belangrijkste conclusies

- Gedurende het laatste decennium bleef in Nederland het percentage HCC patiënten dat chirurgische resectie onderging gelijk, terwijl het percentage patiënten behandeld met locoregionale therapie of sorafenib significant toenam.
- Er waren significante regionale verschillen ten aanzien van het gebruik van locoregionale therapieën voor HCC.

- Er was geen trend richting centralisatie van de diagnostiek en behandeling van HCC in Nederland ondanks de aanbeveling van de Nederlandse richtlijn.
- In landen met een lage incidentie zoals Nederland kan de uitkomst na resectie of sorafenib behandeling voor HCC verschillen tussen diverse ziekenhuistypen (academische versus niet-academische ziekenhuizen).

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- van Meer S, Lieveld FI, van Erpecum K.J. Patients' misconceptions about surveillance for hepatocellular carcinoma: Education is needed. J. Hepatol. 2016 (Epub ahead of print)
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Curriculum vitae

Suzanne van Meer werd geboren op 13 januari 1988 in Arnhem. Na haar middelbare school periode op het Stedelijk Gymnasium in Arnhem begon zij in 2006 aan de studie geneeskunde aan de Universiteit Utrecht. Tijdens haar studie deed Suzanne een co-schap gynaecologie in Paramaribo, Suriname. In het 4de jaar van de studie werd haar interesse voor wetenschap gewekt door een wetenschappelijke stage op de afdeling hematologie in het Children's National Medical Center (Washington DC, USA). In de laatste 2 jaar van haar studie heeft Suzanne zich zowel op klinisch als wetenschappelijk gebied verdiept in de Maag-, Darm- en Leverziekten. Na haar afstuderen in december 2012 startte zij haar promotietraject met als onderwerp hepatocellulair carcinoom onder leiding van prof. dr. P.D. Siersema, dr. K.J. van Erpecum en prof. dr. R.A de Man op de afdeling Maag-, Darm- en Leverziekten van het Universitair Medisch Centrum Utrecht (UMCU). In februari 2016 is Suzanne begonnen met de opleiding tot Maag-, Darm- en Leverarts, waarvan zij momenteel de vooropleiding interne geneeskunde doorloopt in het UMCU (opleider prof. dr. H.A.H. Kaasjager).