New Insights in Hepatocellular Carcinoma

Carlijn Witjes

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CAROLINE DOROTHE MARJOLEINE WITJES

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PART I

INTRODUCTION

Chapter 1

General introduction

INTRODUCTION

Risk factors

Hepatocellular carcinoma (HCC) is the fifth most common cancer worldwide and the third most common cause of cancer mortality [1-4]. HCC is one of the few cancers with well-defined major risk factors [1,5]. Worldwide, in 80% of the cases HCC develops in cirrhotic livers, and cirrhosis is the strongest predisposing factor [1,6,7]. The incidence is high in Asia and parts of Africa [5-13]. Geographical differences in incidence reflect variations of the main causal factors such as hepatitis B virus infection (HBV) which is common in Asia and Africa [14]. HBV infection leads to the development of HCC through direct and indirect pathways. Being an oncogenic virus, it can cause HCC in the absence of cirrhosis, as it has the ability to integrate into the host genome affecting cellular signalling and growth control [15].

Another unique feature of HCC is that it is a vaccine preventable disease. Implementation of a nationwide HBV vaccination programme for newborns in Taiwan has convincingly demonstrated a drop in the incidence of HCC in the years thereafter [16]. In Japan, Europe, and the United States about 60% of the patients with HCC are attributed to chronic hepatitis C virus (HCV) infection, 20% are attributed to chronic HBV infection and about 20% are equally divided between cryptogenic and alcoholic liver disease [17].

Hepatitis C as an infection transmitted by blood or plasma products before 1990 and is an important factor in the development of HCC in western countries [18]. HCV causes HCC mainly through indirect pathways: chronic inflammation, cell deaths, and regeneration. In the pathogenesis the concurrent presence of the use of alcohol, the presence of diabetes and overweight play also an important role as a co-factor in HCV infection [15,17].

Alcohol use is increasing in many countries, suggesting that alcohol induced liver disease will continue to be a common cause of HCC throughout the World [19]. Moreover, obesity constitutes a significant risk of cancer mortality in general and an increasingly recognized risk factor for hepatocellular cancer in particular [20]. Longitudinal case studies indicate that obesity-associated steatosis and secondary non-alcoholic fatty liver disease more in particular non-alcoholic steatohepatitis (NASH) derives this association. A condition that can lead to cirrhosis and HCC alone, or may act synergistically with other disorders such as hepatitis C [20].

Screening

To decrease cancer-related mortality, entrance in a surveillance program of patients with established cirrhosis is recommended, to detect early HCC [21]. Surveillance is recommended for patients with cirrhosis in whom potentially effective treatment for HCC can be offered if tumours can be detected early [22]. The available data on tumour

growth suggest that the interval from undetectable lesions to a 2 cm diameter lesion is approximately 4-12 months [23]. Until 2012 screening for HCC was applied with the combination of the serum alfa-fetoprotein (AFP) level and ultrasonography at 6 months intervals [24].

Clinical features

Patients with HCC present with one or more of several clinical features including right upper quadrant pain, weight loss and early satiety [25]. However, more and more hepatocellular cancers are now detected at an asymptomatic stage because of growing awareness of these tumours in patients with chronic liver disease and cirrhosis [21]. Physical findings in patients with HCC generally reflect the severity of the underlying chronic liver disease and cirrhosis [25].

Diagnosis

The diagnosis of HCC is based on the combination of clinical, laboratory, imaging and pathology examinations [1]. Imaging characteristics of HCC are dependent on size with nodules >2 cm showing a characteristic pattern which is determined by the dominant arterial supply of the tumour. HCC in the cirrhotic liver is characterized by hyperdense enhancing nodules, caused by an increase in arterial supply during the early phase of contrast enhancement when compared with background of liver parenchyma. During the later phase the density becomes lower than the surrounding hepatic parenchyma because of the lack of portal venous supply, the so called wash-out phase.

HCC in a non-cirrhotic liver is characterized by a large dominant, hyperdense enhancing mass. It is usually larger than HCC found in cirrhotic liver [11,26]. It can be difficult to differentiate HCC from a large hepatic adenoma or fibrolamellar HCC based on imaging findings alone [27].

A decision to biopsy a focal liver lesion suspected for HCC should be discussed by a multidisciplinary team, including a hepatobiliary and transplant surgeon because there is a risk of needle tract seeding that is estimated at 2,7% [28]. There is no indication for biopsy of a focal lesion in a cirrhotic liver when the patient is: not a candidate for any form of therapy because of serious co-morbidity; in case of decompensated cirrhosis and the patient is on the waiting list for liver transplantation, when the patient is a candidate for resection that can be performed with an acceptable morbidity and mortality risk.

Staging systems

A staging system should separate HCC patients into groups with homogeneous prognosis, and serve to select appropriate treatment. Because prognostic modelling in HCC is more complex, tumour node metastasis staging has been modified repeatedly. Cancer of the Liver Italian Program (CLIP) and the Barcelona-Clinic Liver Cancer staging (BCLC)

include aspects of performance status, tumour extent, liver function and treatment and provides the best prognostic stratification of patients with HCC [1,29-31]. However, none has received universal acceptance.

Treatment

Curative

Liver transplantation is the only definitive treatment for both HCC and the underlying liver disease. Dependent on the aetiology of the liver disease the initial liver disease may recur in the transplanted liver. Antiviral therapy at present can e.g. completely prevent HBV recurrence but in patients with HCV 30% will develop cirrhosis in their graft after 5-years of follow-up [32]. The selection policy for transplantation for HCC patients follows the Milan criteria: one nodule less than 5 cm or two-three nodules up to 3 cm each. These criteria have been extensively validated with regard to their prognostic value [33].
Resection is reserved to a single HCC with preserved liver function [32].
Percutaneous ablation includes several techniques used to destroy malignant tissue. This technique is relatively inexpensive, requires a very short hospitalization time and seldomly presents complications. RFA is the best option for early, (un)resectable HCC [32].

Palliative

Palliative treatment can be proposed to selected patients with HCCs not amenable to curative treatment, after careful evaluation of residual liver function and co-morbidities. They include transarterial chemoembolization (TACE) or transarterial embolization (TAE) alone [32]. Sorafenib has demonstrated a median increase survival of 3 months in Child-Pugh class A patients with advanced HCC [34].

Due to patient diversity, HCC patients continue to create challenging clinical problems. An interdisciplinary approach with surgeons, gastroenterologists, radiologists, oncologists, and pathologists if therefore required.

This thesis includes studies that address various aspects of HCC: epidemiology, screening, staging and outcome. The thesis is, in addition to the introduction (***Chapter 1***) and the discussion and conclusions (***Chapter 11***), divided into four parts: part II – trends in hepatobiliary cancer, part III- screening for hepatocellular carcinoma, part IV - optimization of staging and part IV – outcome.

TRENDS IN HEPATOBILIARY CANCER

HCC is the most common primary liver cancer, and it is estimated that worldwide, each year 500 000 new patients are being diagnosed with this cancer [1]. Other low-endemic countries reported increasing incidence rates for liver cancer and HCC due to improved detection, by improvement of imaging modalities [7,32]. Because of these changes in

detection, changes in the incidence and survival of HCC and other primary liver tumours are also expected to have occurred in the Netherlands. Therefore, a population-based study was performed to determine whether the reported changes in incidence were also observed in the Netherlands, including all patients with a primary liver tumour. Trends in incidence, treatment and survival were observed in all patients with HCC between 1989 and 2009 (***Chapter 2***).

Improved imaging techniques have facilitated the diagnosis of liver malignancies, changes in intrahepatic cholangiocarcinoma (ICC) incidence and survival can occur [32]. Therefore, trends in incidence, and survival were studied in patients with ICC between 1989 and 2008 (***Chapter 3***).

SCREENING FOR HEPATOCELLULAR CARCINOMA

Until 2012 screening for HCC was widely applied with alpha-fetoprotein (AFP) and ultrasonography in patients at risk e.g. patients with cirrhosis and patients chronically infected with hepatitis B virus [32]. However, AFP has a low sensitivity when used as a screening test [32]. Due to lack of an efficacious test, new markers have been investigated. To define the present state of the art for HCC screening, ***Chapter 4*** offers a review of the literature discussing biomarkers most likely to be introduced as new instrument for HCC screening.

As chronic hepatitis and cirrhosis are known risk factors for HCC development, these patients are defined as an acceptable population to screen on regular basis [1,32]. The natural history of HCC development suggests that chronic hepatitis and cirrhosis induce premalignant steps [15]. However, HCC occasionally appears in the absence of chronic liver disease or cirrhosis [11]. It is suggested that HCC in non-hepatitis and non-cirrhotic livers are the result of a malignant transformation of hepatocellular adenoma [35,36]. This hypothesis could include the co-existence of both hepatocellular adenoma and HCC within the same tumour. The possible relationship between the presence of HCC in the non-cirrhotic liver and a hepatocellular adenoma as precursor lesion was explored (***Chapter 5***).

OPTIMIZATION OF STAGING

The tests used for diagnosing HCC include different imaging modalities (ultrasonography, computed tomography (CT) or magnetic resonance imaging (MRI)), biopsy and AFP serum levels [32]. Dynamic contrast-enhanced MRI has a higher sensitivity in detecting HCC than CT [32]. After detection and treatment HCC can recurrence. Various factors

associated with fatal recurrence after orthotopic liver transplantation and resection for HCC have been well documented [37-39]. The most important ones are vascular invasion, differentiation grade, tumour size, and multifocal tumours as long-term survival is strongly correlated with these findings. However, identification of especially vascular invasion and differentiation grade are by means of histological examination often done within the resected specimen [32]. If the presence of vascular invasion and the type of differentiation grade can be evaluated with a preoperative imaging, without a biopsy, long term-survival might be predicted more accurately preoperatively and subsequently the treatment strategy for some patients might be adapted. Therefore we explored whether contrast-enhanced MRI has the potential to predict outcome for patients with HCC (***Chapter 6***).

Accurate staging is important to determine the prognosis and to guide therapy [32]. According to the AASLD guidelines nodules larger than 1 cm found on ultrasound screening of a cirrhotic liver should be investigated further with either 4-phase multidetector CT scan or dynamic contrast enhanced MRI [32]. In absence of consensus on international guidelines as to which patients require preoperative staging by bone scintigraphy and thorax CT, many clinicians continue to routinely perform these staging investigations in all patients, especially in those at risk for postoperative complications. However, the rising costs of healthcare and (in particular) of cancer care challenge the appropriateness of current practices and emphasise the need for more cost-effectiveness analyses. The positive yield of a routine bone scintigraphy in patients with early-stage hepatoma evaluated for liver transplantation was reported to be very low [39]. To assess the role of baseline scintigraphy and its benefit for eligible patients to receive treatment with curative intent, a review of our clinical practice was performed (***Chapter 7***).

OUTCOME

HCC typically develops in the presence of underlying cirrhosis, particularly in high-endemic areas. In low-endemic, like the Netherlands, HCC as well appears in absence of underlying liver cirrhosis [8-10]. Many investigators tried to report on prognostic factors for HCC in a non-cirrhotic liver. However, they still included patients positive for serological markers of hepatitis B or C virus infection, alcohol abuse or hematochromatosis. These are known risk factors for the development of cirrhosis and eventually HCC [14,15,19]. In HCC patients with no underlying cirrhosis, the 'normal' quality of the non-tumoral liver parenchyma might make this type of HCC a different entity, with a different aetiology, clinical presentation, management and prognosis [40]. In ***Chapter 8*** we aimed to identify the factor(s) predictive for survival and recurrence of HCC in the non-cirrhotic liver for patients able to receive treatment with curative intent.

The liver represents one of the three most common sites of metastasis, and primary hepatic cholangiocarcinoma and HCC often share overlapping morphological appearances [6]. The diagnosis of HCC might be difficult in the absence of risk factors e.g. cirrhosis or hepatitis B/C virus infection. One of the challenges in histopathological diagnosis of hepatocellular mass lesions is to distinguish HCC (particularly well-differentiated) from hepatocellular adenoma [35]. Complicating the diagnostic process is that pathologists are frequently asked to handle and diagnose liver needle core biopsies with various biopsy artefacts. As an overview of the immunoprofile of HCC in non-cirrhotic livers has not been presented before, we established the role of the immunohistochemical staining pattern in HCC in the non-cirrhotic liver as a diagnostic or prognostic test (***Chapter 9***).

If HCC appears in a cirrhotic liver, in many patients HCC occurs against the background of a chronic viral infection. Worldwide approximately 400 million people are chronically infected with HBV [41,42]. In Rotterdam, the Netherlands, the prevalence of hepatitis B virus infection (hepatitis B core antibodies) was 20% [43]. In the last 15 years, reliable quantification of HBV DNA over a large dynamic range has become feasible. Several hospital-based and community-based studies have subsequently found significant associations between the level of serum HBV DNA and the risk to develop liver cirrhosis or HCC [42]. The impact of viral load on survival of HCC patients after surgery with curative intent may be overshadowed by tumour-related factors or stage of the liver disease at detection of HCC. It has been hypothesized that anti-viral therapy for HCC patients with active HBV replication, along with HCC treatment might reduce the recurrence rates for HBV-associated HCC in high endemic areas. Unfortunately the effect of anti-viral therapy on HCC outcome in low-endemic areas was not discussed [44]. ***Chapter 10*** attempt to determine whether HBV DNA levels at time of HCC appearance is associated with overall survival in a low-endemic area.

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PART II

TRENDS IN HEPATOBILIARY CANCER

Chapter 2

Hepatocellular carcinoma in a low-endemic area: rising incidence and improved survival

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ABSTRACT

Objective To examine recent trends in incidence and outcome among patients with hepatocellular carcinoma (HCC) in an unselected population in Western Europe.

Methods Data from the nationwide Netherlands Cancer Registry were used to estimate trends in incidence for all 6514 patients newly diagnosed with primary liver cancer between 1989 and 2009. Trends in incidence, treatment, and relative survival according to sex and age were estimated in 5143 patients with HCC, also using the European Standardized Rates (ESR).

Results The ESR for all primary liver cancers combined increased significantly between 1989 and 2009 as did the ESR for HCC among men (estimated annual percentage change: 2.2%, 95% confidence interval: 1.6–2.7) and for women aged below 60 years, suggesting etiological influences in these groups. Especially, the nonhistologically confirmed HCC incidence increased. More patients underwent surgery for HCC, from 9% in 1989–1994 to 23% in 2005–2009, as well as chemotherapy and/or irradiation, from 6 to 11% in the same period. At the end of the study period, only 66% of patients received noncancer-related HCC therapy, that is, best supportive care, compared with 85% in 1989–1994. The 1 and 5-year relative survival for patients with HCC increased significantly ($P < 0.001$ and $P < 0.001$).

Conclusion In as much as the modest increase in the incidence of HCC was a matter of better detection, due to improved imaging techniques, which may have affected the overall relative survival for HCC patients, the increasing trend in survival is likely to be, in the absence of other explanations, due to better treatment of the underlying liver cirrhosis.

INTRODUCTION

Worldwide, hepatocellular carcinoma (HCC) is the fifth most common cancer and the third most common cause of cancer mortality. It is estimated that worldwide, each year, 500 000 new patients are being diagnosed with HCC [1]. In industrialized countries, HCC is an uncommon type of cancer [2,3]. However, the incidence rates for liver cancer and HCC have been increasing in low-endemic areas [4].

The increase in the incidence in other countries is partly due to improved detection, causing previously diagnosed cancers to be detected. Improvement of imaging modalities during the last decades was responsible for better characterization of liver lesions. Together with the screening program that has been developed for patients with liver cirrhosis, HCC can be detected with a smaller size [5]. The screening program in the Netherlands might influence not only the incidence but also the treatment and survival of patients with HCC.

Because of these changes in detection, changes in the incidence and survival of liver cancer are also expected to have occurred in the Netherlands. Therefore, as a follow-up to our previous analysis [3], we conducted a population-based study in the Netherlands of all patients diagnosed with liver cancer between 1989 and 2009. For HCC only, trends in incidence, treatment, and survival, according to sex and age, were studied.

PATIENTS AND METHODS

Data collection

Incidence and treatment data on liver tumors from 1989 to 2009 were provided by the population-based Netherlands Cancer Registry (NCR), a nationwide registry that was started in 1989 and is maintained by regional comprehensive cancer centers [6]. The NCR is based on notification of all newly diagnosed malignancies in the Netherlands by the automated pathological archive. Additional sources are the national registry of hospital discharges, haematology departments, and radiotherapy institutions. Information on patient characteristics, such as sex and date of birth, and tumor characteristics, such as date of diagnosis, localization (International Classification of Diseases for Oncology), histology, and primary treatment, is obtained routinely from the medical records [7]. The quality of the data is high, due to thorough training of the data managers, good access to care, and computerized consistency checks. The infrastructure of the Netherlands healthcare system and the notification procedure used have made it possible to establish a cancer registry, in which completeness is estimated to be at least 95% [8,9]. In the case of multiple tumors, the same rules were applied as those recommended by the International Association of Cancer Registries [10]. The information on vital status

was initially obtained from municipal registries and from 1995 onwards from the nationwide population registries network. These registries provide complete coverage of all deceased Dutch residents.

For the present study, all patients with a primary liver tumor (C22.0 and C22.1) diagnosed during 1989–2009 in the Netherlands were included (n=6514). Primary liver cancer was classified as HCC, cholangiocarcinoma, neuroendocrine tumors, blastoma, sarcoma, or tumors not otherwise specified. The latter category includes HCC and cholangiocarcinoma without pathological confirmation.

Age was divided into three groups, that is 60 years or younger, 60–74, and 75 years or older. The study period was divided into four unequal categories, namely 1989–1994, 1995–1999, 2000–2004, and 2005–2009. Treatment was scored as follows: surgery including partial liver resection, radiofrequent ablation (RFA) or liver transplantation, chemotherapy (systemic or regional), and irradiation therapy or other/no therapy.

Patients younger than 15 years and older than 95 years were excluded from the survival analysis, as well as cases diagnosed at autopsy.

Statistical analyses

Annual incidence and mortality rates for the period 1989–2009 were calculated per 100 000 person-years, using the annual mid-year population size as obtained from Statistics Netherlands. Rates were age standardized to the European standard population [European Standardized Rates (ESR)]. Changes were evaluated by calculating the estimated annual percentage change (EAPC) and the corresponding 95% confidence interval (95% CI).

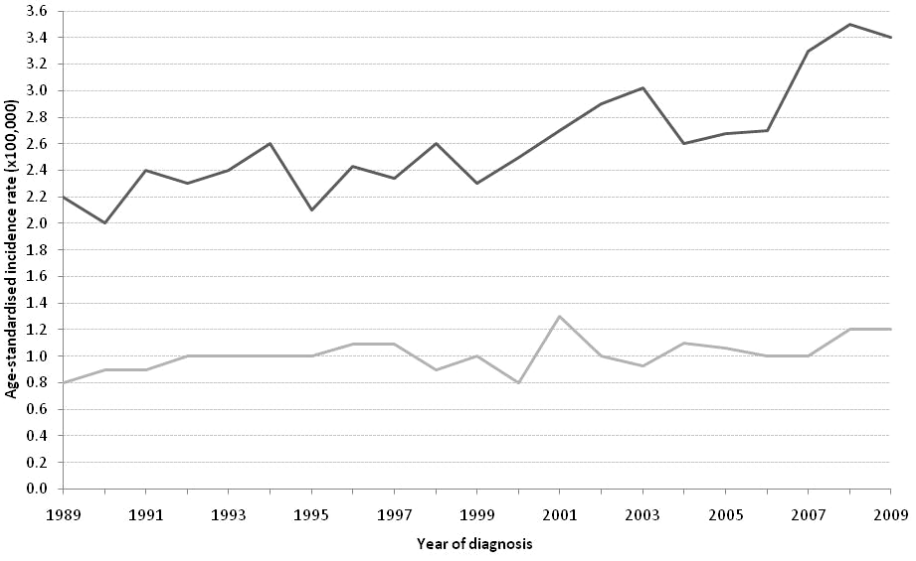
Because of changes in coding during the study period, it was impossible to examine the mortality trends. Proportions of patients receiving different types of treatment (surgery, chemotherapy, radiotherapy) were calculated according to age group and period. Follow-up of the vital status of all patients was calculated as the time from diagnosis to death, censoring due to loss to follow-up, or until 1 February 2010. Traditional cohort-based relative survival analysis was used for the period 1989–2009, which represents the actual survival of patients diagnosed during this period. As follow-up was complete until February 2010, the 3-year and 5-year relative survival of patients diagnosed in the period 2005–2009 could not be calculated using the cohort-based method. Therefore, we used period-based relative survival analysis. Survival trends within 1989–2009 were evaluated by a Poisson regression model [11]. SAS software (SAS system 9.2, SAS Institute, Cary, North Carolina, USA) was used to perform the statistical analyses.

RESULTS

Incidence

Between 1989 and 2009, a total of 6514 patients were diagnosed with primary liver cancer in the Netherlands. The incidence of liver cancer was approximately three times as high in men compared with women (Fig. 1). For both sexes, a significant increase in the incidence of primary liver cancer was observed: ESRs for men increased from 2.2 per 100 000 in 1989 to 3.0 in 2009 (EAPC: 2.1%, 95% CI: 1.5–2.7) and that for women from 0.8 per 100 000 in 1989 to 1.1 in 2009 (EAPC: 1.0%, 95% CI: 0.2–1.8; Fig. 1).

HCC was the predominant histology (n=5143), comprising 79% of all liver cancers, followed by intrahepatic cholangiocarcinoma (17%, n=1110). The remaining 4% (n=261) were neuroendocrine tumors, blastomas, or sarcomas (Fig. 2). The distribution of primary liver cancers remained stable, besides an increase in blastomas, cholangiocarcinoma, and nonhistologically confirmed HCC.

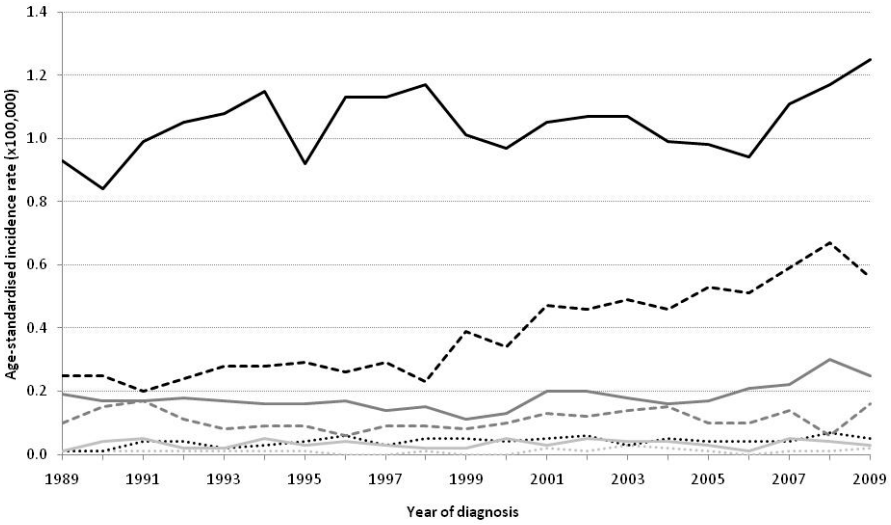


Numbers new diagnosed per year

Gender	Year																				
	1989	1990	1991	1992	1993	1994	1995	1996	1997	1998	1999	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009
Males	147	139	164	159	170	185	153	180	178	197	178	197	216	240	256	227	237	239	300	323	330
Females	75	79	83	98	91	97	93	95	105	84	97	82	128	101	98	109	108	103	111	130	132

Gender	EAPC (95%CI)
Males	2.1 (1.5, 2.7)*
Females	1.0 (0.2, 1.8)*

Figure 1 Age-standardised incidence rates (ESR) for all primary liver cancers according to gender in the Netherlands (1989-2009)
* Significant trend



Morphology		EAPC(95%CI)
Hepatocellular carcinoma (HCC)	———	0.6 (-0.1, 1.3)
Cholangiocarcinoma	———	1.6 (0.1, 3.1)*
Neuro-endocrine	·····	2.7 (-1.4, 6.7)
Blastoma	·····	5.8 (2.3, 9.2)*
Sarcoma	———	1.6 (-2.0, 5.3)
Non specified – HCC	– – – –	5.6 (4.6, 6.6)*
Non specified – Cholangiocarcinoma	– – – –	0.2 (-2.0, 2.4)

Morphology	Numbers new diagnosed per year										
	1989	1990	1991	1992	1993	1994	1995	1996	1997	1998	1999
HCC	171	161	178	197	214	226	192	218	231	228	232
Cholangiocarcinoma	46	49	55	50	40	43	42	42	43	42	33
Neuro-endocrine	2	1	1	1	2	1	2	1	0	2	0
Blastoma	1	1	5	5	2	4	5	7	4	6	7
Sarcoma	2	6	8	4	3	8	5	7	5	3	3

Morphology	Numbers new diagnosed per year									
	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009
HCC	219	264	266	275	263	279	276	326	363	364
Cholangiocarcinoma	45	65	59	63	56	52	59	69	72	84
Neuro-endocrine	1	3	1	5	4	2	0	2	2	3
Blastoma	5	6	7	4	7	5	5	5	8	6
Sarcoma	9	6	8	7	6	6	2	9	8	5

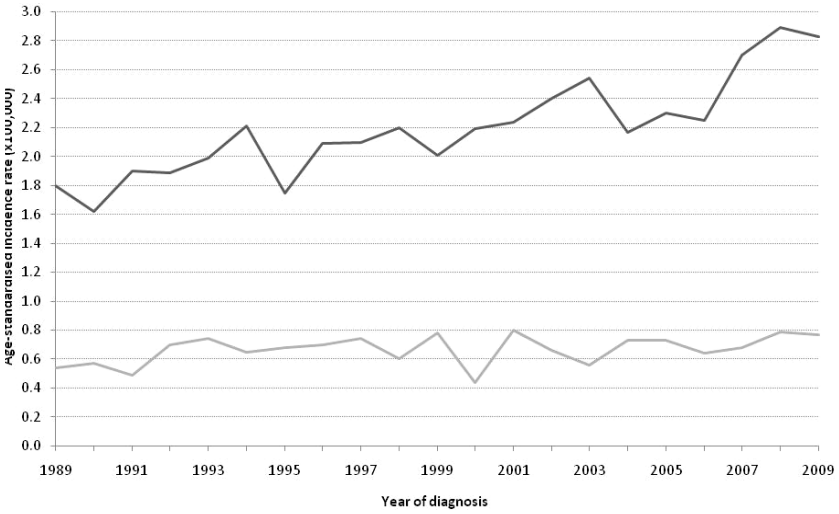
Figure 2: Age-standardised incidence rates (ESR) of morphologies of all primary liver cancers, both sexes, the Netherlands (1989-2009)

* Significant trend

Between 1989 and 2009, 5143 new patients were diagnosed with HCC in the Netherlands, with an increasing incidence rate among men and a slightly but insignificantly increasing incidence rate among women (Fig. 3a). In 2009, the incidence (ESR) of HCC in men was 1.8 and that in women was 0.5 per 100 000. Figure 3b shows the age-specific trends of HCC for men and women. The age-specific trends for women aged 60 years and over remained unchanged. A significantly increasing trend was observed among men in all age groups. The annual number of new male HCC cases ranged from 28 to 83 cases for the age group 0–60 years, 54 to 143 cases for 60–74 years, and 18 to 69 cases for age 75 years and older, ranging in women from six to 27, 14 to 38, and 16 to 36 cases, respectively.

Treatment

Between 1989 and 2009, 16% (n=804) of the total HCC population underwent a partial liver resection, RFA or orthotopic liver transplantation. The local treatment rate with curative intent increased from 19 to 23% in 2005–2009. An increasing number of patients with HCC received palliative therapy, that is, chemotherapy and/or irradiation from 6% in 1989–1994 to 11% in 2005–2009. Over time, fewer patients received noncancer-related therapy; best supportive care decreased from 85 to 66% (Table 1).



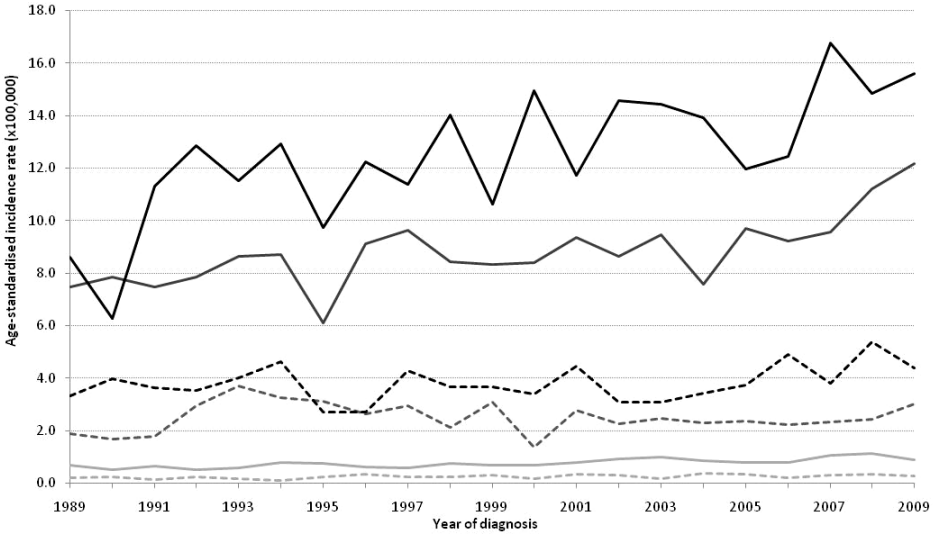
Numbers new diagnosed per year

Gender	Year																				
	1989	1990	1991	1992	1993	1994	1995	1996	1997	1998	1999	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009
Males	122	110	131	132	144	160	129	154	159	169	157	173	183	201	216	189	204	203	251	274	277
Females	49	51	47	65	70	66	63	64	72	59	75	46	81	65	59	74	75	73	75	89	87

Gender	EAPC (95%CI)
Males	2.2 (1.6, 2.7)*
Females	1.0 (-.01, 2.2)

Figure 3A: Age-standardised incidence rates (ESR) for hepatocellular carcinoma according to gender in the Netherlands per 100,000

* Significant trend



Age	EAPC(95%CI)
Males <60	2.9 (1.8, 3.9)*
Males 60-74	1.7 (0.8, 2.5)*
Males ≥75	2.6 (1.3, 3.8)*
Females <60	2.6 (0.3, 5.0)*
Females 60-74	0.2 (-1.6, 2.1)
Females ≥75	0.9 (-0.4, 2.3)

Figure 3B: Age-standardised incidence rates (ESR) for hepatocellular carcinoma according to gender and age groups in the Netherlands, both sexes (1989-2009)

* Significant trend

Table 1: Time trends of treatment patterns for patients with hepatocellular carcinoma in the Netherlands during the period 1989-2009

Therapy	**Period of diagnosis ^**			
	1998-1994 n(%)	**1995-1999 n(%)**	**2000-2004 n(%)**	**2005-2009 n(%)**
Surgery	102(9)	136(12)	194(15)	372(23)
Chemotherapy/ irradiation	65(6)	47(4)	66(5)	172(11)
Other/ no therapy	980(85)	918(83)	1027(80)	1064(66)
Total	1147	1101	1287	1608

^ unequal period clustered

Source: Netherlands Cancer registry

Survival

The relative survival rates for patients with HCC between 1989 and 2009 improved (Table 2): the 1-year survival rate increased from 20 to 37% and the 5-year survival rate increased from 5 to 14% (both P<0.001). The survival rate increased significantly for men and women aged younger than 60 years and for men and women aged 60 years and older.

The relative survival rates for patients with HCC according to clinical characteristics and treatments are shown in Tables 3 and 4. The 1-year, 3-year, and 5-year survival rates for HCC patients treated surgically increased from 59, 41, and 36% in the period of 1989–1994 to 84, 63, and 49%, respectively, during 2005–2009 (all P<0.001). The 1-year survival rates for HCC patients receiving chemotherapy and/or irradiation showed an increase from 32% in the period of 1989–1994 to 51% in the period of 2005–2009 (P=0.03). The 1-year and 3-year survival rates for HCC patients receiving another or no therapy increased from 15 and 4% in the period of 1989–1994 to 19 and 6%, respectively, during 2005–2009 (P=0.004 and 0.001).

Table 2: Relative survival of patients with hepatocellular carcinoma in the Netherlands, according to period of diagnosis

Period of diagnosis^	N	Survival %(SE)			
		6-months	1-year	3-years	5-years
1989-1994	1068	32(1.5)	20(1.3)	8(0.9)	5(0.8)
1995-1999	1053	38(1.5)	26(1.4)	12(1.1)	9(0.9)
2000-2004	1242	41(1.4)	30(1.3)	15(1.1)	11(0.9)
2005-2009	1567	49(1.3)	37(1.3)	20(1.1)	14(1.1)
p-value		<0.001	<0.001	<0.001	<0.001

^ unequal period clustered
Source: Netherlands Cancer registry

Table 3: Relative survival of patients with hepatocellular carcinoma according to age <60 or ≥60 years and therapy in the Netherlands in the period 1989-2009

Therapy 1989-2009	Survival %(SE) age < 60 years					Survival %(SE) age ≥ 60 years				
	N	6-months	1-year	3-years	5-years	N	6-months	1-year	3-years	5-years
Surgery	358	88(1.8)	81(2.2)	61(2.8)	51(3.1)	429	84(1.9)	75(2.3)	52(3.0)	37(3.4)
Chemotherapy/ irradiation	151	64(4.0)	43(4.1)	10(3.0)	**	194	69(3.6)	48(3.9)	14(3.3)	**
Other/ no therapy	854	29(1.6)	17(1.3)	7(0.9)	5(0.8)	2944	30(0.9)	18(0.7)	5(0.5)	3(0.4)

** n<10

Table 4: Time trends of relative survival of patients with hepatocellular carcinoma and therapy in the Netherlands during 1989-2009

Therapy	Period of diagnosis^	N	Survival % (SE)			
			6-months	1-year	3-years	5-years
Surgery	1989-1994	100	74 (4.5)	59 (5.0)	41 (5.1)	36 (5.1)
	1995-1999	130	82 (3.5)	75 (4.0)	50 (4.6)	38 (4.6)
	2000-2004	190	83 (2.8)	79 (3.1)	60 (3.8)	49 (4.0)
	2005-2009	367	91 (1.6)	84 (2.1)	63 (3.1)	49 (3.4)
p-value			<0.001	<0.001	<0.001	<0.001
Chemotherapy/ irradiation	1989-1994	64	54 (6.4)	32 (6.0)	**	**
	1995-1999	46	75 (6.5)	49 (7.5)	12 (4.9)	**
	2000-2004	63	69 (5.9)	45 (6.4)	17 (4.9)	**
	2005-2009	172	68 (3.8)	51 (4.2)	17 (3.9)	**
p-value			0.15	0.03	0.01	n.a.
Other/ no therapy	1989-1994	904	26 (1.5)	15 (1.2)	4 (0.7)	2 (0.5)
	1995-1999	877	30 (1.6)	17 (1.3)	6 (0.9)	4 (0.8)
	2000-2004	989	31 (1.5)	19 (1.3)	6 (0.8)	3 (0.6)
	2005-2009	1028	31 (1.5)	19 (1.3)	6 (0.8)	3 (0.6)
p-value			0.01	0.004	0.001	0.001

** n<10

^ unequal period clustered

DISCUSSION

The incidence of primary liver cancer has increased for both sexes in the Netherlands between 1989 and 2009. Because the terms HCC and primary liver cancer are often used interchangeably, it is possible that the studies conducted in low-endemic areas used a different definition of liver cancer and therefore included several morphologies to study the incidence [2]. Because HCC is low endemic in the Netherlands, it is more accurate to describe primary liver cancers separately. In this way, populations based on Western-world standards can be compared with high-endemic areas reliably.

Incidence

The incidence of HCC increased for men and remained unchanged among women (Fig. 3a). Another study in a low-endemic area found an increasing incidence of HCC over time as well [12]. The apparent changes in HCC incidence as described worldwide are very pronounced. Computed tomography and high-resolution MRI of the liver have facilitated the diagnosis of liver malignancies, especially the imaging of HCC. In 2005, guidelines were published in the Netherlands recommending biopsy, only if the vascu-

lar profile on the image is not characteristic or the nodule is detected in a noncirrhotic liver [5]. Because of the improved imaging techniques, HCC can be diagnosed without a biopsy, which possibly explains an increasing incidence of nonhistologically confirmed HCC.

The age-specific trends of HCC for men can be the result of an increase in the past in the incidence of HCV infections, which require two or three decades to develop into liver cirrhosis and typically precede HCC [12]. The prevalence of HCV in the general Dutch population was estimated at 0.1% in 1995/1996, but within low-endemic countries, pockets with higher endemicity for viral hepatitis do exist [13]. Other causes of the observed overall increases in HCC, mainly affecting low-incidence areas, might be the higher prevalence of nonalcohol fatty liver disease, which can be considered a precursor lesion of nonalcoholic steatohepatitis. Nonalcoholic steatohepatitis progresses to cirrhosis in 20% of cases [14].

Treatment and survival

The overall 5-year survival rates of patients with HCC increased as well as the percentage of patients who underwent surgery. This phenomenon could be explained by the promising RFA methods [15]. The increase in the application of chemotherapy and/or irradiation in our study could be explained by the introduction of transarterial embolization combined with injection of chemotherapeutic agents into the hepatic artery. In 1995, chemoembolization was the preferred palliative treatment for unresectable HCC [16]. Sorafenib for HCC was only introduced in 2009 in the Netherlands.

The survival rates for HCC patients who were able to undergo surgery or who received no specific treatment increased, as well as the 1-year, 3-year, and 5-year survival rates for HCC patients who received chemotherapy and/ or irradiation. Survival rates might improve after changes in tumor-related therapy. For HCC, however, both survival after resection or orthotopic liver transplantation and the treatment outcome of chemotherapy and/or irradiation are influenced markedly by the underlying liver disease [17]. The increasing trend in the survival of patients who did not receive a specific treatment is likely to be, in the absence of other explanations, due to better treatment of the underlying liver cirrhosis.

In contrast, surveillance programs have become more common, also in the Netherlands, which enables the detection of HCC at a smaller size [5]. Surveillance is recommended in patients at high risk for developing HCC, for example, with chronic hepatitis B carriers, hepatitis C cirrhosis, or other cirrhosis (level I evidence) [5]. Such patients might live longer after the diagnosis of HCC without an effect of treatment on date of death [17]. Because of early diagnosis of HCC, treatment may affect the outcome of disease and bring prolonged survival. As over 60% of the patients with HCC in the Netherlands had

underlying cirrhosis [18], the improved survival is likely to be affected by this lead time bias.

In conclusion, we found an increased age-standardized incidence of HCC among all male age groups and among women younger than 60 years in the Netherlands between 1989 and 2009. Overall and treatment-specific survival improved for HCC patients. The modest increase in the incidence of HCC is apparently a matter of better detection and affected the overall relative survival for HCC patients.

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Chapter 3

Intrahepatic cholangiocarcinoma in a low-endemic area: Rising incidence and improved survival

Accepted HPB

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ABSTRACT

Background: To explore trends in incidence and survival of patients with intrahepatic cholangiocarcinoma (ICC) an unselected population in Western Europe was studied.

Methods: All patients newly diagnosed with ICC between 1989 and 2009 were selected from the Netherlands Cancer Registry (n=809). Trends in incidence, treatment and relative survival were calculated according to gender and age. Follow-up for vital status was complete until January 1st 2010.

Results: The incidence rates of ICC increased significantly between 1999 and 2009, especially in the age group 45-59 years (estimated annual percentage change +3.0%, 95% confidence interval 0.2-5.8). In the other age groups ICC incidence remained stable. Patients diagnosed with TNM stage I mainly underwent surgery (68%), the majority of the patients with stage II, III and IV received best supportive care (73%). One-year relative survival for patients with ICC increased significantly from 24% in 1989-1994 to 28% in 2005-2009 (p=0.03), corresponding 3-year relative survival improved from 4% to 8% (p=0.02). Three-month and one-year relative survival for patients with ICC receiving surgery was 91% and 71%.

Discussion: The incidence of ICC rose between 1999 and 2009, especially in the age group 45-59 years, suggesting etiological influences. Survival rates have improved during the study period.

INTRODUCTION

Intrahepatic cholangiocarcinoma (ICC) the malignancy arising form the ductal epithelium of the biliary tree has a poor prognosis.[1,2] Perhaps because ICC is rare diagnosis, few reports on the epidemiology of ICC are available, and information on survival trends of ICC patients are only available from single institution case series, mostly describing patients who underwent a surgical resection.[3-5] Such studies do not reflect the outcome of an unselected group of patients. Because improved computed tomography (CT) and high-resolution magnetic resonance imaging (MRI) of the liver have facilitated the diagnosis of liver malignancies, changes in ICC detection and survival are expected to occur as is reported by two studies from the United States.[6,7] Therefore, a population-based study in the Netherlands of all patients diagnosed with ICC between 1989 and 2009 was performed and trends in incidence and survival according to gender, age and treatment were studied.

METHODS

Data collection

Incidence and treatment data on ICC (ICD-10 code C22.1) from the period 1989-2009 were provided by the population-based Netherlands Cancer Registry (NCR) which is managed by the Comprehensive Cancer Centres the Netherlands and South.[8] The NCR is based on notification of all newly diagnosed malignancies in the Netherlands by the automated pathological archive (PALGA). Additional sources are the national registry of hospital discharges and radiotherapy institutions. Information on patient characteristics, such as gender, and date of birth, and tumour characteristics, such as date of diagnosis, localisation (International Classification of Diseases for Oncology (ICD-O-3), histology, stage (Tumour Lymph Node Metastasis (TNM) classification), and primary treatment, are obtained routinely from the medical records.[9] The quality of the data is high, due to thorough training of the data managers, good access to care and computerized consistency checks. The infrastructure of the Netherlands health care system and the notification procedure used have made it possible to establish a cancer registry in which completeness is estimated to be at least 95% of the country.[10,11] In the case of multiple tumours, the same rules were applied as those recommended by the International Association of Cancer Registries.[12] The information on vital status was initially obtained from municipal registries and from 1995 onwards from the nationwide population registries network, providing complete coverage of all deceased Dutch residents.

ICC arises from the intrahepatic bile duct epithelium while extra-hepatic cholangiocarcinomas and Klatskin tumours involve the biliary tree within the hepatoduodenal

ligament.[13] To limit potential misclassification bias patients with a non-classified tumour (histological or clinical), an extra-hepatic cholangiocarcinoma or a Klatskin tumour were excluded (n=301).

Age was divided into five groups, i.e. <30, 30-44, 45-59, 60-74, and ≥75 years. The study period was divided into four categories, namely 1989-1994, 1995-1999, 2000-2004, and 2005-2009. TNM stage was determined postoperatively, clinical stage was used in case pathological TNM was missing. Treatment was scored as follows: surgery with curative intent including partial liver resection, chemotherapy (systemic or regional)/ irradiation therapy or no specific anti-cancer therapy i.e. best supportive care.

Patients younger than 15 years and older than 95 years were excluded from the survival analysis, as well as patients who had the diagnosis made at autopsy.

Statistical analyses

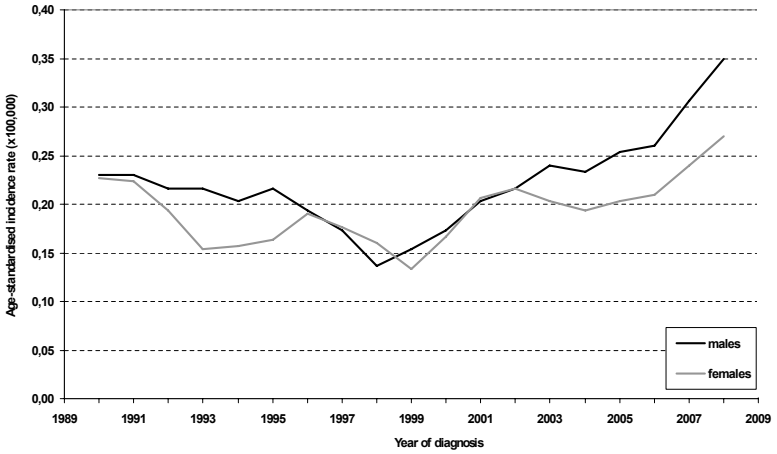
Annual incidence rates for the period 1989-2009 were calculated per 100,000 person-years, using the annual mid-year population size as obtained from Statistics Netherlands. Rates were age-standardised to the European standard population (European Standardised Rates (ESR)). Changes were evaluated by calculating the estimated annual percentage change (EAPC) and the corresponding 95% confidence interval (95% CI). Data regarding mortality trends were not available. Follow-up of vital status of all patients was calculated as the time from diagnosis to death, lost to follow-up or until the 1st of January 2010. The cohort-based method for relative survival analysis was used. Survival trends within 1989-2009 were evaluated by a poisson regression model.[14] SAS software (SAS system 9.2, SAS Institute, Cary, NC) was used to perform the statistical analyses.

RESULTS

Incidence

During the period 1989-2009 809 patients were diagnosed with ICC of whom 785 (97%) were pathologically confirmed. Median age was 68 years (range 23-95) and did not change over time. The age-standardized incidence rate (ESR per 100,000) was similar for males and females. The overall incidence of ICC decreased from 0.22 per 100,000 in 1989 to 0.12 in 1999 (EAPC -5.0%, 95%CI -9.2, -0.7) and thereafter increased to 0.35 per 100,000 in 2009 (EAPC +9.4%, 95% CI 4.6, 14.3). Figure 1 shows the incidence rates by gender.

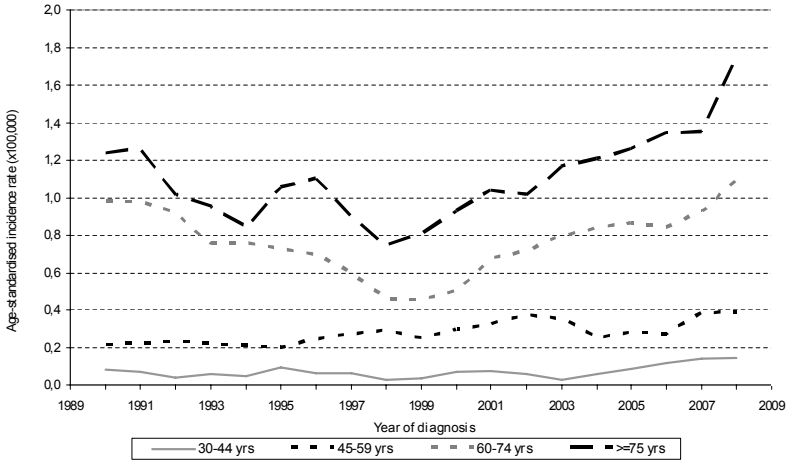
Figure 2 displays in five age groups the trends of ICC for both genders combined. A significantly increasing trend was observed in the age group 45-59 years (EAPC +3.0%, 95% confidence interval 0.2-5.8). The average annual number of new patients diagnosed with ICC (both sexes) was 0.3 (range 0-1) patients for age group 0-29 years, 2.9 (range



Period	Estimated annual percentage change (95%CI)
1989-1998	-5.0 (-9.2, -0.7)*
1999-2009	+9.4 (4.6, 14.3)*

Figure 1: Trends (*three year moving averages*) in age-standardised incidence rates (ESR) for intrahepatic cholangiocarcinoma according to gender, in the Netherlands (1989-2009)

* Significant trend



Age group	30-44 yrs	45-59 yrs	60-74 yrs	≥75 yrs
Estimated annual percentage change (95%CI) period 1989-2009	2.7 (-2.6, 7.9)	**3.0 (0.2, 5.8)***	0.7 (-2.1, 3.5)	1.8 (-0.7, 4.3)

Figure 2: Trends (*three year moving averages*) in age-specific incidence rates (ESR) for intrahepatic cholangiocarcinoma in the Netherlands, 1989-2009

* Significant trend; Age group < 30 yrs: n<10

0-8) patients for 30-44 years, 9.2 (range 3-16) patients for 45-59 years, 16.8 (range 4-31) patients for 60-74 years and 11.4 (range 6-22) patients for age 75 years and over.

Treatment

Data regarding treatment and TNM stage between 1989 and 2009 were available in 511 patients and are summarized in table 1.

Table 1: Treatment according to TNM stage in patients with intrahepatic cholangiocarcinoma in the Netherlands between 1989 and 2009.

TNM stage	N	Treatment n(%)		
		Surgery	Chemotherapy and/or irradiation*	No specific anti-cancer therapy
I	31	21(68)	0(0)	10(32)
II	47	15(32)	4(8)	28(60)
III	121	35(29)	19(16)	67(55)
IV	312	7(2)	49(16)	256(82)

*patients were treated according to hospital protocols, no exact chemotherapy and/or irradiation treatment regimes were available from the registry over time

Survival

Data regarding survival between 1989 and 2009 were available in 760 patients and missing in 49 patients. The relative survival rates for patients with ICC between 1989 and 2009 improved (Table 2). The short term (3-months, 6-months and 1-year) survival proportions for ICC patients who underwent surgery were 91%, 81% and 71%, respectively. The long term (3-year and 5-year) survival proportions for ICC patients who underwent surgery were 40% and 34%, respectively. For ICC patients receiving chemotherapy and/or irradiation corresponding short term numbers were 93%, 74% and 51%, respectively. For ICC patients receiving no anti-cancer therapy the short term rates were 39%, 23% and 12%, respectively. There were no long term survivors.

Table 2: Relative survival of patients with intrahepatic cholangiocarcinoma in the Netherlands, since 1989 according to period of diagnosis

Period of diagnosis	N	Survival % (SE)			
		3-months	6-months	1-year	3-year
1989-1994	174	50 (3.9)	33 (3.7)	24 (3.3)	4 (1.6)
1995-1999	137	45 (4.3)	30 (4.0)	17 (3.2)	7 (2.3)
2000-2004	181	55 (3.7)	37 (3.6)	24 (3.2)	11 (2.4)
2005-2009	268	56 (3.1)	41 (3.1)	28 (2.9)	8 (2.6)
p-value for trend		0.04	0.02	0.03	0.02

DISCUSSION

Incidence

This population-based study demonstrates an increase of ICC incidence in the Netherlands between 1999-2009 and improvements in survival. The increasing ICC incidence confirms and expands the previous findings on hepatocellular carcinoma reported in low endemic areas.[6,15] The question is whether the rising incidence rate of ICC is a true increase or a reflection of improved diagnostic tests resulting in more detected tumours (e.g. MRI, multiphase CT, positron emission tomography (PET) scan and endoscopic retrograde cholangiopancreatography) which became only available at larger scale since 2002 in the Netherlands. In absence of generally used serum markers better imaging techniques might cause an increased detection or reclassification of hepatobiliary tumours. ICC arises from the intrahepatic bile duct epithelium while extra-hepatic cholangiocarcinomas and Klatskin tumours involve the biliary tree within the hepatoduodenal ligament.[13] To limit potential misclassification bias, patients with a non-classified tumour (histologically or clinically), an extra-hepatic cholangiocarcinoma or a Klatskin tumour were not included. The number of patients (97%) with ICC histologically confirmed is therefore rather high.

Tumours previously described as unclassified might be classified as ICC today.[16] In case of misdiagnosis one would expect the incidence of at least one hepatobiliary tumour to decrease upon the rise of ICC. This has not happened, as the incidence of hepatocellular carcinoma also increased, and therefore the increasing incidence of ICC seems to be real.[15] Increased detection of cancer is usually associated with an increase in the proportion of patients with early-stage cancers in all age-groups. Therefore, it would have been interesting to study trends according to disease-stage. Unfortunately due to changes in TNM-staging classification during the study period, it was not possible to draw any conclusion from trends by disease-stage.

If the increase of ICC is a true reflection of an increased frequency, as can be suggested, the reason for this increase is unknown.[6,17] Several medical conditions are potentially related to ICC, including biliary cirrhosis, cholecystitis, alcoholic liver disease, liver cirrhosis, type II diabetes mellitus, chronic pancreatitis, hepatitis C viral infection (HCV), hepatitis B viral infection (HBV), obesity, non-alcohol fatty liver disease (NAFLD), non-alcoholic steatohepatitis (NASH), primary sclerosing cholangitis (PSC), primary biliary cirrhosis (PBC) and inflammatory bowel diseases including ulcerative colitis.[18] One possible explanation, for the observed increases, supported by recent studies, might be an increase of HCV and/or HBV related ICC.[19] However, details on presence of HCV and/or HBV in this population were not available, preventing us from further investigating these hypotheses. Chronic inflammation, cholestasis, and chronic liver damage are associated with malignant transformation of the biliary epithelium.[18] Perhaps ICC

increasing incidence might be influenced by an increasing prevalence of PSC.[6,16,17] PSC-related cancers are reported to peak after the 4th or 5th decade of life.[18,20] In this study rising incidence occurred predominantly in patients aged from 45 to 59 years which is possibly explained by an increasing prevalence of PSC.[7] Though data regarding changes in prevalence of risk factors for ICC were not analyzed, the increasing incidence of ICC is likely to be a true phenomenon rather than a reflection of improved diagnosis or a detection bias.[11] Other explanations of the observed trends may be misclassification and changes in registration and/or diagnostic practices over time, therefore these data need to be interpreted with caution. The distinction between intrahepatic and extrahepatic cholangiocarcinoma is somewhat arbitrary, especially if it concerns a hilar cholangiocarcinoma. But, as extrahepatic cholangiocarcinoma in the Netherlands is five to six times more common than ICC, misclassification of only a small proportion of the extrahepatic tumours as intrahepatic has a relatively large influence on the incidence of ICC.

Treatment and survival

Tumour resection is the only potential cure for ICC, mostly patients in TNM stage I are able to receive this therapy. Preoperative evaluation includes an assessment of patients' fitness for surgery, evaluation of presence of metastatic disease and analysing the possibility to create a resection margin free from cancer.[21] If any of these conditions are not fulfilled, surgical therapy is not indicated and palliative modalities are recommended.

Aljiffry et al. reviewed the literature according to outcome after surgical resection for ICC and found 5-year survival rates ranging from 15-40%, comparable with the 5-year survival rate of this study of 34%.[13] Because only a few patients received surgery, trends in survival over time could not be calculated for surgery.

Following complete surgical resection, a strategy aimed at optimizing local control with postoperative radiation alone or in combination with chemotherapy may theoretically provide benefit.[22] However, the available literature consists mainly of uncontrolled small series, and many reports consist of a mix of bile duct cancers, gallbladder cancer, ampullary cancer, and either pancreatic or hepatocellular cancers; as a result, the benefit of any type of adjuvant therapy remains uncertain.[5,23] In general, no single drug or combination has consistently led to objective tumour shrinkage or an increased median survival beyond the expected 8 to 15 months.[5,23] It is interesting to see that patients who received chemotherapy/irradiation had a similar short term survival rate, especially 3-months and 6-months (93% and 74%) compared with patients who underwent surgery (91% and 81%). Studies on palliative chemotherapy on patients with ICC have reported a median survival time between 6 and 15 months.[13] The survival benefit for patients able to receive surgery was increased with duration of follow-up, as the long term survival in patients receiving surgery is much better compared to patients receiving chemotherapy/irradiation.

As the benefit of any type of adjuvant therapy remains doubtful, the improved survival can be attributed to improved palliative treatments (stenting and drainage), improved overall supportive medical care, or both.

The improved survival could indicate lead-time bias related to early detection as patients with associated factors are controlled more often. For instance patients with PSC who have a lifetime risk of ICC which ranges from 8 to 20% and therefore these data need to be interpreted with caution.[16]

In conclusion, this population-based analysis appears to demonstrate an increasing incidence of ICC since 1999. Overall survival of patients with ICC appears to be improved, suggesting possible influences of improved imaging techniques, a better patient selection for surgery, or improved surgical techniques. Due to limitations both these statements should be interpreted with caution. This study demonstrates that even though improvements in ICC survival were achieved over time, patients with these tumours continue to have a very poor prognosis. Despite several advances over the last decades, ample opportunity for improvement still remains.

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PART III

SCREENING FOR HEPATOCELLULAR CARCINOMA

Chapter 4

Recently introduced biomarkers for screening of hepatocellular carcinoma: a systematicreview and meta-analysis

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ABSTRACT

Purpose Early detection of hepatocellular carcinoma (HCC) is essential for improved prognosis and long-term survival. To date, screening for HCC depends on serological testing (alpha-fetoprotein, AFP) and imaging (ultrasonography), both of which are not highly sensitive. A meta-analysis was performed to discuss recent developments in biomarkers that may be effective in screening for HCC.

Methods A systematic search of PubMed, Embase, and Web of Science was performed for articles published between January 2005 and October 2010, and focusing on biomarkers for HCC in urine, serum, or saliva. Data on sensitivity and specificity of tests were extracted from each included article and displayed with a summary ROC. A meta-analysis was carried out in which the area under the curve for each biomarker was used to compare the accuracy of different tests.

Results In seven well-defined studies, three biomarkers were identified for potential use, namely, Golgi protein 73 (GP73), interleukin-6 (IL-6), and squamous cell carcinoma antigen (SCCA). Comparison with AFP showed that GP73 was superior ($p = 0.006$; 95 % CL -0.23, -0.12), IL-6 was similar ($p = 0.66$; 95 % CL -0.31, 0.25), and SCCA was inferior to AFP ($p = 0.001$; 95 % CL 0.12, 0.23).

Conclusion GP73 is a valuable serum marker that seems to be superior to AFP and can be useful in the diagnosis and screening of HCC. Although GP73 may improve the detection and treatment of one of the most common malignancies worldwide, additional research is required.

INTRODUCTION

The incidence of hepatocellular carcinoma (HCC) is rising in many countries [1]. Alpha-fetoprotein (AFP) levels and ultrasonography are widely applied for HCC screening. However, AFP alone has a sensitivity of 60 % at a cut-off value of 20 ng/mL, and ultrasonography has a sensitivity of 65–80 % with a specificity ≥90 % when used as a screening test [1].

The lack of efficacious tests necessitates investigation for new HCC markers. Recent studies have focused on tests that can detect HCC, including tests for DCP, also known as prothrombin induced by vitamin K absence II (PIVKA II), the ratio of glycosylated AFP (L3 fraction) to total AFP, alpha fucosidase, glypican 3, and HSP-70. However, as sensitivity and specificity values of these serological markers were low, they proved to be inadequate for HCC screening purposes, even when combined [1].

General criteria for effective disease screening have been proposed by the World Health Organization (WHO). These criteria are as follows: the disease screened for should represent a major cause of death, the natural history of the disease should be well characterized, screening for the disease should be cost-effective, and the screening test should be acceptable to the population. In addition, facilities for diagnosis and treatment should be available, and there should be a treatment for the disease that improves the outcome if the disease is detected at an early stage [2]. These WHO criteria are met for HCC [1, 3].

To define the present state-of-the-art technology for HCC screening, we initiated a systematic review and metaanalysis, and discuss biomarkers most likely to be introduced as new instruments for HCC screening.

MATERIALS AND METHODS

Literature search strategy

A systematic search of PubMed, Embase, and Web of Science was performed for articles published between January 2005 and October 2010 (cut-off date 1 October 2010) and relevant to HCC biomarkers in urine, serum, or saliva. In 2005, Bruix et al. [1, 4] published the AASLD guidelines, which they updated in 2011. However, with respect to screening tests, their paper did not report on new findings [1]. Therefore, in the present study a new literature search was initiated based on the search terms listed in Table 1.

Table 1: Terms used in the systematic search for the present review

Database	Search terms
Pubmed	(hepatocellular carcinoma[mesh] OR hepatoma*[tw] OR liver cell neoplasm*[tw] OR hepatocellular neoplasm*[tw] OR liver cell cancer*[tw] OR hepatocellular cancer*[tw] OR liver cell tumo*[tw] OR hepatocellular tumo*[tw] OR liver cell carcinom*[tw] OR hepatocellular carcinom*[tw]) AND (biological markers[mesh] OR Biomarker*[tw] OR Biological Marker*[tw] OR Biologic Marker*[tw] OR Biochemical Marker*[tw] OR Immunologic Marker*[tw] OR Immune Marker*[tw] OR Laboratory Marker*[tw] OR Serum Marker*[tw] OR Clinical Marker*[tw]) AND (blood[mesh] OR blood[sh] OR blood[tw] OR serum*[tw] OR plasm[tw] OR plasma[tw] OR urine[mesh] OR urine[sh] OR urine*[tw] OR saliva[tw]) NOT (animals[mesh] NOT humans[mesh]) AND
limits	Publication date: 2005-3000 OR entrance date: 2005-3000 AND Language: English OR Dutch
Embase	((('liver cell' OR hepatocell*) NEAR/3 (neoplasm* OR cancer* OR tumo* OR carcinom*)):ti,ab,de) AND (marker/exp OR (biological NEAR/3 marker*):ti,ab,de OR biomarker*:ti,ab,de) AND (blood/exp OR blood:ti,ab,de OR serum*:ti,ab,de OR plasm:ti,ab,de OR plasma:ti,ab,de OR urine*:ti,ab,de OR saliva:ti,ab,de)
limits	Publication date: 2005-present AND Human Language: English OR Dutch
Web of Science	(hepatoma* OR liver cell neoplasm* OR hepatocellular neoplasm* OR liver cell cancer* OR hepatocellular cancer* OR liver cell tumo* OR hepatocellular tumo* OR liver cell carcinom* OR hepatocellular carcinom*) AND (Biomarker* OR Biological Marker* OR Biologic Marker* OR Biochemical Marker* OR Immunologic Marker* OR Immune Marker* OR Laboratory Marker* OR Serum Marker* OR Clinical Marker*) AND (blood OR serum* OR plasm OR plasma OR urine* OR saliva) NOT (animal* NOT human*)
limits	Publication date: 2005-present AND Language: English

Literature screening

Studies were evaluated for their relevance to our present topic. Study selection was accomplished through four levels of study screening (C.D.M.W. in consensus with S.M.A) (Fig. 1). At level 1, studies were excluded for the following reasons: review, letters, case reports, editorials, and comments. At level 2, abstracts of all the studies accepted at level 1 were reviewed for relevance. The full text was obtained for relevant papers, as well as any citations for which a decision could not be made from the abstract. At level 3, inclusion required a control group with ≥10 cirrhotic patients (hepatitis B and/or hepatitis C and/or alcohol abusers), ≥10 patients with a HCC, and ≥10 confirmed healthy persons. Finally, at level 4, those studies that tested the biomarker in a second independent population were included together with the studies that were a continuation of studies included at level 3. All studies without repeated measurements, as validation of their method, were excluded.

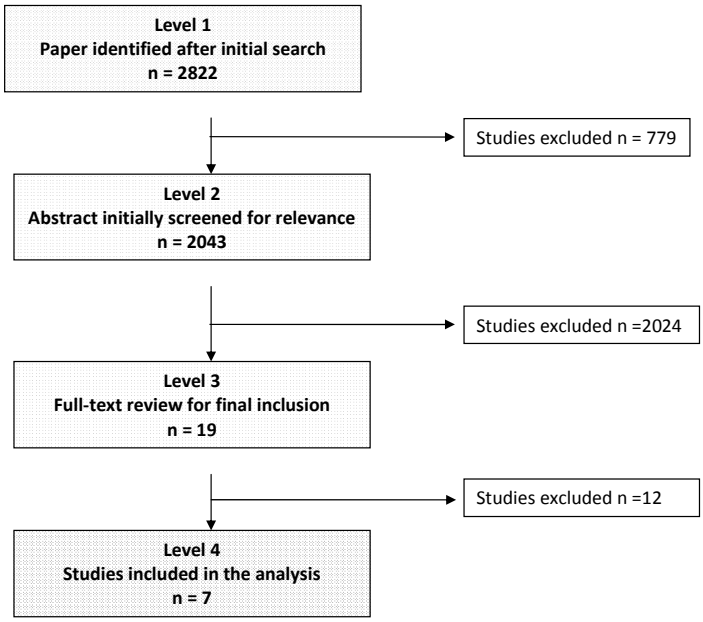

Figure 1: Flow diagram showing selection of the seven articles

Data extraction and critical appraisal

From each included article, we extracted data on study design, study population, and test results. The level of evidence of each article was scored using the Oxford Centre for Evidence-based Medicine Level of Evidence scale [5].

Data on sensitivity and specificity were extracted from each included article. If percentages were not reported, the sensitivity and specificity at several cut-off points were taken from the ROC curve in the included manuscripts.

Statistics

Sensitivities and specificities of the included studies were logistically transformed, and a linear regression line was fitted through the resulting points. This line was then backtransformed to obtain the summary ROC (sROC) curve, according to the method described by Littenberg and Moses in 1993 [6]. A conventional ROC curve describes the impact of threshold in a single patient population. The sROC curve, a compact description of the accuracy of the diagnostic test, describes the test in many populations. Note that we did not extrapolate the curve past the range of empiric data.

The area under the curve (AUC) for the biomarkers of the included studies was taken from the reports. For each biomarker, the pooled AUC was calculated using the inverse standard errors as weights. This pooled AUC, together with their similarly pooled

standard error, was used to compare the accuracy of the diagnostic tests. AFP was considered as a reference for comparison to the other markers and was compared with the pooled AUC of each new biomarker using Student's t test. SAS software (SAS system 9.2, SAS Institute, Cary, NC, USA) was used to perform the statistical analyses. A result was considered statistically significant at a p value of <0.05.

RESULTS

Among 2,822 articles identified by the initial search, seven were within the scope of the study (Fig. 1) [7–13]. Two articles described Golgi protein 73 (GP73) as a HCC biomarker [7, 8], two described interleukin-6 (IL-6) [9, 10], and three described squamous cell carcinoma antigen (SCCA) [11–13]. All identified studies provided level 2b evidence on the Oxford Level of Evidence scale and included a control group with ≥10 cirrhotic patients (hepatitis B and/or hepatitis C and/or alcohol abusers), ≥10 patients with a HCC, and ≥10 confirmed healthy persons [5].

GP73

GP73, also named Golgi phosphoprotein 2 (GOLPH2), is a 400-amino acid, 73 kDa transmembrane glycoprotein that normally resides within the cis-Golgi complex [7]. Marrero et al. tested GP73 in the sera of 352 patients, of whom 144 had HCC, 152 had cirrhosis, and 56 did not have any disease [7]. At the optimal cut-off point of 10 relative units (RU), the sensitivity of GP73 was 62%, with a specificity of 88%.

A recent study by Mao et al. tested GP73 in the sera of 4,217 subjects: 789 with HCC, 427 who were HBV or HCV carriers, 614 with cirrhosis, and 1,690 healthy controls [8]. GP73 sensitivity was 74.6% and specificity was 97.4% at an optimal cut-off value of 8.5 RU. The sROC of GP73 in these studies is shown as the gray dotted line in Fig. 2.

IL-6

IL-6 is a pleiotropic cytokine playing a central role in hematopoiesis, and in the differentiation and growth of a number of cells with different histological origins [9, 10]. The expression of IL-6 on hepatocytes, its upregulation by hepatitis B virus X protein, and its increased hepatic expression in liver cirrhosis have made IL-6 an intriguing cytokine to study in HCC [9].

Porta et al. [9] studied IL-6 in the sera of 90 patients: 30 with HCC, 30 with cirrhosis, and 30 healthy subjects. At the cut-off of 12 pg/mL, they found a sensitivity of 73% with a specificity of 87%.

Hsia et al. [10] also studied IL-6 in the sera of 128 patients, of whom 26 patients had HCC, 50 had chronic HBV or HCV infection, and 29 were without any disease (healthy

controls). The authors found a sensitivity of 46% with a specificity of 95% for IL-6 at a cut-off of 3 pg/mL. The sROC of IL-6 of these studies is shown as the black straight line in Fig. 2.

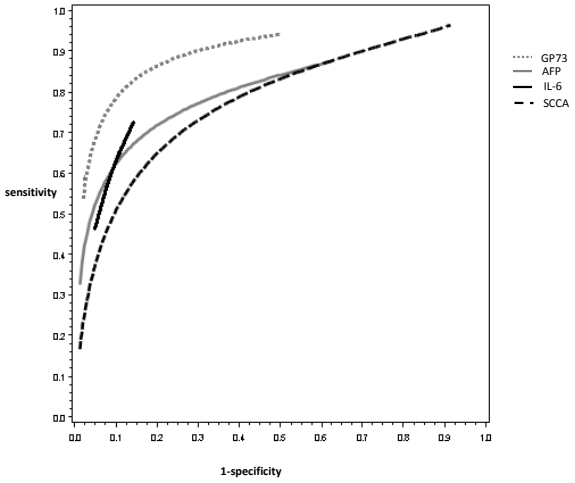

Figure 2: The sROC with the sensitivity and 1-specificity of GP73, AFP, IL-6, and SCCA

GP73=Golgi protein 73; AFP=Alpha-fetoprotein; IL-6=Interleukin-6 ; SCCA= Squamous cell carcinoma antigen

SCCA

SCCA, a component of the high molecular weight serine protease inhibitors named serpins, is physiologically expressed in the squamous epithelia [11–13]. Increased levels have been detected in several epithelial cancers such as those of the head, neck, cervix, and lung [11–13].

Giannelli et al. [11] tested SCCA in the sera of 251 patients: 120 with HCC, 90 with cirrhosis, and 41 healthy subjects. At an SCCA cut-off of 0.368 ng/mL, the sensitivity was 84% with a specificity of 48%.

In 2007, Giannelli et al. [12] reported on serum SCCA testing in 961 patients at a cut-off of 3.8 ng/mL; a sensitivity of 42% with a specificity of 83% was found.

In 2008, Hussein et al. [13] evaluated SCCA in the sera of 94 patients, including 49 patients with HCC, 30 with chronic liver disease without HCC, and 15 healthy persons. They used several cut-off points for SCCA: 100% sensitivity and 7% specificity were found at cut-off 0.3 ng/mL; 78% sensitivity and 84% specificity, at cut-off 1.5 ng/mL; and 39% sensitivity and 100% specificity were found at cut-off 3.5 ng/mL. The sROC of SCCA in these three studies is shown as the black dotted line in Fig. 2.

AFP

Under physiological conditions, AFP is a fetal-specific glycoprotein with a molecular weight of around 70 kDa. It is synthesized primarily by cells of the embryonic liver, of the vitelline sac, and of the fetal intestinal tract in the first trimester of pregnancy [14]. The serum concentration of AFP declines rapidly after birth, and its expression is repressed in adults [14]. In the pathological state of chronic liver disease, particularly, that associated with a high degree of hepatocyte regeneration, AFP can be expressed in the absence of cancer [14]. All studies compared the performance characteristics of their biomarker to those of AFP in differentiating HCC from non-malignant chronic liver disease [14]. The seven articles defined within the scope of this study all tested serum AFP in their population. The sensitivity and specificity for AFP are summarized in Table 2. The sROC of AFP of the seven studies is shown as the gray straight line in Fig. 2.

Table 2: Characteristics and outcome measures of the included studies describing serum AFP levels in the patients tested.

Authors	Year published	No. of patients	Cut-off value AFP (ng/ml)	Sensitivity (%)	Specificity (%)
Marrero et al.[6]	2005	352	112	25	97
Mao et al.[7]	2010	4,217	35	58	85
Porta et al.[8]	2008	90	12.8	63	88
Hsia et al.[9]	2007	128	20	62	88
Giannelli et al.[10]	2005	251	12.6	45	87
Giannelli et al.[11]	2007	961	18.8	41	94
Hussein et al.[12]	2008	94	7.7	90	93

AFP = alpha-fetoprotein

SROC

The sROC is a method for summarizing discrepant data on the accuracy of diagnostic technologies; it summarizes the central tendency of a set of accuracy reports.

Comparing the 'gold standard' with the three new biomarkers displayed that GP73 was superior to AFP ($p = 0.006$; 95 % CL -0.23, -0.12), IL-6 was similar to AFP ($p = 0.66$; 95 % CL -0.31, 0.25), and SCCA was inferior to AFP ($p = 0.001$; 95 % CL 0.12, 0.23).

DISCUSSION

This systematic review has attempted to correlate recently discovered biomarkers for HCC screening with AFP. Our findings suggest an advantage of GP73 over AFP as a serum marker for HCC screening. In our review process, many papers were excluded because of

limitations in study design: mostly because of a poor definition of the underlying etiology and the absence of a healthy control group. Due to our rigorous selection criteria, the review was

limited to seven studies with level 2b evidence, all testing their biomarker in serum [5]. Preferably, HCC screening should be performed using a non-invasive diagnostic test. Although the field of tumor markers in HCC is rapidly evolving, no ideal marker tested with a proper study design currently exists.

Since HCC is among the cancers with the worst prognosis, early diagnosis and treatment are essential for effective treatment [1]. The use of serological markers in patients at highest risk for developing HCC may decrease HCC mortality. However, for many years AFP has been the only standard serum marker for the detection of HCC, despite its unsatisfactory sensitivity [1]. Therefore, several new biomarkers (such as GP73, IL-6, and SCCA) have been investigated for their diagnostic accuracy and potential clinical application.

Giannelli et al. reported SCCA to be a good biomarker for discriminating early HCC from liver cirrhosis. Combining the three studies reporting on SCCA in a metaanalysis, we found SCCA to be inferior to AFP ($p = 0.001$).

In this systematic review, our meta-analysis of publications reporting on IL-6 showed that the accuracy of IL-6 was similar to that of AFP ($p = 0.66$).

Recent studies have identified serum GP73 as a potential biomarker for HCC. The study of Marrero et al. showed GP73 to be promising but had a small sample size [7]. A second study performed in medical centers in China and the USA showed GP73 to be a valuable tumor marker for HCC [8]. Combining both studies in our meta-analysis showed GP73 to be superior to AFP ($p = 0.006$). Although GP73 appears to be a better marker than AFP for diagnosing HCC, additional research is required that focuses on GP73.

Mao et al. found the elevation of serum GP73 to be modest in virus carriers, moderate in patients with cirrhosis, and dramatic in patients with HCC [8]. This indicates that the performance of GP73 might depend on the etiology of the underlying disease. This is important if one wants to differentiate between non-malignant disease and early HCC in; for instance, patients with chronic viral hepatitis. The authors also claimed tumor recurrence to be correlated with an elevated GP73 level in the blood [8]. Thus, besides being an interesting screening test, GP73 might also be useful as a surveillance test. The role of intrahepatic metastasis of the original tumor versus the development of de novo tumors could not be tested by Mao et al. The authors found no effect based on tumor size and tumor differentiation on the serum levels of GP73 [8].

The small amount of data available per paper on differences in etiology, tumor recurrence, and tumor development (numbers of tumors) precluded us from establishing the performance of GP73 in relation to these three parameters.

It would be interesting to examine whether combined measurements of GP73 and AFP further increase the sensitivity for detection of HCC. Although GP73 is a promising marker, more studies are warranted, especially because this protein is detected by Western blot analysis which hampers its reliability and availability in clinical use. Further studies are needed to analyze and validate early-stage HCC markers. Recently, Shang et al. [15] evaluated osteopontin as a marker of early-stage HCC. Although this study has some limitations, it is an important first step in the evaluation of new markers of early-stage

HCC [15]. The next step should be large-scale validation to determine whether osteopontin is superior to GP73 and to analyze whether osteopontin in combination with GP73 complements screening tests.

In conclusion, GP73 is a valuable serum marker that is superior to AFP and can be useful in the diagnosis and screening of HCC. GP73 may improve the detection and treatment of one of the most common malignancies worldwide. More studies are needed to further elucidate the influence of the etiology of disease on the signal strength of GP73.

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Chapter 5

The likelihood of hepatocellular adenoma as a risk factor for hepatocellular carcinoma in a non-cirrhotic liver

Adapted to: Hepatocellular adenoma as a risk factor for hepatocellular carcinoma in a non-cirrhotic liver: a plea against'. Accepted Gut

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ABSTRACT

Objective: Hepatocellular adenoma (HCA) is a rare benign tumour, with a small risk of malignant transformation into hepatocellular carcinoma (HCC). This study explores whether an HCA component or a transition zone can be demonstrated in HCC in a non-cirrhotic liver, to confirm the concept of adenoma-carcinoma transition.

Design: Paraffin-fixed liver tissue slides of patients diagnosed with HCC in a non-cirrhotic liver were retrieved from the archives of pathology department. Pre-operative images were analyzed, and recurrence and survival rates calculated.

Results: Between January 2000 and May 2011, 48 patients with 52 HCCs in a non-cirrhotic liver were operated. Median age at diagnosis was 65 (range 21-82) years, 24 patients (50%) were male. The median tumour size was 10 cm (range 2-24 cm), median body mass index was 23 (range 18-34). All lesions were without any HCA component or signs of a transition zone. In 9 patients HCA immunohistochemical staining and revision of the pre-operative imaging was performed because of doubt between HCA and well-differentiated HCC. No HCA component or transition zone was found on routine stains or by immunohistochemical staining.

Conclusion: Using extensive immunohistochemical staining no HCA components or transition zones from benign to malignant could be demonstrated in a large cohort of patients who developed HCC in a non-cirrhotic liver. We conclude that it is highly unlikely that HCC in a non-cirrhotic liver originates from a liver adenoma.

INTRODUCTION

Hepatocellular adenoma (HCA) is a rare benign liver tumour that most frequently occurs in young women and carries a small risk of malignant transformation into hepatocellular carcinoma (HCC) [1-4]. HCC is the fifth most common cancer in the world and the third most common cause of cancer-related mortality [5,6]. Natural history of HCC development suggests that chronic hepatitis and cirrhosis induce common premalignant steps whatever the origin of the disease [5]. HCC occasionally develops in the absence of chronic liver disease or cirrhosis [7]. It is questioned whether these tumours have a specific aetiology and a common pathway of carcinogenesis. It is suggested that HCC in non-hepatitis and non-cirrhotic livers may be the result of a malignant transformation of HCA [3,8]. This hypothesis assumes the co-existence of both HCA and HCC within the same tumour at a certain moment. The exact point at which the switch to malignant transformation occurs remains unknown [4]. Progression from a precursor lesion might be a multistep process, accompanied by a transition zone from benign hepatocytes towards dysplastic and malignant hepatocytes. Hypothesizing that HCC may arise from HCA is based on the assumption that at a certain moment residual HCA or a transition zone with dysplastic changes (as found in colorectal cancers) is present within the malignant liver lesion [9].

As to date, no reports can be found in literature supporting this concept and we doubt whether this mechanism is indeed of any relevance for the pathogenesis of HCC in non-cirrhotic livers. This study explores whether an HCA component or a transition zone can be demonstrated within HCC lesion in non-cirrhotic livers.

MATERIAL AND METHODS

Patients

The Erasmus University Medical Centre Rotterdam is a tertiary referral centre for focal liver lesions. From January 2000 to May 2011, 48 patients who were radiologically diagnosed with HCC in a non-cirrhotic liver were treated by surgical resection, i.e. segment or wedge resection and hemihepatectomy. A total of 52 lesions were surgically excised. Clinical data for age, gender, body mass index (BMI), aetiology, biochemical parameters and serum tumour markers alpha-fetoprotein (AFP) were retrospectively collected. Only patients with complete data at the time of resection and who were negative for HCC risk factors (i.e. hepatitis C viral infection, hepatitis B viral infection, alcohol abuse, haemochromatosis, metabolic syndrome or cirrhosis of any other cause) were included. Patients with a fibrolamellar HCC were excluded.

After treatment, patients had a follow-up using contrast-enhanced computed tomography (CT) or magnetic resonance imaging (MRI) combined with determination of serum AFP at 3 and 6 month intervals for up to 2 years post-treatment. After 2 years, follow-up was continued annually up to 5 years. HCC recurrence was defined as the occurrence of a focal liver lesion with HCC characteristics with or without increase of AFP, enlargement of lymph nodes in the liver hilum, or suspected extrahepatic lesions, showing growth at follow-up.

Histopathological and immunohistochemical analysis

Paraffin-fixed liver tissue slides were retrieved from the archives of the pathology department. From each tumour at least one sample per 1 cm tumour diameter was included. For each lesion, macroscopic characteristics (size, number of tumours and surgical resection margin) and microscopic features were evaluated (F.J.W.K.). Non-tumoral liver tissue was evaluated for fibrosis and cirrhosis according to the classification of the METAVIR group as follows: F0 (no fibrosis); F1 (portal fibrosis), F2 (few porto-central bridges), F3 (many porto-central bridges) and F4 (cirrhosis) [10]. Steatosis was graded according to Kleiner et al.: Grade 1 (5-33% of the hepatocytes), grade 2 (33-66% of the hepatocytes), grade 3 (more than 66% of the hepatocytes). Steatosis, if not observed or <5% was graded as grade 0 [11]. Lesions were evaluated for the presence of a fibrous tumour capsule and presence of necrosis and steatosis. The tumours were defined as HCC if nuclear polymorphia, a lack of reticulin pattern and presence of a capsula was demonstrated. Tumour differentiation grade was determined according to the Lauwers classification, and was based on the areas of tumour showing the highest grade [12]. Vascular invasion was defined as microscopic tumour invasion identified in the branches of the hepatic portal vein which was contiguous to the tumour [13]. Patients who had prior treatment with radiofrequency ablation or transarterial (chemo) embolization were only included for analysis if vital tumour tissue was found to characterize the lesion. A transition zone was defined as an area within the tumour lesion where benign hepatocytes were found in close relationship with dysplastic or malignant hepatocytes.

Immunohistochemistry was performed on selected paraffin sections of the tumour. Immunostaining included L-FABP (polyclonal antibody, 1:100 dilution, Bio-Connect), SAA (monoclonal mouse antibody, 1:200 dilution, Dako), CRP (mouse monoclonal antibody, 1:1600 dilution, Bio-Connect), GS (monoclonal mouse antibody, 6/glutamine syn., B&D, CC1-standard) and β-catenin (monoclonal mouse antibody, clone 14, Autostainer, PT high, Dako). A nuclear staining for β-catenin, focal or diffuse, was considered positive. L-FABP was considered positive when the tumour tissue was negative compared to the non-tumoral liver tissue. For each immunohistochemical staining a positive and negative control was used for control.

Imaging

The available pre-operative liver imaging was analysed independently by two experienced radiologists (F.E.J.A.W. and R.S.D.). They reviewed the images (CT or MRI) performed prior to the operation independently while blinded to the clinical history and pathologic diagnosis. Each radiologist was asked to make a diagnosis for every lesion detected; they had to report their certainty of diagnosis (uncertain, doubt, definite).

Statistical analysis

Continuous variables were summarised as median with ranges, and categorical variables were summarised as frequency and percentages. The Mann-Whitney U test, t-test or the χ^2-test were used whenever appropriate. Statistical significance was considered at a p-value < 0.05. The Kaplan-Meier method was used to calculate the survival rate.

RESULTS

Clinical features

Of 241 patients with HCC in a non-cirrhotic liver 48 patients (24 male; 50%) with 52 lesions were shown to be free of any known risk factor for the development of HCC. Lesions had a median size of 10 cm (range 2-24 cm). Median age at diagnosis was 65 (range 21-82) years, median BMI was 23 (range 18-34) and median AFP at diagnosis was 6 ng/ml (range 2-2,745,000). Of the 48 patients treated with surgical resection, 15 (31%) had prior treatment with radiofrequency ablation or transarterial (chemo) embolization as downstaging therapy.

During follow-up 17 patients (35%) had recurrence of HCC after a median period of 6 (range 2-42) months. The 1, 3 and 5-year survival rate was 86%, 64% and 37%, respectively.

Pathological features

Non-tumoral liver characteristics

Non-tumoral liver tissue was available in all cases. Pathological analysis per patient of the non-tumoral liver tissue showed steatosis grade 0 in 28 (58%) patients, steatosis grade 1 in 11 (23%) patients or grade 2 in 8 (17%) patients, and steatosis grade 3 in 1 (2%) patient. No fibrosis (F0) was present in 36 (75%) patients, whereas all others had F1 (n=11; 23%) or F2 (n=1; 2%).

On preoperative imaging, 42 HCC patients were found to have a single HCC node in the liver. Multiple HCCs (2-3 nodules) were found in 10 patients. Non-tumoral liver tissue didn't show dysplastic hepatocellular lesions.

Tumour characteristics

All lesions radiologically diagnosed as HCC were histologically confirmed. In 45 patients (94%) the surgical resection margins were free of tumour. Differentiation according to the Lauwers classification resulted in 12 well-differentiated (23%), 33 moderately-differentiated (64%) and 7 poorly-differentiated (14%) tumours. A total of 33 lesions (63%) had presence of vascular invasion. Capsule was present in 24 lesions (46%), 27 lesions (52%) revealed tumour necrosis, and in 22 lesions (43%) steatosis was present in the tumour.

In none of the tumours was a HCA component detected, and no transition zone from HCA into HCC was found in any of the patients.

Immunohistochemical staining and radiological diagnosis

In 9 patients with 9 nodules there was some doubt between the diagnosis HCA and well-differentiated HCC when analyzing the hematoxilin & eosin (HE) stained slides. In these cases an acinar growth pattern, a tumour capsule and a high mitotic activity were less often seen. In these patients, immunohistochemical stainings were used to differentiate between HCA and HCC. Table 1 summarises the results of the immunohistochemical staining tests of these 'doubtful' HCCs. The immunohistochemical staining profile of HCA is also presented in Table 1 [14]. After extended immunohistochemical staining there were no signs of a HCA component in the HCC lesion or a transition zone. Table 1 also presents the diagnosis made by the two radiologists after revising the pre-operative imaging. In these 9 patients there was no HCA component found on radiological examination.

Table 1: Histological immunostaining and radiological characteristics of patients with 'doubtful' HCC and the immunostaining profile of HCA lesions.[14]

Staining	Hepatocellular carcinoma (n=9)	Hepatocellular adenoma (n=57) [14]
Glutamine synthetase positive	4 (44)	5 (9)
β-catenin positive	2 (22)	7 (12)
CRP positive	8 (89)	36 (63)
SAA positive	3 (33)	36 (63)
L-FABP positive*	4 (44)	11 (19)
Radiologist 1	Certain HCC (n=9)	-
Radiologist 2	Certain HCC (n=8) Doubt HCC (n=1)**	-

Data are numbers (%)

* the tumour being negative compared to the non-tumoral liver tissue; ** another imaging modality was required for decision-making

DISCUSSION

This study explored whether a HCA component or a transition zone is present in HCC which develops in a non-cirrhotic liver. Although HCA is suggested to have a small risk of malignant transformation into HCC, so far a HCA component or a transition zone in HCC which would strengthen this hypothesis was not demonstrated. Therefore, we conclude that it is highly unlikely that HCC in a non-cirrhotic liver arises from HCA.

In a recent review by Stoot et al. malignant transformation was reported in 4.2% of the HCA cases and in 4.5% of the resected HCA cases [15]. Most case series reporting on malignant transformation of HCA have a limited sample size and report only on resected adenomas. This may have led to an overestimation of the true risk. Furthermore a selection bias of reported studies cannot be excluded. In the present study, because it is possible that HCA lesions were resected before transformation into HCC was initiated, we included patients at the end of the transformation process, i.e. lesions of highly differentiated HCC in non-cirrhotic livers [4]. If indeed HCA has the ability to transform into a malignant lesion a stepwise progression would be expected, comparable to the adenoma-carcinoma progression sequence in colorectal cancer [2,9,16]. Cases of HCA transformed into HCC are described with a pattern of a nodule within a nodule or two tumours lying next two each other without a transition zone [2,8,15-17]. However, a randomly scattered HCC throughout the HCA or absence of a thin fibrous capsule was not mentioned [18]. Without an explicit description of these features, we cannot assume that the two nodules are related to each other. We had the unique opportunity to study a large cohort of patients resected for HCC in a non-cirrhotic liver. In the 48 patients with 52 lesions, there were no signs of HCA or a transition zone from HCA into HCC. No premalignant lesions could be demonstrated. There are some cases in which it might be difficult to distinguish HCA from well-differentiated HCC. HCC or borderline tumours in which the differential diagnosis between adenoma and HCC is debatable, are hypothesized to have a higher risk of malignant transformation [18]. It is reported that β-catenin activated HCA is associated with an enhanced risk of malignant transformation and progression into HCC [19]. Several studies have identified mutations of β-catenin gene in HCAs and reported that activated β-catenin mutations deregulate the β-catenin pathway [15].

In the present study, in 9 of the 52 cases the differential diagnosis included HCA as well as a highly-differentiated HCC. The specimens of these patients were studied using additional immunohistochemical staining. Ruling out the potential diagnosis of HCA on the absence of validated markers for HCA is daring, as different types of adenoma are likely to vary in the potential rate of malignant progression. However, this staining did not provide any argument for a history of HCA and was not similar to the pattern found in HCA lesions (Table 1). A median of 10 (range 2-23) paraffin-fixed slides per tumour

seems representative. However, as this is a retrospective study, there is no guarantee that the entire tumour tissue is included in the paraffin-fixed slides which are evaluated by one observer. In all 9 lesions the definite diagnosis was established as HCC, and not HCA. In addition, reviewing the radiological images all lesions were diagnosed as HCC; only in one case did one of the radiologists request a second imaging modality. None of the lesions was diagnosed as a non-malignant lesion, which might have led to a different management.

In conclusion, this study explored the hypothesis that HCC in non-cirrhotic livers may result from malignant transformation of HCA. Using extensive immunohistochemical staining we found no HCA components or transition zones to confirm this hypothesis. Therefore, we conclude that it is highly unlikely that a HCA in a non-cirrhotic liver will degenerate into a malignancy, and that data that aim to legitimise resection are biased and may lead to overtreatment.

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PART IV

OPTIMIZATION OF STAGING

Chapter 6

Histological differentiation grade and microvascular invasion of hepatocellular carcinoma predicted by dynamic contrast-enhanced MRI

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ABSTACT

Purpose: To explore the potential use of magnetic resonance imaging (MRI) in predicting the outcome for patients with hepatocellular carcinoma (HCC), imaging characteristics were correlated with pathological findings and clinical outcome.

Materials and Methods: With permission from the Ethical Board, clinical data and tissues of resected HCC patients were collected, including the preoperative MRI. The role of MRI characteristics on recurrence and survival were evaluated with univariate and multivariate analyses.

Results: Between January 2000 and December 2008, 87 patients with 104 HCCs were operated on. Microvascular invasion was present in 55 lesions (53%). HCC was characterized as well differentiated in 15 lesions (14%), as moderate in 50 lesions (48%), and as poorly differentiated in 34 lesions (33%). Due to preoperative treatment in five lesions (5%) no vital tumor was left. In 85 lesions (88%) washout of contrast was noted. Of the 87 patients, 28 (32%) with 37 lesions developed HCC recurrence; these patients had microvascular invasion significantly more often and a moderate or poorly differentiated tumor ($P < 0.001$ and $P = 0.025$, respectively). MRI more often showed washout when HCC was moderately or poorly differentiated ($P < 0.001$) or microvascular invasion was present ($P = 0.032$).

Conclusion: Differentiation grade and microvascular invasion are significantly associated with the presence of washout demonstrated on dynamic contrast-enhanced MRI.

INTRODUCTION

Hepatocellular carcinoma (HCC) is the fifth most common cancer in the world and the third most common cause of cancer-related mortality (1). The tests used to diagnose HCC include different imaging modalities, biopsy, and alpha-fetoprotein (AFP) levels (2). Dynamic contrast-enhanced magnetic resonance imaging (MRI) has a higher sensitivity in detecting HCC (81%) compared with computed tomography (CT) (68%) and is widely used as preoperative imaging (3). Typical imaging characteristics of HCC on MRI are a large (>2 cm) hyperintense lesion on T2-weighted images, arterial enhancement and washout in the venous phase, and a pattern of "rapid rising and rapid descending" (4,5). An enhancing capsule is also often noticed.

Various risk factors for fatal recurrence after orthotopic liver transplantation (OLT) or resection for HCC have been well documented. The most important ones are microvascular invasion, differentiation grade, tumor size, and multifocal tumors (6–14). Identification of microvascular invasion and differentiation grade by histological examination of resected specimens is important because long-term survival is strongly correlated with these findings (9).

If microvascular invasion and differentiation grade can be evaluated by imaging and without biopsy, long-term survival might be predicted more accurately preoperatively and the treatment strategy for some patients might be adapted.

To explore the potential use of MRI in predicting the outcome for patients with HCC, in this study we correlated imaging characteristics with pathological findings and clinical outcome.

MATERIALS AND METHODS

The local Institutional Ethical Review Board granted permission for analysis of the preoperative MRI and all the histological specimens. All patients with HCC operated on in our center from January 2000 to December 2008 with at least one preoperative MRI were included in this study. The most recent MRI prior to the operation or preoperative downstaging treatment was analyzed. The preoperative diagnosis of HCC was based on imaging and clinical and laboratory findings. Diagnosis was histologically confirmed after surgery. After diagnostic MRI and before surgery, tumor progression was followed by ultrasound or contrastenhanced CT every 3 months (2). During preoperative follow-up no changes in tumor size were noted. Treatment with curative intent was defined as a complete surgical resection, including wedge resection, segment resection, hemihepatectomy, and OLT.

Not included in the study were patients who received lobectomy for palliative treatment (n = 1) and patients whose preoperative radiologic evaluation did not include an MRI (n = 49).

The medical charts of the included patients were analyzed. Data on age, gender, etiology, biochemical parameters, and serum tumor markers were reported. All patients were classified according to the Child-Pugh and BCLC scoring system. Slices of the resected specimens from these patients were blindly scored by one pathologist. The MRI scans were blindly scored by two radiologists (6 years abdominal interest, and 1 year abdominal fellow) with special interest in liver imaging. In case of different findings, consensus was defined afterwards. After treatment, patients had a follow-up using contrast-enhanced CT or MRI combined with determination of serum AFP at 3 and 6 month intervals for up to 2 years posttreatment. After 2 years, follow-up was continued annually up to 5 years. In the presence of liver cirrhosis, lifetime follow-up was pursued.

HCC recurrence was defined as the appearance or reappearance of a lesion with vascular enhancement in the treated area or elsewhere on imaging, ie, local recurrence, a new lesion in the liver, enlargement of lymph nodes in the liver hilum, or suspected extrahepatic lesions and an increase of serum AFP.

MRI

All patients had a protocolized contrast-enhanced MR scan using a 1.5T unit (Philips Medical Systems, Best, the Netherlands; or General Electric, Signa, Milwaukee, WI). A four-channel phased-array body coil was used. The scan sequence started with a single-shot fast spin echo (SSFSE) (repetition time [TR] msec, echo time [TE] msec 832/80–120; flip angle 90^0) with varying TE values (short TE = 80 and long TE = 120) fat-suppressed T2-weighted FSE (TR/TE 3000/80, flip angle 90^0), and T1-weighted in-phase and opposed-phase spoiled gradient recalled (SPGR) sequences (shortest/TE 4.6 msec and 2.3 msec, respectively; flip angle 90^0). After administration of an intravenous gadolinium-chelate (28 ml [double dose]; Magnevist [gadopentetate dimeglumine], Schering, Berlin, Germany), at 3 ml/s using a power injector, dynamic imaging with 3D T1-weighted fat-suppressed SPGR (General Electric) or 2D T1-weighted SPGR (Philips) images were performed in at least three phases (arterial, portal, and delayed). Imaging parameters: TR 5.8 msec, TE 1.94 msec, flip angle 15.0^0, NSA 0.5, matrix 256 x 192, field of view (FOV) 38.0 cm, slice thickness 4.0 mm, acquisition time each phase 20–23 seconds, scanned in breath-hold. Arterial phase was timed using a test bolus technique. The portal and delayed phases were performed at 45 and at 120 seconds, respectively, after the acquisition of the arterial phase. In addition, a delayed-phase 3D T1-weighted SPGR sequence was performed at least 4 minutes after contrast injection. Slice thickness of 4–7 mm was used in all sequences.

No adverse reactions were reported during the entire study. A total of 13 patients were scanned elsewhere using an identical protocol, and images were reviewed at our institution by the same radiologists.

MRI Analysis

All preoperative MRI examinations were transferred to a picture archiving and communication system (PACS) and workstation (AquariusNET; Terarecon, San Mateo, CA). Examinations with major respiratory artifacts were excluded (n = 8). The liver was evaluated according to the presence and type of cirrhosis, and presence of portal hypertension and ascites. All visible lesions were categorized as benign, malignant, or possibly malignant. Benign lesions were not further analyzed. Characteristics of the malignant lesions were assessed. Tumor size (longest diameter), number, shape, capsule, and margin were evaluated in the arterial, portal, and venous phase. The shape of the tumor was scored as round, lobular, or irregular. Washout was reported as present when the tumor had the typical features (eg, arterial enhancement wash-in and portal venous or delayed loss of enhancement) compared to the surrounding liver parenchyma washout (5).

Intensities were compared with the liver parenchyma and defined as hypointense, isointense, or hyperintense. The presence of fat was noted at the T1 opposed-phase images.

The dynamic MRI data were analyzed on a Workstation (AquariusNET). Tumor signal intensity of the individual lesions were measured using a region of interest (ROI) manually placed by the same investigator. The size of the ROI was as large as possible, thereby avoiding necrotic areas. A circle of at least 2 cm^2 was drawn in the tumor. The ROI was manually placed in the lesion at the position of interest. This position was automatically copied to all sequences. After copying, the position of the ROI was checked and in case of a wrong position the position of the ROI was corrected manually.

The signal intensity values derived from the ROI were plotted against time in a tumor-intensity curve (TIC). Per individual lesion, 4–12 images were used to create the TIC. The increase in signal intensity during contrast enhancement was assessed by percentage. No clinical, laboratory, or pathological information other than the presence of HCC was provided during image analysis.

Patients with a recent MRI, ie, a maximum interval of 6 months (26 weeks) between the MRI and treatment, were included in the analysis in which radiological findings were compared with histological characteristics.

Surgical Pathology Parameters

All slices of the resected specimens from patients were analyzed by a pathologist with expertise in liver pathology. Features including the presence or absence of fibrosis or cirrhosis and dysplasia in the surrounding liver tissue, type of tumor, differentiation grade, microvascular invasion, and the surgical resection margin were recorded. The outcome of the histological examination was defined as the gold standard. The histological grade was determined according to the Lauwers classification (11), and was based on the areas of tumor showing the highest grade. Microvascular invasion was defined as microscopic tumor invasion identified in the hepatic portal vein which was contiguous to the tumor (8).

Statistical Analysis

The Kaplan–Meier method was used to calculate recurrence rate; the difference was tested using the logrank test. Cox regression univariate analysis was used to assess the importance of prognostic factors (age, gender, etiology, AFP, tumor size, presence of cirrhosis, presence of dysplasia, tumor differentiation grade, and presence of microvascular invasion) on recurrence. Multivariate Cox regression analysis was performed to assess the independent prognostic factors. Strong colinearity between differentiation grade and microvascular invasion made interpretation of the results of one multivariate Cox regression analysis impossible. Therefore, two models are presented: Model 1: differentiated grade and Model 2: presence of microvascular invasion. Both models were adjusting for age, gender, level of AFP, and tumor size.

Differences in radiological variables between histological differentiation grade and histological microvascular invasion were compared using the Mann–Whitney U-test, t-test, or the x2-test whenever appropriate. Statistical significance was considered at $P < 0.05$.

The tumor intensity was visualized in a TIC. The shape of the patient specific intensity curve was fitted with a nonlinear regression model: a first-order opencompartment model [15]. Differences between the TIC were assessed comparing the patient-specific estimated parameters of the nonlinear regression model.

RESULTS

Clinical Characteristics

From January 2000 through December 2008, 597 patients visited our hospital for treatment of HCC. Of these, 100 patients were able to receive surgical curative treatment having received at least one preoperative MRI. No vital tumor was left in five patients (tumor differentiation could not be assessed on histological analysis because of exten-

sive tumor necrosis), and in eight patients MRI was not included for analysis because of artifacts; these 13 patients were excluded from the analysis.

A total of 87 patients were included with a total of 104 HCCs. Their median number of tumors was 1 (range 1–6). The median time between the preoperative MRI and the operation was 10 (range 1–126) weeks. Of the 87 patients with HCC, 62 (71%) received resection and 25 (29%) underwent OLT. Of the 62 patients treated with resection, 6 (10%) had prior treatment with radiofrequency ablation (RFA) or transarterial(chemo) embolization (TACE). Of the 25 patients receiving OLT, 13 (52%) underwent RFA or TACE as bridging to transplantation.

Radiological Characteristics

Of the 104 HCCs, 97 (93%) were depicted on MRI. Seven HCCs were too small to detect with MRI. They were found in explanted livers and were detected by histological examination only; these seven HCCs were recorded as missing data. Morphology of the HCCs was predominantly round-shaped in 74 lesions (76%), versus lobular in 20 lesions (21%); three lesions (3%) had an irregular contour. In 85 lesions (88%) there was washout of contrast present during the portal or late venous phase. A total of 82 lesions (85%) showed capsular enhancement.

Pathological Characteristics

In 101 lesions (94%) the diagnosis of HCC was confirmed. In three lesions (3%) focal areas of cholangiocellular differentiation in HCC were noted. In three tumors (3%) the surgical resection margin was not free of tumor. Differentiation according to the Lauwers classification resulted in 15 well-differentiated lesions (14%), 50 moderately differentiated (48%), and 34 poorly differentiated (33%) tumors. Five nodules (5%) did not display enough vital tumor due to preoperative treatment with RFA or TACE. A total of 55 lesions (53%) had microvascular invasion.

Recurrence

During follow-up 28 patients had recurrence of HCC after a median period of 10 (range 1–24) months. Patients with recurrence of HCC were characterized by higher preoperative AFP serum levels and larger tumor size. In addition, they more often had a moderate or poorly differentiated tumor and presence of histological microvascular invasion (Table 1).

Strong colinearity between differentiation grade and microvascular invasion made interpretation of the results of one multivariate Cox regression analysis impossible. Therefore, two models are presented. Model 1: adjusting for age, gender, level of AFP, and tumor size, a moderately and/or poorly differentiation grade was shown to be significantly associated with recurrence of HCC (Fig. 1A). The recurrence rate of well,

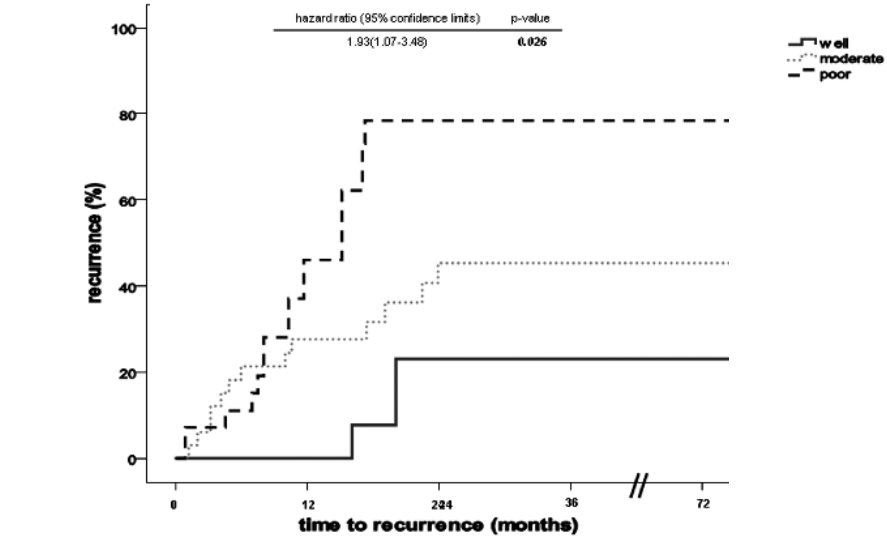
moderate, and poorly differentiated HCC was 15%, 40%, and 61%, respectively, 2 years after treatment with curative intent. Model 2: adjusting for age, gender, level of AFP, and tumor size, the presence of microvascular invasion was shown to be significantly associated with recurrence of HCC (Fig. 1B). The recurrence rate of HCC without, suspected, and with microvascular invasion was 4%, 66%, and 69%, respectively, 2 years after treatment with curative intent.

Table 1: Summary of patient characteristics of the hepatocellular carcinoma patients with and without recurrence after treatment with curative intent.

patient characteristic	**no recurrence (n=59)***	**recurrence (n=28)***	**p-value****
age (years)	57 (21-82)	55 (1-78)	0.766
gender (♂)	48 (81)	19 (68)	0.199
etiology			0.363
hepatitis B viral infection	9 (15)	4 (14)	
hepatitis C viral infection	17 (29)	3 (11)	
alcohol	12 (20)	9 (32)	
none	21 (36)	12 (43)	
AFP (ng/ml)	8 (1-17700)	34 (2-121000)	<0.001
histological characteristic			
tumor size (mm)	43 (3-170)	90 (20-190)	0.003
presence of cirrhosis	36 (61)	12 (43)	0.140
radical resection (R0)	56 (97)	27 (96)	0.848
dysplasia			0.549
none	33 (57)	16 (57)	
small and/or large cell	24 (41)	12 (43)	
unclassified	1 (2)	-	
differentation			0.025
well	11 (19)	2 (7)	
moderately	29 (50)	14 (50)	
poorly	13 (22)	12 (43)	
unclassified	5 (9)	-	
presence of microvascular invasion	5 (10)	6 (21)	<0.001

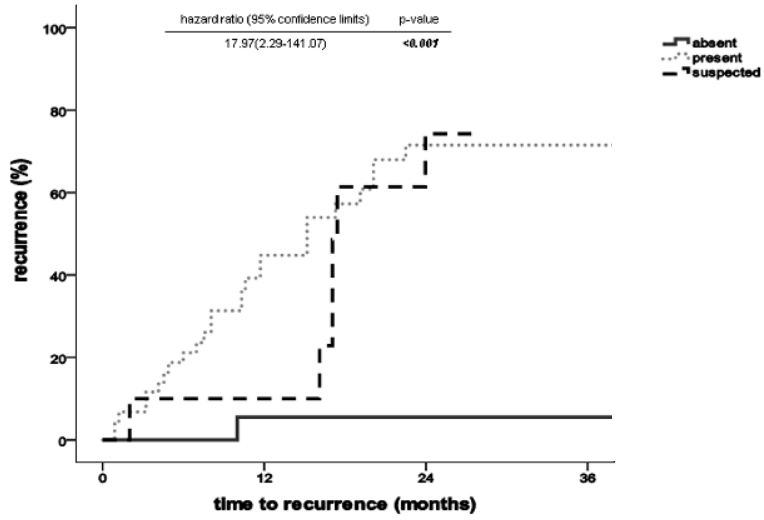
* numbers (%) or median (range)

** score test univariate Cox regression



differentiation grade		number at risk			
well	13	13	11	10	5
moderate	44	27	17	11	5
poor	25	12	6	4	3

Figure 1A: Recurrence of HCC after treatment with curative intent comparing patients with a well, moderate and poorly-differentiated HCC



microvascular invasion		number at risk		
absent	26	23	21	15
present	41	18	7	7
suspected	11	8	3	1

Figure 1B: Recurrence of HCC after treatment with curative intent comparing patients in the absence of microvascular invasion, suspected for presence of microvascular invasion and in the presence of microvascular invasion

Survival

The 1- and 3-year survival rate of patients without, suspected, or with microvascular invasion was 92%, 88%, and 82%, respectively, and 57%, 68%, and 40%, respectively ($P < 0.001$).

Patients with well-differentiated HCCs had a better overall survival compared with those with moderate and poorly differentiated HCCs. The 1- and 3-year survival rates for patients with well-differentiated HCC was 100% and 92%, respectively, 72% and 52% for patients with moderately differentiated HCC, and 76% and 56% for patients with poorly differentiated HCC ($P = 0.021$).

MRI Characteristics for Pathological Differentiation and Vascular Invasion

Radiological findings were compared with histological characteristics. Only patients with a recent MRI ($n = 76$) were included in this analysis. Histological characteristics associated with recurrence of HCC, HCC differentiation grade, and microvascular invasion were analyzed to determine which radiological characteristics correspond to these pathological characteristics, which were considered the gold standard. Patients with a well-differentiated HCC had a radiological capsular enhancement significantly less often and less often washout compared with patients with a moderate or poorly differentiated HCC ($P = 0.003$ and $P < 0.001$) (Table 2).

Patients with HCC without microvascular invasion on microscopic evaluation had a radiological capsular enhancement significantly less often and less often washout compared with patients with microvascular invasion suspected or present ($P = 0.040$ and $P = 0.032$) (Table 3).

Tumor - Intensity Curve

The increase in tumor signal intensity during the contrast enhancement sequences was assessed in a curve. The shape of the patient-specific tumor–intensity curve was fitted with a nonlinear regression model (15). Comparing the shape of the TIC of HCCs with microvascular invasion with the shape of the TIC of HCCs without microvascular invasion, by comparing the patient-specific estimated parameters of the nonlinear regression model, showed no significant difference ($P = 0.405$). The TIC shape did not differ between the different histological grades ($P = 0.120$). The shape of the TIC of the HCC with and without recurrence showed no significant difference ($P = 0.880$).

DISCUSSION

In this study we attempted to correlate morphologic presence of microvascular invasion and differentiation grade with enhancement features of HCC on MRI. Even though treat-

Table 2: Summary of the radiological characteristics of the well, moderately and poorly-differentiated hepatocellular carcinoma (HCC).

	HCC differentiation grade			
radiological characteristic	**well (n=13)***	**moderate (n=34)***	**poor (n=29)***	**p-value**
morphology				0.138
round	12 (91)	22 (65)	21 (72)	
irregular	1 (9)	-	2 (7)	
lobular	-	12 (35)	6 (21)	
presence of fat	2 (15)	15 (44)	9 (31)	0.211
presence of a capsule	6 (46)	32 (94)	25 (86)	0.003
T1				
low signal	7 (54)	27 (79)	24 (83)	0.179
inhomogeneous aspect	6 (46)	23 (68)	21 (72)	0.543
T2				
high signal	11 (85)	32 (94)	27 (93)	0.565
inhomogeneous aspect	6 (46)	25 (74)	20 (69)	0.410
dynamic phase				
presence of wash-out	5 (38)	30 (88)	27 (93)	<0.001

* numbers (%)

Table 3: Summary of the radiological characteristics of the hepatocellular carcinoma (HCC) with and without microvascular invasion.

	histological microvascular invasive HCC			
radiological characteristic	**present (n=45)**	**suspected (n=12)**	**absent (n=19)**	**p-value**
morphology				0.903
round	31 (69)	9 (75)	15 (79)	
irregular	2 (4)	-	1 (5)	
lobular	12 (27)	3 (25)	3 (16)	
presence of fat	12 (27)	4 (33)	9 (47)	0.167
presence of a capsule	40 (89)	11 (92)	11 (58)	0.040
T1				
low signal	39 (87)	7 (58)	12 (63)	0.246
inhomogeneous aspect	28 (62)	10 (83)	11 (58)	0.210
T2				
high signal	42 (93)	10 (83)	17 (89)	0.491
inhomogeneous aspect	29 (64)	10 (83)	11 (58)	0.243
dynamic phase				
presence of wash-out	40 (89)	10 (83)	11 (58)	0.032

* numbers (%)

ment options with curative intent are emerging and are currently providing a better perspective, HCC continues to have a dismal prognosis based on recurrence of disease after curative treatment. Histological presence of microvascular invasion and a moderate or poor differentiation grade are accepted as independent predictors of poor survival (7,8,11,12,14). In the present study, after adjusting for age, gender, level of AFP, and tumor size, the histological differentiation grade and presence of microvascular invasion were significantly associated with recurrence of HCC (Fig. 1a,b).

Based on our observations, the presence of washout is important because it might be an indication of microvascular invasion and/or a moderate or poorly differentiated tumor. Okamoto et al (16) also found a relationship between washout and histological grade. Unfortunately, they did not analyze microvascular invasion, which is strongly correlated with HCC longterm survival. This is in contrast to a recent study showing tumor multifocality on imaging to be the only variable that was significantly correlated with the presence of microvascular invasion (17); these authors found that no other imaging features were useful. However, they only selected patients with small tumors, able to receive liver transplantation.

Our findings might have important clinical implications for the imaging work-up of these patients, as recurrence of HCC after treatment is common. Two years after treatment with curative intent, 69% of our patients with histological microvascular invasion had recurrence of HCC (Fig. 1a); for patients with a poorly differentiated HCC this figure was 61% (Fig. 1b).

Differences in enhancement pattern between benign and malignant lesions in the liver have been reported (5). The enhancement characteristics of the tumor can be visualized by a TIC (18–21). However, the shape of the TIC does not seem to differentiate between recurrence and no recurrence, the various histological differentiation grades, or the presence or absence of microvascular invasion.

Nevertheless, our data show that the presence of washout is an important indicator of microvascular invasion and moderate or poorly differentiated HCC. Moreover, HCC can be visualized with a TIC. Recently, Kitao et al (22) found organic transporting polypeptide 8 (OATP8) to be helpful in HCC diagnosis, but not in predicting HCC outcome. Because current dynamic imaging is not yet able to predict the outcome for patients with HCC, we have to depend on histology. Obtaining tissue for histological analysis may not be ideal, even when discounting the risk of bleeding and needle track seeding when taking a fine-needle liver biopsy (2). It has to be stressed that biopsies of small lesions (<2 cm) may not be reliable (2). Needle placement can be difficult, and on a small biopsy, it may be difficult to distinguish well-differentiated HCC from dysplasia, although new immunohistochemical markers may be useful for this (2,23). A positive biopsy is helpful, but a negative biopsy can never be accepted as conclusive.

There are a number of limitations. First, in this retrospective study we included patients with and without cirrhosis, with only one HCC lesion in many patients. In case of absence of cirrhosis tumors were often large. Based on imaging findings, eg, tumor size, multifocality, and relation to main vessels, patients are scheduled for surgery. There might be a possibility that the parameters analyzed in this study are partly influenced by the imaging findings on which treatment management was planned. Second, because we had a limited number of patients for each differentiation grade the ability to show differences in tumor intensity for several small groups is low.

Future studies should focus on the feasibility of predicting microvascular invasion and the various differentiation grades by improving imaging techniques, eg, diffusion-weighted imaging and hepatobiliary contrast agents to distinguish between the various differentiation grades and microvascular invasion of HCC, while focusing on the presence of washout and capsular enhancement.

In conclusion, moderate to poorly differentiated HCC and microvascular invasion are significantly associated with the presence of a capsule and washout demonstrated on dynamic contrast-enhanced MRI. It is possible to visualize HCC by a TIC. However, at present, the shape of the TIC cannot be used to predict prognosis or to differentiate between the various grades of malignancy, as demonstrated by histological examination.

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Chapter 7

Is bone scintigraphy indicated in surgical work-up for HCC patients?

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ABSTRACT

Background: Patients with hepatocellular carcinoma (HCC) undergo extensive staging investigations when being assessed for surgical resection. The aim of this study was to assess the use and yield of baseline bone scintigraphy in patients with HCC necessitating high-risk surgical resection.

Material and Methods: All patients diagnosed with HCC between 2000 and 2010 within a tertiary referral centre were reviewed. Recurrence and survival rates were compared between patients with and without bone scintigraphy in their preoperative work-up.

Results: A total of 366 patients were diagnosed with resectable HCC. In the work-up for resection 137 (41%) HCC patients underwent bone scintigraphy, showing bone metastases in 3 (2%). There was no significant difference in long-term survival between patients with and without bone scintigraphy. None of the patients with a positive bone scintigraphy died due to skeletal bone metastases. Only one patient had an indication for bone scintigraphy based on clinical suspicion. Two patients were found to have asymptomatic skeletal metastases prior to surgery. Symptomatic skeletal metastases were identified at an estimated cost of €27,008 per case.

Conclusion: Clinically unsuspicious bone lesions turned out to be metastases in two patients with an estimated cost of €27,008 per case. Recurrence rate, disease-free and overall survival showed no significant difference between patients with and without preoperative baseline bone scintigraphy. There is no justification for routine preoperative bone scintigraphy to detect asymptomatic skeletal metastases in patients with resectable HCC.

INTRODUCTION

Hepatocellular carcinoma (HCC) is the fifth most common cancer in the world and the third most common cause of cancer-related mortality [1].

For patients with solitary HCC in combination with the setting of decompensated cirrhosis and for those with early multifocal disease (up to 3 lesions, none larger than 3 cm), a liver transplantation offers the best change for long-term survival [1,2]. In the absence of cirrhosis or with compensated Child A cirrhosis, surgical resection has been reported as a successful alternative [3]. Liver transplantation and surgical resection may both be considered as treatments with curative intent [4]. However, in successfully resected patients, underlying liver cirrhosis may cause high morbidity and even result in mortality due to liver failure. Therefore, in patients with HCC accurate staging is important to define the risks and benefits of resection [4].

In the absence of consensus as to which patients require preoperative staging by bone scintigraphy and computed tomography (CT) of the chest clinicians continue to routinely perform these investigations in all patients, especially in those at risk for postoperative complications. The rising costs of healthcare and (in particular) of cancer care, challenge the appropriateness of current practices and emphasise the need for more cost-effectiveness. The positive yield of a routine bone scintigraphy in liver transplant candidates with early-stage hepatoma was reported to be very low [5]. Based on these results a bone scintigraphy is no longer considered as a prerequisite for liver transplant listing in patients with HCC according to the UNOS guidelines. Only symptomatic patients listed for liver transplantation, clinically suspected of having bone metastases should undergo bone scintigraphy.

To assess the use of baseline bone scintigraphy and its benefit for potentially curable patients, a review of our clinical practice was performed. The influence on disease management, and cost of bone scintigraphy for patients with resectable HCC was evaluated.

METHODS

Clinical characteristics

All patients with HCC diagnosed in our centre from January 2000 to December 2010 were analyzed (n=799). The diagnosis of HCC was based on imaging, clinical and laboratory findings including serum alpha-fetoprotein (AFP). The diagnosis was histologically confirmed if necessary. Treatment with curative intent was defined as a complete surgical resection, also including orthotopic liver transplantation (OLT) and radiofrequency ablation (RFA). HCC treatment was performed according to the Milan criteria [2].

The medical charts of the included patients (n=336) were analyzed. Data on age, gender, etiology, biochemical parameters and serum tumor marker (AFP) were collected. High-risk surgery was defined as a large surgical resection up to an extended hemihepatectomy in a non-cirrhotic liver or surgery close to the hilar main portal branches or hepatic vein trunks. Based on clinical judgment these patients presented with a higher morbidity and/ or mortality risk and therefore, in the work-up for resection, 137 HCC patients (41%) were identified as high-risk surgical procedures and underwent bone scintigraphy. Patients who underwent RFA underwent a bone scintigraphy prior to referral to our tertiary center. During follow-up patients underwent contrast-enhanced CT or magnetic resonance imaging (MRI) combined with determination of serum AFP at 3 and 6-month intervals for up to 2 years post-treatment. A bone scintigraphy was performed during follow-up on indication, *i.e.* in the presence of possible metastases related complaints; bone pain, motor or sensory nerve disturbance, weight loss, and jaundice. After 2 years, follow-up was continued annually up to 5 years. In the presence of liver cirrhosis, lifetime follow-up was pursued. Recurrence or disease was defined as the occurrence of a focal liver lesion with HCC characteristics, enlargement of lymph nodes in the liver hilum, or suspicious extrahepatic lesions and an increase of serum AFP.

Bone scintigraphy

The patients had a bone scintigraphy at baseline, pre-treatment for metastases screening. Total-body images were acquired 2-4 h after injection of 550 MBq or 825 MBq (if the bodyweight exceeded 100kg) ^{99m}Tc-hydroxymethylene diphosponate (Mallinckrodt Medical, Petten, the Netherlands). Bone scintigrams were acquired using a double-head gamma camera (DST SMV; DST Sopha Medical Vision, Brie, France) in the whole-body mode, in 20 minutes. Bone lesions, which showed as hotspots on scintigraphy, were further analyzed by conventional plain radiography, CT or MRI.

Statistical analysis

Variables were compared using the Mann-Whitney U test, t-test or with the χ^2-test whenever appropriate. Statistical significance was set at $p < 0.05$. Survival curves were drawn using the Kaplan-Meier method, the log-rank test was used for determining statistical significance. All analyses were performed using the Statistical Package for Social Sciences 17.0 (SPSS inc., Chicago, IL, USA).

RESULTS

Clinical characteristics

From January 2000 through December 2010, 336 patients were treated for resectable HCC at our tertiary referral center. Of these patients 98 (29%) were women and 238 (71%) were men, with a median age of 60 years (range 1-82).

Bone scintigraphy before curative treatment

At diagnosis 137/336 (41%) patients who were planned to undergo high-risk surgery (a large surgical resection up to extended hemihepatectomy in a non-cirrhotic liver or surgery close to the hilar main portal branches or hepatic vein trunks) underwent bone scintigraphy, showing skeletal metastases in 3 patients (2%) (Figure 1). Two out of these three patients were asymptomatic. One out of these three patients underwent diagnostic work-up for metastatic disease because of suspected positive lymph nodes on the abdominal CT scan. These 3 patients did not require palliative treatment for their skeletal metastases. All died due to tumor progression with liver failure.

In the entire cohort, 2 patients out of 137 patients underwent a bone scintigraphy because of high-risk surgery had an incidental finding of metastatic bone disease. Excluding 1 asymptomatic patient in whom a bone scintigraphy was clinically indicated, 136 bone scintigraphies were routinely performed. The total cost of performing these bone scintigraphies was estimated at €54,015. This translates to an estimated cost of €27,008 per case of unsuspected skeletal metastases detected.

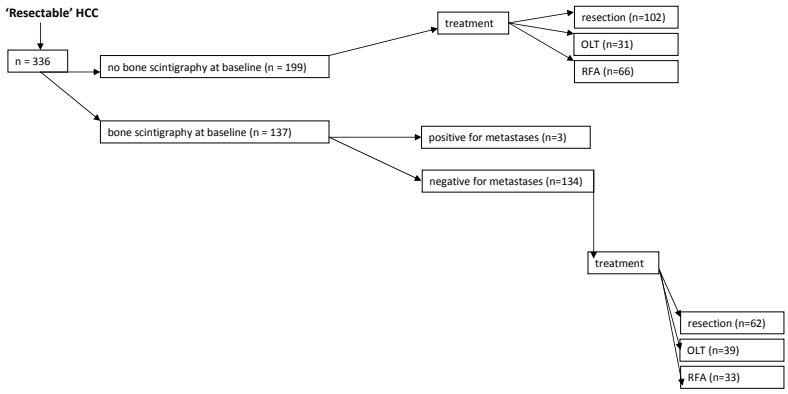


Figure 1: Flowchart for bone scintigraphy in patients with resectable hepatocellular carcinoma and applied treatment.

HCC = hepatocellular carcinoma, OLT = orthotopic liver transplantation, RFA = radiofrequency ablation

Treatment

A total of 333 HCC patients received treatment with curative intent: resection (n=164), OLT (n=70), and RFA (n=99). The baseline clinical characteristics of these patients are summarized in Table 1. Of all 333 patients receiving treatment with curative intent, 134 (40%) had a negative bone scintigraphy, of whom 62 underwent a resection, 39 OLT, and 33 RFA (Figure 1).

Table 1: Summary of the baseline characteristics of the hepatocellular carcinoma patients treated with curative intent.

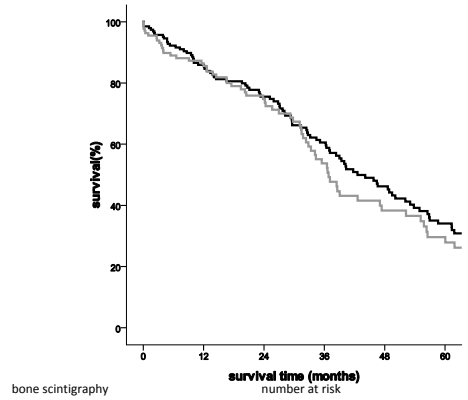
Patient characteristics	Study population n=333*	Bone scintigraphy present n=134*	Bone scintigraphy absent n=199*	Presence vs. absence bone scintigraphy p-value
Age (years)	60 (1-82)	58 (1-78)	60 (10-82)	0.007
Gender (♂)	236 (71%)	96 (72%)	140 (70%)	0.947
Aetiology				
HBV	82 (25%)	40 (30%)	42 (21%)	0.061
HCV	75 (22%)	39 (29%)	46 (23%)	0.336
Alcohol	49 (15%)	36 (27%)	43 (22%)	0.092
None	128 (38%)	39 (29%)	89 (45%)	0.003
AFP (ng/ml)	15 (1-834350)	24 (2-424100)	9 (1-834350)	0.017
Presence of cirrhosis	225 (68%)	99 (74%)	126 (63%)	0.087
Tumour size (mm)	33 (3-193)	33 (10-193)	33 (3-170)	0.278

*number (%) or median(range)
HBV = hepatitis B viral infection; HCV = hepatitis C viral infection; AFP = alfa-fetoprotein

Survival and recurrence

The 1- and 5-year survival rates of patients with a bone scintigraphy were 86% and 30% respectively, and without a bone scintigraphy 86% and 34%, respectively (p= 0.369) (Figure 2). Median overall survival of patients with a bone scintigraphy and without a bone scintigraphy was 37 months and 43 months, respectively.

Out of 110 patients with recurrent of HCC 46 patients (42%) had a bone scintigraphy. Median time to recurrence was 9 months (range 2-102 months). The time to recurrence did not differ between patients with and without bone scintigraphy (p= 0.990) (Figure 3). At 3 years after treatment with curative intent, the recurrence rate of patients with a bone scintigraphy and without a bone scintigraphy was 43% and 40%, respectively. Table 2 summarizes the pattern of disease recurrence (local or distant). Patients with bone metastases had metastases related complains like bone pain, motor or sensory nerve disturbance, weight loss, and jaundice. Of the 14 patients with bone metastases, all died due to tumour progression with liver failure, none of these patient had spinal paralysis induced by bone metastases. Out of these 14 patients, 8 patients (57%) received palliative radiotherapy for their bone metastases.

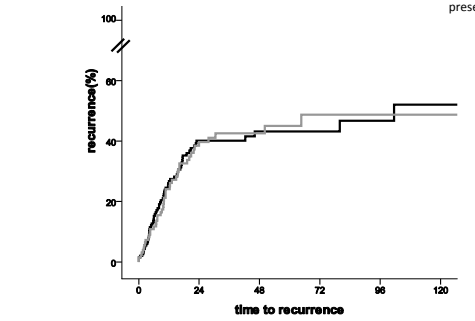


bone scintigraphy	survival (%)			
	1-year	3-year	5-year	
absent	86	61	34	p=0.369
present	86	54	30	

bone scintigraphy	number at risk					
absent	199	130	101	71	47	33
present	134	96	65	37	24	17

Figure 2: Survival of hepatocellular carcinoma after treatment with curative intent comparing patients with and without bone scintigraphy

bone scintigraphy	recurrence rate (%)			
	1-year	3-year	5-year	
absent	26	40	43	p=0.990
present	24	43	45	



bone scintigraphy	number at risk					
absent	199	70	34	18	10	3
present	134	49	23	13	5	3

Figure 3: Recurrence of hepatocellular carcinoma after treatment with curative intent comparing patients with and without bone scintigraphy

Table 2: Localization of hepatocellular carcinoma recurrence after treatment with curative intent

Localization	No. of patients
Local recurrences	30
New lesion in the liver	48
Lung	9
Abdomen	7
Bones	14
Lymph nodes	2

DISCUSSION

In this study the value of bone scintigraphy for asymptomatic HCC patients selected for surgical resection was investigated. The effectiveness of bone scintigraphy was found to be very low, confirming data reported in literature on liver transplant candidates [5].

The skeletal system is a common metastatic site in breast cancer, prostate cancer and lung cancer. Bone scintigraphy is advocated for the detection of metastases as it is considered an optimal examination for preoperative staging [6,7]. Although the incidence of HCC bone metastases has increased, the skeletal system remains an uncommon metastatic sites for HCC [4,8-11]. Symptoms attributable to the corresponding lesions are present in 77% of the HCC patients with bone metastases, manifesting as pain, motor or sensory nerve disturbance, or local bone swelling [12].

Bone scintigraphy may be of clinical importance, as the presence of metastases might change the prognosis or treatment. Patient characteristics at baseline differ regarding age, aetiology and AFP. Young patients are more suitable for high risk surgery, there patients receiving a bone scintigraphy were younger. Due to an aetiology of cirrhosis or chronic hepatitis liver capacity decreases and surgical resection becomes riskier and therefore the screened patients had less often absence of an aetiology [3]. Previous reports displayed that serum AFP level might be higher in patients with cirrhosis compare to patients without cirrhosis, however the presence or absence of cirrhosis did not influenced [10]. In our population baseline characteristics between patients with and without bone scintigraphies did not differ regarding survival. The conclusion that there was no difference in overall survival between the screened group and non-screened group might be biased as the screened patients who were positive for bone metastases were not operated. However, it does speak of lack of worse outcome in the non-screened group who could have had undetected metastases. Moreover, median survival of HCC patients with untreated bone metastases seems similar to survival of HCC patients with treated bone metastases [7,13,14]. The cause of death in HCC patients with bone metastases is seldom related to these metastases [7].

The current staging classification assumes that preoperative evaluation of HCC patients will identify a substantial number with subclinical extra-abdominal disease, thereby avoiding unnecessary treatment of these patients. The data from the current study do not support this assumption. Bone scintigraphy has a well-established role in identifying skeletal metastases. The role of bone scintigraphy in staging patients with HCC able to undergo surgical resection, OLT or RFA is less clear. Of all newly diagnosed patients with 'resectable' HCC, 41% underwent a staging bone scintigraphy. This low rate is the result of a biased indication for bone scintigraphy in our center, as a strict staging protocol was not defined until recently. Most of the patients undergoing a bone scintigraphy had to undergo a high-risk operation, e.g. resection of a large volume of the

cirrhotic liver. It is difficult to determine the justifiable cost to avoid unnecessary treatment using these data. This study shows that costs of €53,221 were generated for bone scintigraphies (n=134) without detection of even 1 patient with subclinical metastases. Therefore, the policy of bone scintigraphy in patients with HCC undergoing treatment with curative intent is not effective. This is in line with the National Comprehensive Cancer Network guidelines who recommend bone scanning on indication and not routinely-only [14]. Because the economic cost of staging investigations in the Dutch healthcare system is difficult to quantify, we approximated the cost of staging scans in our own hospital. We acknowledge the limitation of such an estimation especially because this is a single centre based study without a cost-effective analysis. However, we believe this is reasonable as the cost of a bone scintigraphy in our study (€397.17) compares favourably with that in an American study ($711) [5,16]. Therefore the economic cost of detecting each one of these cases of incidental skeletal metastases is approximately €27,008. This figure would be much higher if the cost of performing a bone scintigraphy was taken from the American model.

The present study has some limitations, related to the single centre and retrospective design which were already mentioned above. Although all patients were recruited prospectively into the database, the clinical indication for bone scintigraphy was based on subjective decisions. This implies a patient selection bias, hence it would have been better if all patients were randomized into a screened group and non-screened group. From a scientific point of view a randomized controlled trial would be interesting to perform, but the clinical relevance to conduct such as study may be discussed.

Despite these limitations, we conclude that baseline bone scintigraphy in patients with HCC is not indicated unless specific bone-related complaints or clinical suspicion of extrahepatic disease is present in patients able to receive treatment with curative intent. We suggest that, in the absence of concluding data in favour of staging investigations, clinical judgement should remain to have an important role.

In case of a resectable HCC a thorough history and clinical examination focusing on skeletal pain, tenderness and/or other signs of systemic disease must indicate the need for a selective bone scintigraphy.

In conclusion, the routine use of a bone scintigraphy to detect metastases in asymptomatic patients included for surgical treatment for resectable HCC is not indicated.

In a large cohort of 336 patients with resectable HCC, unsuspected bone metastases were discovered in only 2 patients. Post-operative HCC recurrence rate, disease-free and overall survival showed no significant difference between patients with a bone scintigraphy and without a bone scintigraphy.

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PART V

OUTCOME

Chapter 8

Increased alfa-fetoprotein serum level is predictive for survival and recurrence of hepatocellular carcinoma in non-cirrhotic livers

Submitted

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ABSTRACT

Background: Hepatocellular carcinoma (HCC) may be diagnosed in the absence of cirrhosis. However, most patients have risk factors for cirrhosis, such as hepatitis B/C virus infection, alcohol abuse or haemochromatosis. Little is known about prognostic factors for survival of HCC with a non-cirrhotic liver and in the absence of any of these well established risk factors.

Methods: Survival rates and risk factors for survival and recurrence were analysed in all patients diagnosed between 2000-2010 with HCC in a non-cirrhotic liver and in the absence of well established risk factors.

Results: Ninety-four patients were analysed. Treatment with curative intent consisted of surgical resection in 43 patients (46%) and RFA in 4 patients (4%). Palliation was offered to 3 patients (3%) by TACE 14 patients (15%) by radio- or chemotherapy, and 30 patients (32%) by best supportive care. In patients treated by curative intent and alive 30 days after treatment (n=40) 1 and 5-year overall survival rates were 95% and 51%. Patients with a high preoperative alfa-fetoprotein (AFP) serum level, the presence of microvascular invasion in the resected specimen, a complicated postoperative course, and a major resection, due to a greater amount of tumour volume, had a significantly worse outcome and a higher recurrence rate. In multivariate analysis high AFP serum level at presentation was significantly associated with recurrence and a worse survival.

Conclusion: HCC presenting in a non-cirrhotic liver in the absence of well established risk factors has a poor prognosis. Increased AFP serum levels are significantly associated with clinical outcome.

INTRODUCTION

Hepatocellular carcinoma (HCC) is the fifth most common cancer in the world and the third most common cause of cancer-related mortality [1]. The incidence of HCC is high in Asia and parts of Africa, whereas it is low but increasing in the Western world even in the Netherlands [1-5].

HCC is the most common primary tumour of the liver and typically develops in the presence of underlying cirrhosis, particularly in high-endemic areas [1]. In low-endemic areas in 5-40% of HCC patients no cirrhosis is diagnosed [6-10]. Although many investigators have reported on prognostic factors for HCC in a non-cirrhotic liver, they still included patients positive for serological markers of hepatitis B or C virus infection, alcohol abuse or hemochromatosis. These are known well established risk factors for the development of cirrhosis and eventually HCC [11].

In HCC patients with no underlying cirrhosis, the 'normal' quality of the non-tumoral liver parenchyma might make this type of HCC a different entity, with a different aetiology, clinical presentation, management and prognosis [12].

The aim of this retrospective study was to asses clinical presentation, surgical outcome, and prognostic factors of our serie of patients who had HCC without presence of underlying cirrhosis or well established risk factors for cirrhosis and HCC.

METHODS

Clinical characteristics

All patients with HCC diagnosed in a tertiary reference centre between January 2000 and December 2010 were analyzed. Patients included (n=94) had no underlying cirrhosis and none of the well established risk factors for cirrhosis or HCC as determined by negative hepatitis B and C serological markers (anti-HCV negative, HBsAg negative, anti-HBc negative), a negative history of alcohol abuse (ones a week over 6 glasses of alcohol [13]), absence of haemochromatosis or a metabolic liver disease. Preoperatively, HCC was diagnosed by means of imaging criteria in two imaging techniques. In case HCC was diagnosed, treatment was discussed in a multidisciplinary meeting according to the AASLD guidelines [1]. In all patients alpha-fetoprotein (AFP) serum levels were assessed. The AFP cut-off point for our hospital is 9 ng/ml. Data on age, gender, size and number of lesions, and presence and extent of extrahepatic disease were collected.

Patients were primarily assessed for surgical resection, those not amenable for this option were considered for radiofrequency ablation (RFA). Treatment with curative intent was defined as a complete surgical resection, including wedge resection, segment resection, hemihepatectomy and RFA. Presence of a conventional HCC in a non-

cirrhotic liver was postoperatively histological confirmed in all patients. Patient with a fibrolamellar hepatocellular carcinoma were excluded. Tumour differentiation grade was determined according to the Lauwers classification [14].

Postoperative complications were reported up to 30 days post-surgery and were grouped as complicated (pharmacological treatment or intervention needed) or uncomplicated. Patients not amenable for curative therapy were offered palliative options, consisting of either transarterial chemo-embolization (TACE), radiotherapy, palliative systemic therapy or best supportive care.

Follow-up

After surgical resection, patients had a follow-up using contrast-enhanced computed tomography (CT) or magnetic resonance imaging (MRI) combined with determination of serum AFP at 3 and 6-month intervals for up to 2 years post-treatment. After 2 years, follow-up was continued annually for up to 5 years. HCC recurrence was defined as the appearance of a new focal liver lesion with HCC characteristics, enlargement of lymph nodes in the liver hilum, or suspected extrahepatic lesions. The diagnosis was histologically confirmed if necessary. Data regarding survival of patients that were lost to follow-up was obtained from the general practitioner or the civil registration systems.

Statistical analysis

Variables were compared using the Mann-Whitney U test, t-test or with the χ^2-test, as appropriate. Statistical significance was considered at a p-value < 0.05. Receiver operating characteristic (ROC) plots were used to define the optimum cut-off point for serum AFP. Multivariate Cox regression analysis was performed to assess the independent prognostic factors. According to the literature the patient characteristic 'recurrence of disease' has a high negative impact on survival, therefore the analysis was performed within a model adjusting for recurrence. Within this model, preoperative biochemical and postoperative tumour characteristics with a p-value <0.05 in univariate analysis together with the baseline characteristics age and gender were included to determine the independent contribution of each variable. Survival curves were drawn using the Kaplan-Meier method. Differences between Kaplan-Meier curves were tested using the log-rank test. All analyses were performed using the Statistical Package for Social Sciences 17.0 (SPSS, Chicago, IL, USA).

RESULTS

Patient characteristics

Ninety-four patients with HCC without underlying cirrhosis and with no established risk factors for HCC were identified. Of these, 46 patients (49%) were female and the median age was 62 (range 10-87) years. Table 1 summarises biochemical and radiological characteristics.

Table 1: Biochemical and radiological characteristics of hepatocellular carcinoma (HCC) patients without well established risk factors.

Characteristics*	
Albumin (g/l)	41 (27-52)
ASAT (U/l)	50 (16-897)
ALAT (U/l)	40 (6-461)
AP (U/l)	159 (55-2024)
Bilirubin (umol/l)	10 (4-199)
AFP (ug/l)	12 (1-834350)
Number of tumours	1 (1-7)
Radiological tumour size (mm)	98 (5-250)
TNM	
Stage I	28 (30)
Stage II	16 (17)
Stage IIIA	12 (13)
Stage IIIB	6 (6)
Stage IV	32 (34)

ASAT: aspartate aminotransferasis; ALAT: alanine aminotransferasis; AP: alkaline phosphatise; AFP: alfa-fetoprotein
*median (range) or numbers (%)

Outcome

Forty-three patients (46%) underwent a surgical resection and 4 (4%) underwent RFA. Out of 43 patients who underwent a surgical resection, 3 patients died within 30 days after surgery; 2 patients died due to a myocardial infarct and 1 patient died because of an eventful postoperative course secondary to a bleeding. Median time to death was 44 months (range <1-128).

After complete surgical resection (n=40) of a histologically confirmed conventional HCC in a non-cirrhotic liver the 1, 3 and 5-year survival rate was 95%, 60% and 51%, respectively. Of the 17 patients with an increased serum AFP (>9 ng/ml), 13 (76%) had a normalized serum AFP after surgical resection. The 4 patients without a normalized serum AFP had HCC metastasis within 5 months after surgery. Fifteen (38%) out of 40 patients treated by surgical resection had recurrence of HCC during follow-up. Median

time to recurrence was 8 (range 1-20) months. Of the patients treated with a minor resection (n=12) 3 patients had recurrence of HCC. In case of wedge resections HCC recurrence was found in the ipsilateral hemi-liver.

Table 2 presents the operative characteristics and postoperative histological characteristics of the patients treated with surgical resection.

In 47 patients (50%) not amenable for curative treatment, palliative treatment was considered. They received either TACE/TAE (3 patients; 3%), radiotherapy or systemic chemotherapy (14 patients; 15%), or best supportive care (30 patients; 32%).

Table 2: Surgical and pathological parameters in HCC patients undergoing surgical resection

Parameters*	**(n = 40)**
Type of resection	
Minor resection	
Segment	11 (28)
Major resection	
Left hemihepatectomy	10 (25)
Right hemihepatectomy	16 (40)
Extended left hemihepatectomy	1 (2)
Extended right hemihepatectomy	2 (5)
Mortality and morbidity postoperative	
Uncomplicated	25 (63)
Complicated	15 (37)
Fibrosis	
F0	19 (48)
F1	17 (42)
F2	4 (10)
Steatosis	
G0	22 (55)
G1	15 (38)
G2	2 (5)
G3	1 (2)
Microvascular invasion	
Presence	22 (55)
Absence	17 (43)
Undefined	1 (2)
Differentiation grade	
good	8 (20)
moderate	22 (55)
poor	8 (20)
undefined	2 (5)

*median (range) or numbers (%)

Factors associated with survival and recurrence of HCC after surgical resection

The optimal AFP cut-off point selected by ROC-plot in 40 patients treated with a surgical resection was between 8.0 ng/ml and 9.5 ng/ml. Patients with a poor survival after surgical resection more often underwent a major resection, had presence of microvascular

invasion, a moderately or poorly-differentiated tumour, a complicated postoperative course, an increased preoperative serum AFP, and recurrence of disease (Table 3). After adjusting for recurrence, in the Cox regression analysis an increased preoperative AFP serum level was shown to be significantly associated with a worse survival (Table 4A).

Table 3: Biochemical, radiological, surgical and pathological characteristics associated with overall survival following surgical resection (n=40) (univariate analysis).

Characteristics	Survivors	Non-survivors	p-value
AFP (ng/ml)			0.010
≤9	74%	26%	
> 9	47%	53%	
TNM			0.345
stage I/II	62%	38%	
stage III	62%	38%	
Number of tumours			0.134
1	59%	41%	
>1	83%	17%	
Radiological tumour size (mm)			0.514
≤5	80%	20%	
5-10	56%	44%	
>10	57%	43%	
Postoperative			0.008
Uncomplicated	72%	28%	
Complicated	50%	50%	
Type of resection			0.030
minor	82%	18%	
major	55%	45%	
F0	68%	32%	0.512
F1	53%	47%	
F2	75%	25%	
G0	55%	45%	0.176
G1	63%	27%	
G2	50%	50%	
G3	100%	0%	
Microvascular invasion (histology)			0.035
presence	55%	45%	
Absence	76%	24%	
Differentiation grade (histology)			0.048
good	87%	13%	
moderate	59%	41%	
Poor	40%	60%	
Recurrence			<0.001
Present	13%	87%	
Absent	82%	18%	

AFP: alfa-fetoprotein

Table 4A: prognostic factors associated with overall survival in multivariate analysis (n=40)

Characteristics	Hazard ratio (95% confidence limits)	p-value
Age (years)	0.98 (0.94-1.02)	0.263
Gender	0.41 (0.80-2.07)	0.280
AFP (ng/ml)*	1.21 (1.02-1.45)	0.034
Differentiation grade good vs. moderate vs. poor	1.98 (0.45-8.72)	0.370
Microvascular invasion absence vs. present	3.55 (0.69-18.33)	0.130

AFP: alfa-fetoprotein
* Continues variable

Table 4B: prognostic factors associated with recurrence in multivariate analysis

Characteristics	Hazard ratio (95% confidence limits)	p-value
Age (years)	0.97 (0.93-1.01)	0.120
Gender	1.29 (0.39-4.65)	0.702
AFP (ng/ml)*	1.29 (1.02-1.63)	0.031
Differentiation grade good vs. moderate vs. poor	1.05 (0.21-5.10)	0.957
Microvascular invasion absence vs. present	7.03 (1.03-47.97)	0.047

AFP: alfa-fetoprotein
* Continues variable

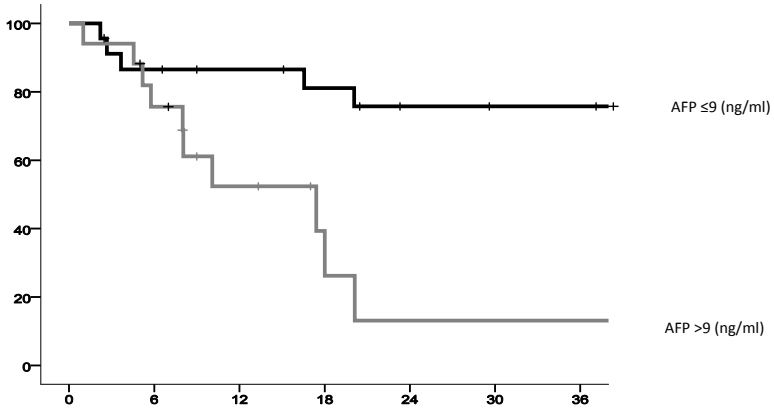

Figure 1: Overall survival of hepatocellular carcinoma patients without well established risk factors starting at 30 days after surgical resection comparing normal vs. increased serum levels of alfa-fetoprotein (AFP)

Patients with recurrence of HCC were characterized by higher preoperative AFP serum levels (p=0.003) and presence of microvascular invasion (p=0.005).

In the Cox regression analysis an increased preoperative AFP serum level and presence of microvascular invasion were shown to be significantly associated with appearance of HCC recurrence (Table 4B).

The 1 and 3-year survival rate of patients with a high and a low AFP serum level was 53% and 86%, and 21% and 75%, respectively (p=0.010) (Figure 1).

DISCUSSION

In this study we evaluated the outcome of HCC treatment in patients without well established risk factors for cirrhosis or HCC. Several studies have explored clinicopathological characteristics in HCC patients without cirrhosis [1,11,15]. Although they studied HCC in the absence of cirrhosis, they often included patients with well established risk factors for cirrhosis or HCC or analyzed a small group of patients [11,16]. This selection bias will influence overall survival as it is known that, for example, chronic hepatitis B influences survival in HCC patients [17]. We were able to include a large group of patients, all non-cirrhotic HCC patients without well established risk factors, and had the opportunity to study this unique population.

Diagnosis

In the absence of a screening program developed for patients with liver cirrhosis, early detection in an asymptomatic stage is seldom seen and diagnosis of non-cirrhotic HCC is usually made at an advanced stage with large tumours [18]. By then many patients have already developed an unresectable tumour, either because of tumour size, anatomical localization, or the presence of multiple bilobare intrahepatic metastases [19]. In the present study, nearly 50% of the patients referred with HCC without liver cirrhosis and well established risk factors were beyond curative surgical options. The studied population is homogenises for conventional hepatocellular carcinoma, none of the patients had a fibrolamellar hepatocellular carcinoma.

Survival and recurrence

In our population of HCC patients without well established risk factors the survival rates were comparable with patients with a Child-Pugh A liver cirrhosis treated with surgical resection (5-year survival rate 50%), with similar associated factors [1,20]. As the optimal AFP cut-off point selected by ROC-plot was between 8.0 ng/ml and 9.5 ng/ml, the AFP cut-off point of for hospital (9 ng/ml) was an adequate cut-off point. Although, AFP as serological marker for HCC in screening and surveillance in patients with cirrhosis is

debated [21], AFP seems a useful marker in patients with HCC without well established risk factors, especially when AFP serum level is increased. It is likely that the 4 patients without normalization of AFP who had metastases within 5 months of surgery already had metastatic disease at surgery that was not identified by imaging due to small size.

According to the literature little is known about the value of AFP serum level in patients with HCC without well established risk factors. Our study displays the presence of an increased AFP serum level preoperatively to be the most important factor associated with a worse outcome and recurrence of HCC in multivariate analysis. In case AFP serum level is increased in patients who have to undergo a high-risk operation, e.g. an extended hemihepatectomy or in patients with many comorbidities it might be worth reconsidering the high-risk treatment.

Therapy considerations

If a surgical resection is performed with curative intent in a non-cirrhotic liver, 38% of the patients suffer from recurrence of disease. Solitary recurrence might benefit from repeated resection, but in most patients recurrence after primary resection will be multifocal because of intra-hepatic dissemination from the primary tumour [1]. Out of 15 patients with recurrence after treatment with curative intent, 3 had recurrence after a local surgical resection. Their disease recurrence appeared as a tumour in the ipsilateral hemi-liver, whereas the contralateral hemi-liver was free of tumour recurrence. Thus, in HCC patients with a well-preserved liver function, there might be a preference to perform a major resection because, after minor resections, patients tend to more often have recurrence in the ipsilateral hemi-liver. In the absence of well established risk factors, the presence of a small satellite tumour, too small to detect on imaging, might cause tumour recurrence in the ipsilateral liver lobe.

Until now liver transplantation is a therapy considered for cirrhotic patients only. In patients with underlying cirrhosis treated with liver transplantation 5-year survival rates up to 70% are reported [1]. This higher survival rate makes transplantation a treatment option for HCC patients without well established risk factors worth considering [19]. The Milan criteria and the algorithms used for HCC in cirrhotic livers are probably not applicable to HCC in a non-cirrhotic liver without well established risk factors, because it is a different disease with (probably) different aetiology and pathogenesis [22,23]. Based on these findings we propose the following treatment strategy for patients with unresectable HCC in a non-cirrhotic liver without well established risk factors. Patients with extrahepatic spreading or gross vascular involvement should be strictly excluded for liver transplantation. If biopsy is performed and the presence of a moderate or poorly-differentiated tumour with microvascular invasion is noted, caution is needed, especially when the preoperative serum AFP level is high. A large tumour size per se

(outside the Milan criteria) should not be seen as a strict contraindication for considering liver transplantation in patients with HCC without well established risk factors.

This study evaluating a single institution's experience of HCC patients without well established risk factors is influenced by confounding factors, as the data were acquired retrospectively and might be influenced by patient selection. However, as patients with HCC without underlying cirrhosis and HCC are relatively rare, no previous study was able to describe such a large cohort. Our study might be based on a small amount of patients (n=40), still we analyzed the largest cohort presented so far. Although the study population is unique, because some of our conclusions are based on a small number of patients (patients with local recurrence: n=3) experiences from other centres are needed. However, based on our observations, the absence of well established risk factors seems to have less impact on final outcome parameters in HCC patients. One can speculate whether the presence of cirrhosis is an independent prognostic parameter, or whether the overall assessment of HCC and the recurrence of HCC after treatment with curative intent, determine the final outcome in patients with HCC. Therefore, expansion of treatment options by liver transplantation in non-cirrhotic patients is worth re-considering.

In conclusion, this retrospective analysis of a large cohort of HCC patients without well established risk factors shows that they were diagnosed with an advanced stage of disease. If surgical resection is performed, good results can be achieved. The higher the preoperative AFP serum level is the worse the outcome. Patients might benefit from major resections as disease recurrence often appears in the ipsilateral liver lobe instead of the contralateral liver lobe. Although cirrhosis is absent, liver transplantation as a treatment option for HCC patients without well established risk factors is worth considering and should be further explored.

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Chapter 9

Hepatocellular carcinoma in non-cirrhotic livers; an immunohistochemical study

Submitted

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ABSTRACT

Background: Hepatocellular carcinoma (HCC) typically develops in cirrhotic livers. The diagnosis of HCC might be difficult in absence of risk factors e.g. cirrhosis or hepatitis B/C virus infection. We aimed to explore whether immunohistochemical staining of HCC in a non-cirrhotic liver contributes to diagnostic and prognostic characteristics, and whether these immunohistochemical characteristics differ from HCC in a cirrhotic liver.
Methods: Paraffin embedded, formalin fixed tissue slides from live resection specimens of patients with HCC in a non-cirrhotic liver were analyzed.
Results: From January 2000 through April 2011, 47 patients with 50 HCCs in a non-cirrhotic liver were operated. The tumours were stained positive for AFP in 15 cases (30%), for CD34 in a diffuse pattern in 44 (88%), for CK7 in 22 (44%), for CK19 in 6 (12%), for GPC3 in 20 (40%), for GS in 32 (62%) and for β-catenin in 16 (32%). Moderate or poorly differentiated HCC more often expressed β-catenin, and GPC3 and showed a higher percentage of MIB-1 positive hepatocytes. More patients with presence of vascular invasion presented with a positive expression of MIB-1 in the tumour. A positive AFP immunohistochemical staining was significantly related with a high pre-operative AFP serum level (p=0.001). The larger the tumour the more often diffuse CD34 staining was present (p=0.037). None of the immunohistochemical stainings was associated with a worse overall survival. Of the patients treated with a surgical resection 17 had recurrence of HCC and these patients more often had a positive CK19 staining (p=0.048). There was similarity in immunohistochemical expression of several markers comparing HCC in a non-cirrhotic liver with HCC in a cirrhotic liver.
Conclusion: Immunohistochemical expression of several markers in HCC in a non-cirrhotic and HCC in a cirrhotic liver are comparable. Immunohistochemical markers β-catenin, GPC-3 and MIB-1 are markers helpful in establishing HCC differentiation grade, without being a predictor for overall survival.

INTRODUCTION

Hepatocellular carcinoma (HCC) is the fifth most common cancer in the world and the third most common cause of cancer-related mortality [1]. The incidence of HCC is high in Asia and parts of Africa, whereas it is low but increasing in the Western world [2-4]. HCC constitutes a major health problem and its relevance is expected to increase in the near future [4]. HCC is by far the most common primary malignant tumour of the liver and typically develops in a cirrhotic liver, particularly in high-endemic areas [1]. In low-endemic areas 5-40% of the HCC patients has a non cirrhotic liver [5-10]. The diagnosis of HCC might be difficult in absence of risk factors e.g. cirrhosis or hepatitis B/C virus infection. The liver represents one of the three most common sites of metastasis, and primary hepatic cholangiocarcinoma and HCC often share overlapping morphologic appearances [3]. One of the challenges in histopathological diagnosis of hepatocellular mass lesions is to distinguish HCC (particularly well-differentiated) from hepatocellular adenoma [11]. Moreover, complicating the diagnostic process is that pathologists are frequently asked to handle and diagnose liver needle core biopsies with various biopsy artefacts.

Serum alpha-fetoprotein (AFP) level remains the best available marker for the diagnosis of HCC clinically, although it does not fulfill the requirements of an ideal tumour marker [12-14]. However, only about 25-40% of the HCCs in a cirrhotic liver are positive for AFP by immunohistochemistry [15]. It is not clear, however, how expression of AFP immunohistochemistry correlates to HCC in a non-cirrhotic liver.

As to our knowledge, an immunohistochemical profile of HCC in non-cirrhotic livers has not been presented before, neither from a clinical perspective, nor from a pathological perspective. Only a few immunohistochemical markers are investigated for the distinction between benign and malignant hepatocellular lesions [15]. Being representative for a low-endemic area we initiated the present study to investigate the expression of immunohistochemical markers in HCC in a non-cirrhotic liver and studied an unique population. We analyzed in the current study the expression of AFP, CD34, cytokeratine 7 (CK7), cytokeratine 19 (CK19), glypican-3 (GPC3), (antigen) Ki67 (MIB-1), glutamine synthetase (GS) and β-catenin in patients with HCC arising in a non-cirrhotic liver. We established the role of the immunohistochemical staining pattern as a diagnostic or prognostic test and compared whether this immunohistochemical profile differs from the immunohistochemical profile of HCC in a cirrhotic liver.

MATERIAL AND METHODS

Cases

The Erasmus University Medical Centre Rotterdam in the Netherlands is a tertiary referral centre for focal liver lesions. Yearly over 200 liver resections are being performed of which a minority in patients with HCC in non-cirrhotic livers. In the present study all patients (n=47) with HCC in a non-cirrhotic liver who had a surgically resection between January 2000 and April 2011 were included. These patients were demonstrated to be negative for HCC risk factors i.e. hepatitis C viral infection, hepatitis B viral infection, alcohol abuse or hemochromatosis. Patients with a fibrolamellar HCC were excluded.

Survival time was determined from the date of the histopathological diagnosis to the end of the study on 1 April 2011 with a median survival time of 45 months (range 1-129 months). Confirmation regarding survival of patients that were lost to follow-up was obtained from the general practitioner or the civil registration systems.

HCC recurrence was defined as the occurrence of a focal liver lesion with HCC characteristics, enlargement of lymph nodes in the liver hilus, or suspected extrahepatic lesions which were, if necessary, pathologically confirmed.

Histopathology

Paraffin embedded formalin fixed liver tissue slides were retrieved from the archives of the department of pathology, Erasmus MC Rotterdam. For each lesion microscopic features were evaluated (F.J.W.K.). Tumour differentiation grade was determined according to the Lauwers classification, and was based on the areas of tumour showing the poorest differentiation grade [17]. Non-tumoral liver tissue was evaluated for fibrosis/cirrhosis according to the classification of the METAVIR group as follows: F0 (no fibrosis); F1 (peri-portal fibrosis), F2 (few porto-central bridges), F3 (many porto-central bridges) and F4 (cirrhosis) [18]. Steatosis was graded according to Kleiner et al.: Grade 1 (5-33% of the hepatocytes), grade 2 (33-66% of the hepatocytes), grade 3 (more than 66% of the hepatocytes). Steatosis, if not observed or <5% was graded as grade 0 [19].

Immunohistochemistry

Immunohistochemistry was performed on representative sections of the tumour. Immunostaining for AFP (rabbit polyclonal antibody, Dako), CD34 (mouse monoclonal antibody, clone QBEnd/10, Neomarkers, CC1-Standard), GPC-3 (mouse monoclonal antibody, clone Ig12, Santa Cruz, Biotechnology, CC1-Mild), CK19 (mouse monoclonal antibody, clone RCK108, Dako M0888, Benchmark Ultr. Ventana, CC1-standard), CK7 (mouse monoclonal antibody, OV_TL 12/30, Biogenex, CC1-standard), MIB-1 (mouse monoclonal antibody, clone Ki67, M7240, Dako), GS (monoclonal mouse antibody, 6/glutamine syn., B&D, CC1-standard) and β-catenin (monoclonal mouse antibody, clone 14, Autostainer,

PT high, Dako) was performed in these cases. In case CD34 staining was not diffusely positive it was graded as negative. CK7 and CK19 staining was graded as positive in cases with >5% positive cells. A nuclear staining for β-catenin, focal or diffuse, was considered positive. For each immunohistochemical staining a positive and negative control was used. To compare immunohistochemical staining of HCC in a non-cirrhotic liver to the immunohistochemical profile of HCC in a cirrhotic liver a control group was used, including 41 cases with 65 HCCs in a cirrhotic liver. In all cases we performed immunohistochemical staining of AFP, CD34, GPC-3, CK19, CK7, MIB-1, GS and β-catenin.

Statistical analysis

Continuous variables were summarized as median with ranges and categorical variables were summarized as frequency and percentages. The Mann-Whitney U test, t-test or the χ^2-test were used whenever appropriate. Statistical significance was considered at a p-value < 0.05. The Kaplan-Meier method was used to calculate the survival rate, the difference between Kaplan-Meier curves was tested using the log-rank test. All analyses were performed using the Statistical Package for Social Sciences 17.0 (SPSS, Chicago, IL, USA).

RESULTS

Clinical, pathological and immunohistochemical features

From January 2000 through April 2011, 47 patients with 50 lesions were treated by surgical resection of HCC in a non-cirrhotic liver. Clinical and pathological characteristics are summarized in Table 1. These patients were demonstrated to be negative for HCC risk factors i.e. hepatitis C viral infection, hepatitis B viral infection, alcohol abuse or hemochromatosis. In all cases of HCC absence of cirrhosis was confirmed histologically.

Differentiation according to the Lauwers classification resulted in 12 well-differentiated (24%), 31 moderately-differentiated (62%) and 7 poorly- differentiated (14%) tumours. Vascular invasion was present in 30 (60%) tumours. The immunohistological staining pattern of HCC in a non-cirrhotic liver and HCC in a cirrhotic liver as conducted in the present study l and in literature are displayed in table 2.

Comparison with clinicopathological characteristics

The relationship between the expression of AFP, CD34, CK7, CK19, GPC3, MIB-1, GS and β-catenin and the differentiation grade are given in table 3A. The relationship between the expression of AFP, CD34, CK7, CK19, GPC3, MIB-1, GS and β-catenin and presence of vascular invasion are given in table 3B. Patients with a moderate or poorly differentiated HCC had a significantly higher prevalence of β-catenin, GPC3 and MIB-1 expression.

Table 1: clinical and pathological characteristics of patients with hepatocellular carcinoma in a non-cirrhotic liver

Characteristic	n=47
Clinical	
Age (years)	65 (21-82) *
Gender (male)	23 (50) **
Level of serum AFP (ng/mL)	6 (2-217800) *
Pathological	
Number tumours	1 (1-3) *
Tumour size (mm)	110 (17-240) *
Fibrosis grade 0/I/II	35 (75)/ 11 (23)/ 1 (2) **
Steatosis grade 0/I/II/III	27 (58)/ 11 (23)/ 8 (17)/ 1 (2) **

*median (range)
**number (%)
AFP= alpha-fetoprotein

Table 2: Staining pattern immunohistochemical markers in hepatocellular carcinoma in a non-cirrhotic liver and in hepatocellular carcinoma in a cirrhotic liver according to our hospital and the literature

Staining		Hepatocellular carcinoma in a non-cirrhotic liver [Rotterdam]	Hepatocellular carcinoma in a cirrhotic liver [Rotterdam]	Hepatocellular carcinoma in a cirrhotic liver [Literature]
AFP	Positive	30%	35%	25-40% [15]
β-Cat	Positive	32%	25%	20-35% [20,21]
CD34	Positive	88%	95%	88% [22]
GPC-3	Positive	40%	85%	76-83% [11,13,23]
GS	Positive		52%	60% [24]
	Positive diffuse	42%		
	Positive focally	20%		
CK7	Positive	44%	59%	22-40% [25,26]
CK19	Positive	12%	6%	10-17% [25,26]
MIB	1-10% positive	22%	17%	51% [27]
	≥10% positive			33% [27]
	10-30% positive	28%	26%	
	30-50% positive	28%	34%	
	>50% positive	22%	23%	

AFP= alpha-fetoprotein; β-Cat= β-catenin; GPC-3= glypican-3; CK7= cytokeratine 7; CK19=cytokeratine 19; MIB-1=antigen Ki67; GS= glutamine synthetase

More patients with presence of vascular invasion presented with a higher prevalence of MIB-1 expression in the tumour.

A positive AFP immunohistochemical staining was significantly related with an increased pre-operative AFP serum level (p=0.001). The larger tumour size the more often

Table 3A: Staining pattern immunohistochemical markers in hepatocellular carcinoma in a non-cirrhotic liver according to the differentiation grade

		Differentiation grade			
Staining		**Good**	**Moderate**	**Poor**	**p-value***
AFP	Positive	1 (8%)	10 (32%)	4 (57%)	0.074
	Negative	11 (91%)	21 (68%)	3 (43%)	
B-Cat	Positive	0 (0%)	15 (48%)	1 (14%)	0.005
	Negative	12 (100%)	16 (52%)	6 (86%)	
CD34	Positive	11 (92%)	28 (90%)	5 (71%)	0.344
	Negative	1 (8%)	3 (10%)	2 (29%)	
GPC-3	Positive	0 (0%)	15 (48%)	5 (71%)	0.003
	Negative	12 (100%)	16 (52%)	2 (29%)	
GLUL	Positive diffuse	2 (17%)	17 (55%)	2 (29%)	0.183
	Negative	6 (50%)	10 (32%)	3 (43%)	
	Positive weak	4 (33%)	4 (13%)	2 (29%)	
CK7	Positive	7 (58%)	13 (42%)	2 (29%)	0.421
	Negative	5 (42%)	18 (58%)	5 (71%)	
CK19	Positive	0 (0%)	4 (13%)	2 (29%)	0.175
	Negative	12 (100%)	27 (87%)	5 (71%)	
MIB	1-10	6 (50%)	5 (16%)	0 (0%)	0.034
	10-30	3 (25%)	9 (29%)	2 (28%)	
	30-50	3 (25%)	10 (32%)	1 (14%)	
	>50	0 (0%)	7 (64%)	4 (57%)	

*univariate analysis $\chi 2$-test

AFP= alpha-fetoprotein; β-Cat= β-catenin; CD34= antigen CD34; GPC-3= glypican-3; CK7= cytokeratine 7; CK19=cytokeratine 19; MIB-1=antigen Ki67; GS= glutamine syntetase

CD34 staining was positive in a diffuse pattern (p=0.037). None of the immunohistochemical stainings was associated with a poor overall survival. After a median follow-up of 9 months (range 2-42 months) 17 patients had recurrence of HCC, these patients more often had a positive CK19 staining (p=0.048).

DISCUSSION

The biological behaviour of HCC in a non-diseased liver, as defined by the absence of well known risk factors for cirrhosis, has been subject for debate as to define this HCC as an entity different from HCC in a diseased liver parenchyma [28]. HCC in a non-cirrhotic liver might have a different pathogenesis with a different clinical, histological and his-

Table 3B: Staining pattern immunohistochemical markers in hepatocellular carcinoma in a non-cirrhotic liver according to the presence of vascular invasion

		Vascular invasion			
Staining		**No**	**Yes**	**Suspect**	**p-value***
AFP	Positive	3 (16%)	12 (40%)	0 (0%)	0.158
	Negative	16 (84%)	18 (60%)	1 (100%)	
B-Cat	Positive	5 (26%)	11 (37%)	0 (0%)	0.591
	Negative	14 (74%)	19 (63%)	1 (100%)	
CD34	Positive	18 (95%)	25 (83%)	1 (100%)	0.456
	Negative	1 (5%)	5 (17%)	0 (0%)	
GPC-3	Positive	4 (21%)	15 (50%)	1 (100%)	0.061
	Negative	15 (79%)	15 (50%)	0 (0%)	
GLUL	Positive diffuse	8 (42%)	13 (43%)	0 (0%)	0.344
	Negative	5 (26%)	13 (43%)	1 (100%)	
	Positive weak	6 (32%)	4 (13%)	0 (0%)	
CK7	Positive	12 (63%)	10 (33%)	0 (0%)	0.082
	Negative	7 (37%)	20 (67%)	1 (100%)	
CK19	Positive	2 (11%)	4 (13%)	0 (0%)	0.893
	Negative	17 (89%)	26 (87%)	1 (100%)	
MIB	1-10	8 (42%)	3 (10%)	0 (0%)	0.009
	10-30	8 (42%)	6 (20%)	0 (0%)	
	30-50	2 (11%)	11 (37%)	1 (100%)	
	>50	1 (5%)	10 (33%)	0 (0%)	

***univariate analysis** χ2-test
AFP= alpha-fetoprotein; β-Cat= β-catenin; CD34= antigen CD34; GPC-3= glypican-3; CK7= cytokeratine 7; CK19=cytokeratine 19; MIB-1=antigen Ki67; GS= glutamine syntetase

tochemical presentation, which might result in a different management and prognosis [28]. In low-endemic areas HCC in a non-cirrhotic liver may contribute to the overall survival of HCC patients to a larger extent as compared to high-endemic areas where HCC in cirrhotics dominate. It may be questioned whether the prognosis of HCC in absence of cirrhosis is more favourable as compared to the prognosis reported in literature for HCC in cirrhotics. Factors that may influence prognosis of patients with HCC in non-diseased livers are not well described, neither from a clinical perspective, nor from a pathological perspective. Being representative for a low-endemic area we initiated the present study to investigate the expression of immunohistochemical markers in HCC in a non-cirrhotic liver.

The outcome of immunohistological staining of HCC according to our clinic and the literature in a cirrhotic liver or in presence of risk factors e.g. hepatitis B/C viral infection or alcohol abuse, are summarized in Table 2 [11,13,15,20-27]. Although GPC-3 is less often

positive in a HCC in a non-cirrhotic liver, our immunohistochemical data demonstrate a similarity in immunohistochemical expression of several markers comparing HCC in a non-cirrhotic liver with HCC in a cirrhotic liver. These findings suggest that by defining immunohistological characteristics HCC in a non-cirrhotic liver cannot be discriminated from HCC in a cirrhotic liver. The difference in CK7 and CK19 expression between our patients with cirrhosis and the literature can be a result of interpretation as these markers are not always diffusely positive. In our study CK7 and CK19 were scored as positive in case they were focal positive or diffusely positive. The differences in MIB-1 expression can also be explained by interpretation variability as MIB-1 is a percentage that is observer dependent. GPC-3 being less often positive in a HCC in a non-cirrhotic liver might be an affect of the absence of an underlying liver disease as it is reported that GPC-3 has a strong immunoreactivity in hepatocytes in liver biopsies with chronic hepatitis C or cirrhosis [11,29]. Although it can not be stated from the results of this study it may be speculated that accordingly to the lack of differences between the immunohistological characteristics the oncobiological behaviour of HCC in non-cirrhotic and HCC in cirrhotic livers may not differ.

Clinicopathological characteristics

AFP immunohistochemical staining was positive in 30% of the cases. Our results demonstrate a strong correlation between AFP in serum and AFP in tumour specimens as shown by immunohistochemical staining. In addition in those cases in which absence of AFP in immunohistochemical staining was found serum AFP was not increased either. It should be noted that a negative AFP immunohistochemical staining does not exclude HCC, as compared to a negative serum AFP and that this marker should be interpreted with care in case of tumour analysis in non-cirrhotic and cirrhotic patients. Other immunohistochemical markers may be helpful in the differentiation of good, moderate and poorly differentiated tumours and presence of vascular invasion, both parameters known as prognostic features [30-34]. β-catenin, GPC-3 and MIB-1 are significantly more often positive in patients with a moderate or poorly differentiated tumour. Although HCC in a non-cirrhotic liver is less often GPC-3 positive, GPC-3 is a marker that is significantly associated with a moderate or poor differentiation grade. GPC-3 is an oncofetal protein, a member of the glypican family of membrane-bound heparin sulphate proteoglycans, with roles in development and regulation of cellular proliferation and apoptosis in specific tissues [8]. The expression of GPC-3 is detected in the placenta and the fetal liver and reappears in HCC during hepatic carcinogenesis [23]. In addition MIB-1 expression is also increased in HCCs with presence of vascular invasion. In case one has to distinguish well-differentiated HCC from a hepatocellular adenoma on a needle core biopsy β-catenin, GPC-3 and MIB-1 might be helpful.

It was found that CK19 more often was found to be positive in patients who showed recurrence of disease during their follow up. The relevance of this finding should be validated in larger cohort to define its use for clinical management.

In spite of the fact that we had the opportunity to study an unique population and were able to report data from to the best of our knowledge the largest number of cases reported so far, none of the immunohistochemical markers eventually seems to be able to differentiate between patients with a good and a poor survival.

In summary, in the analysis of tumours in non-cirrhotic livers a positive AFP immunohistochemical staining may be helpful and correlates with an increased AFP serum level. However, as in cirrhotic patients absence of AFP serum as well as tumour tissue does not exclude the presence of HCC. Immunohistochemical markers, including β-catenin, GPC-3 and MIB-1 may be helpful in establishing HCC differentiation grade. These markers can not be used as a predictor for prognosis and survival.

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Chapter 10

Quantitative HBV DNA and AST are strong predictors for survival after HCC detection in chronic HBV patients

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ABSTRACT

Background: Hepatitis B virus infection (HBV) is an important co-factor in the development of hepatocellular carcinoma (HCC). We studied whether quantitative HBV DNA at time of HCC detection influences survival of HCC patients.

Methods: All diagnosed HCC cases between 2000 and 2008 at our university-based reference centre were analysed to determine the influence of hepatitis B viral load on overall survival. Clinical and virological findings were evaluated in univariate and multivariate analyses, survival rates were assessed for HCC patients with a high viral load (HBV DNA $\geq 10^5$ copies/ml) and low viral load (HBV DNA $< 10^5$ copies/ml).

Results: HCC was diagnosed in 597 patients, including 98 patients with HBV. The group of 37 patients (38%) who had a high viral load contained more HBeAg-positive patients, had lower serum albumin levels and higher serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels. The one- and five-year survival rates of HCC patients with a high viral load were 58% and 11% and for HCC patients with a low viral load 70% and 35%, respectively. In multivariate analysis a higher AST level and higher viral load were significantly associated with shorter overall survival (HR=2.30; p=0.018, HR=1.22; p=0.015, respectively).

Conclusion: HBeAg positivity, low albumin level or high AST or ALT levels in HCC patients are associated with a higher HBV DNA . HBV DNA level at detection is associated with overall survival of HCC patients. These findings support the concept that after HCC detection adequate suppression of HBV DNA by nucleoside analogue therapy may improve survival.

INTRODUCTION

Hepatocellular carcinoma (HCC) is the fifth most common cancer in the world and the third most common cause of cancer-related mortality [1]. In many patients, HCC occurs against the background of a chronic viral infection. Chronic hepatitis B virus (HBV) infection, chronic hepatitis C viral (HCV) infection and cirrhosis are major aetiologies of HCC [2,3]. Worldwide approximately 400 million people are chronically infected with HBV [4].

In the last 15 years, reliable quantification of HBV DNA over a large dynamic range has become feasible. Several hospital-based and community-based studies have subsequently found significant associations between the level of serum HBV DNA and the risk to develop liver cirrhosis or HCC [5]. After an HCC has developed and surgery is performed, recurrence of HCC is associated with original tumour size, number of tumours, grade of differentiation, level of alpha-fetoprotein (AFP), alcohol consumption and HCV co-infection[6-9].

The impact of viral load on survival of HCC patients after surgery with curative intent may be overshadowed by tumour-related factors or stage of the liver disease at detection of HCC. Understanding the respective role of tumour and viral factors in HCC survival may provoke new treatment strategies to increase HCC survival. It has been hypothesised that anti-viral therapy for HCC patients with active HBV replication along with HCC treatment might reduce the recurrence rates for HBV-associated HCC [10].

A few recent studies have evaluated HBV replication status as a predictor of HCC recurrence [9,11,12]. However, to our knowledge only a few reports from high endemic areas published to date, often with a limited number of patients, have suggested a relation between viral status and prognosis in patients with HBV-associated HCC [13-16]. In the current study in a low endemic area, univariate and multivariate analyses of the prognostic factors, including serum HBV DNA level, were performed to determine whether the HBV DNA levels at the time of HCC appearance are associated with overall survival.

MATERIAL AND METHODS

Study design

A hospital-wide registry, including data from patient files and virological records of all patients diagnosed with HCC at the Erasmus MC in Rotterdam, the Netherlands during the period from 1 January 2000 to 31 December 2008, was used. The diagnosis of HCC was made from radiological and biochemical findings and, if necessary, confirmed by histological examination. Within the group of nodules larger than 2 cm, with the typical features of HCC on a dynamic imaging technique, no biopsy was performed. Nodules

between 1-2 cm were investigated further with two dynamic studies imaging modalities, computed tomography (CT) scan or magnetic resonance imaging (MRI) with contrast. If the appearances were typical of HCC (i.e., hypervascular with washout in the portal/venous phase) in two techniques the lesion was treated as HCC. If the findings were not characteristic or the vascular profile was not coincidental among techniques, the lesion was biopsied, according to the AASLD guidelines [17].

All HBsAg(+) patients were included in this study. Follow-up of HCC recurrence by an alpha-fetoprotein (AFP) test and ultrasound, CT, or MRI was done every three to six months for up to two years after potential curative treatment. After two years, follow-up was continued annually for up to five years after treatment. Recurrence of tumour in the treated area or elsewhere was defined as re-appearance of vascular enhancement [17]. In the presence of underlying liver cirrhosis, lifetime follow-up was performed. If HCC recurred, the size, number, and localisation of the recurrent disease were registered. Verification of living patients was done using information obtained from the general physician or the civil registration.

Biochemical and serological markers

Data were collected on patient age, gender, nucleotide or nucleoside analogue therapy (lamivudine, adefovir, telbuvidine, tenofovir or entecavir or a combination of these drugs), AFP, size and number of lesions, and the presence of lymph node enlargement or metastases. The collected liver parameters included aspartate aminotransferase (AST), alanine aminotransferase (ALT), total bilirubin and albumin. In addition, the Model For End-Stage Liver Disease (MELD) score was calculated. Cirrhosis was diagnosed using established clinical, biochemical, and histological criteria. Patients with cirrhosis were classified according to the Child-Pugh classification.

At time of HCC diagnosis, the serum HBV DNA level was assessed using in-house Taqman PCR (detection limit 400 copies/ml) based on the Eurohep standard, HBeAg (AxSYM, Abbott, Abbot Park, IL, USA) and hepatitis B surface antigen (HBsAg) (AxSYM, Abbott) status were measured [18]. A high HBV load was considered to be HBV DNA $\geq 10^5$ copies/ml, HBV DNA $<10^5$ copies/ml was considered a low viral load [19]. All patients were negative for anti-hepatitis C virus antibody and did not report alcohol abuse at time of diagnosis and commencement of this study.

Statistical analysis

Variables were compared using the Mann-Whitney U test, t-test or with the χ^2 test whenever appropriate. Statistical significance was considered if the p value was <0.05. Univariate analysis was used to assess the importance of prognostic factors on overall survival. Survival curves were drawn using the Kaplan-Meier method. The difference between Kaplan-Meier curves was tested using the log-rank test. The baseline charac-

teristics age, gender, log bilirubin, log albumin, log AST, log ALT, log HBV DNA, log AFP, MELD score, Barcelona Clinic Liver Cancer (BCLC) score, HBeAg and anti-viral therapy were considered.

Multivariate Cox regression analysis was performed with all characteristics with a p value <0.20 in univariate analysis and known factors associated with survival to determine the independent contribution of each variable. Analysis was performed using SPSS software.

RESULTS

Clinical, biochemical and virological data

A total of 597 patients were diagnosed with HCC. Out of these 597 patients, 98 patients (16%) fulfilled the inclusion criteria. The patient characteristics at presentation with HCC are shown in table 1. Median follow-up was 22 months (1-114). One year after presenta-

Table 1: Patient characteristics at first presentation with HCC

Characteristic	(n =98)
Age (years)*	55(23-80)
Gender (male)	79(81%)
Total bilirubin (umol/L)*	18(4-481)
Albumin (g/L)*	38(22-49)
AST (U/L)*	69(21-1278)
ALT (U/L)*	54(19-670)
AFP (ng/ml)*	70(1-652660)
MELD score*	6(6-25)
Non-cirrhotic	22(22%)
Child-Pugh-classification	
A	49(64%)
B	20(26%)
C	7(9%)
HBV DNA $\geq 10^5$ copies/mL	37(38%)
HBeAg‡	24(25%)
Anti-viral (nucleoside or nucleotide analogue) therapy	50(51%)
Number of tumours*	1(1-7)
Tumour size (mm)*	34(8-227)
Metastases	29(30%)
BCLC	
Stage A	30(31%)
Stage B	37(38%)
Stage C	7(7%)
Stage D	24(24%)

*median(range), ‡ positive value

tion, 60% of the patients were still alive, and the five-year survival rate of this cohort was 21%. In 50 patients (51%), treatment with curative intent was initiated; this included surgical resection (wedge resection, segment resection, or hemihepatectomy), liver transplantation or radio frequency ablation. In eight patients (8%) transarterial (chemo) embolisation (TACE) or another therapy such as radiotherapy or systemic chemotherapy with palliative intent was started. The remaining group of 40 patients (41%) received no therapy.

Fifty patients (51%) received oral anti-viral therapy. Nine patients had an increase of HBV DNA during the study period but none of these patients switched from the low HBV DNA group to the high HBV DNA group.

In 21 out of 50 patients (42%) a recurrence of HCC was documented after potentially curative treatment. The median time to recurrence was 12 (1-50) months. Recurrence of HCC presented as local recurrence in four patients (19%), a new lesion in 11 patients (52%) and metastases in six patients (29%).

Factors associated with HBV viral load

Among the 98 patients, 37 (38%) had a high viral load. As expected, the group of patients with a high viral load contained more HBeAg(+) patients, had a lower serum albumin level and had a higher serum AST, ALT, and total bilirubin level compared with the group of patients with a low viral load (table 2). Treatment with curative intent was not significantly different between patients with high and low viral load (p=0.188). Treatment of HCC was independent of the level of HBV DNA (p=0.202). Patients with a higher viral load more often had a recurrence of HCC after treatment with curative intent (p=0.025).

Univariate and multivariate analyses were performed to determine HBV-related predictors for overall survival (table 3). Multivariate Cox regression analysis was performed with all characteristics with a p value <0.20 in univariate analysis and known factors associated with survival to determine the independent contribution of each variable. The strong correlation between AST and HBV DNA made it impossible to join them in one model. Separately, multivariate analysis confirmed both a high AST level and a high viral load (HBV DNA) to be significantly associated with a shorter survival (HR=2.30; p=0.018, HR=1.22; p=0.015, respectively). A higher AFP, a higher MELD score and a higher BCLC classification were also associated with a shorter survival (HR=1.30; p=0.008, HR=1.08; p=0.021, HR=1.95; p<0.001, respectively).

Table 2: Differences between patients with high and low HBV load

Characteristic	HBV DNA <10.5 (n =61)	HBV DNA ≥10.5 (n =37)	p-value
Age (year)*	56(23-77)	54(27-80)	0.650
Gender (male)	49(80%)	30(81%)	0.928
Total bilirubin(umol/L) *	15(4-481)	24(6-372)	0.058
Albumin(g/L) *	39(25-49)	35(22-48)	0.032
AST(U/L) *	62(21-328)	95(33-1278)	0.002
ALT(U/L) *	50(19-356)	67(32-670)	0.039
AFP(ng/ml)*	70(1-652660)	70(2-121000)	0.640
Non-cirrhotic	15(25%)	7(19%)	0.516
MELD score*	6(6-23)	7(6-25)	0.253
HBeAg‡	11(18%)	13(35%)	0.047
Anti-viral (nucleotide or nucleoside analogue) therapy	32(53%)	18(49%)	0.716
Number of tumours*	1(1-7)	1(1-4)	1.000
Tumour size (mm)*	34(8-200)	34(11-227)	0.714
Metastases	15(25%)	14(38%)	0.166
BCLC			0.466
Stage A	21(34%)	9(24%)	
Stage B	23(38%)	14(38%)	
Stage C	5(8%)	2(5%)	
Stage D	12(20%)	12(32%)	

*median(range), ‡ positive value

Table 3: Univariate analysis of factors associated with survival

Variable	hazard ratio (95% confidence limits)	p-value
Age (10 years)	1.00 (0.78-1.27)	0.97
Gender (female:male)	0.80 (0.41-1.57)	0.50
log total bilirubin (10 umol/L)*	1.02 (0.99-1.05)	0.31
log albumin(10g/L)*	0.68 (0.44-1.03)	0.07
log AST(U/L)*	2.30 (1.15-4.60)	0.018
log ALT(U/L)*	0.75 (0.31-1.80)	0.51
log AFP(ng/ml)*	1.32 (1.09-1.60)	0.006
MELD score	1.07 (1.01-1.14)	0.033
HBV DNA	1.22 (1.04-1.43)	0.015
HBeAg‡	1.47 (0.84-2.55)	0.19
Anti-viral (nucleotide or nucleoside analogue) therapy	0.69 (0.42-1.14)	0.15
BCLC	1.95 (1.54-2.48)	<0.001

*continuous value ‡ positive value

Association of serum HBV DNA levels at time of HCC diagnosis and overall survival

The median survival time of HCC patients with a high viral load was 15 months (1-62), and 25 months (1-114) in HCC patients with a low viral load (figure 1). The one-, three- and five-year survival rates of HCC patients with a high viral load were 58%, 32% and 11%, respectively. For HCC patients with a low viral load, the one-, three and five-year survival rates were 70%, 39% and 35%, respectively (figure 1). Patients with higher serum HBV DNA levels at the time of tumour presentation had a shorter overall survival compared with patients with lower serum HBV DNA levels (p=0.05). Including HCC treatment into the total multivariate analysis, a high viral load continued to be significantly associated with a shorter survival (HR=1.18 (95% CL; 1.01 to 1.38); p=0.042).

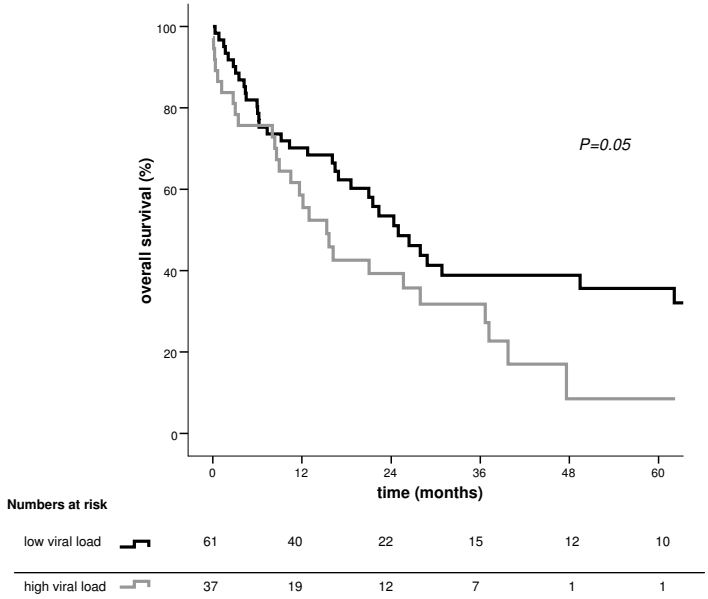

Figure 1: Overall survival of HCC patients with high and low HBV DNA

DISCUSSION

In our study we showed in multivariate analysis that a high AST level and high viral load were two independent factors associated with poor survival. A unique and important finding of this study is that it demonstrates the impact of high viral load on overall survival of HCC patients despite the treatment they received. Consistently, we observed that biochemical profiles indicative of active inflammation in our data were worse in patients who had high viraemia than in patients who had low viraemia, further sup-

porting the theory of the potential carcinogenic process through active inflammation associated with high viraemia.

Localised HCC tumours can be subjected to potentially curative treatments such as surgical resection, liver transplantation or radiofrequency ablation [17]. In our study 50 patients (51%) were able to receive treatment with curative intent. Only 8% of the study population received treatment with palliative intent; this low percentage is due to a limited availability of TACE treatment during the study period. Patients without treatment were often unable to receive treatment due to more advanced liver disease.

In many patients, HCC occurs against a background of advanced fibrosis or cirrhosis [20-22]. Cirrhosis decreases the regenerative capacity of the liver and therefore not every HCC patient is a suitable candidate for local surgical resection. Although many surgical and nonsurgical options have been developed for the treatment of HCC, the prognosis for these patients remains poor. Even in those who receive radical therapy, prevention of post-treatment recurrence remains a medical challenge [23].

Several factors have been reported to be associated with poor survival after surgical resection or local ablation therapies, including tumour characteristics, such as multiplicity, size, AFP levels, portal invasion, surgical tumour findings, parameters related to liver function such as albumin levels, and Child-Pugh classification [11,15].

Taking into account the fact that HCC arises in cirrhotic livers, evaluation of the detailed oncogenic process in patients with cirrhosis is an important subject for cancer prediction [17].

Liver cirrhosis due to hepatitis C virus usually shows a rather steady and constant clinical course, which enables us to estimate the future carcinogenesis rate only from clinical information at the time of the diagnosis of cirrhosis.

However, in contrast to hepatitis C and other risk factors, it is known that HBV-related HCC is less associated with the presence of cirrhosis, and this trend becomes more obvious in younger patients often infected at birth whose duration of infection is not long enough to develop full-blown cirrhosis [23]. This observation has prompted the suggestion that HBV itself has direct carcinogenic potential [23]. The detailed mechanism of HBV-related liver carcinogenesis is still unclear [18,24]. It is possible that active viral replication and HBx-protein expression contribute to the carcinogenic process [11]. Prospective studies have indicated a very strong correlation between the height of the viral load and the risk of developing HCC. Lamivudine therapy in patients with HBV-related compensated cirrhosis reduced the incidence of HCC in patients when viral suppression was sustained, but no previous report has studied the relationship of these viral factors and survival of HCC patients [11,15,25,26].

When we investigate the relationship between hepatocellular carcinogenesis and its affecting and contributing factors, explanatory parameters should include not only tumour-related factors but also data on the extent of the liver disease, as e.g. included

in the Child classification, BCLC and MELD classification. We also suggest including quantitative virological data in this prognostic modelling.

In the current study, patients with a higher viral load also had more elevated liver enzymes. Oral nucleoside or nucleotide analogue therapy has developed over the last years. The profile of drugs such as entecavir or tenofovir combines high efficacy with a low potential for resistance. Therefore, a logical next step is to treat all HBV-related HCC patients with nucleoside or nucleotide analogue therapy. A meta-analysis also suggested a potential efficacy of adjuvant interferon after curative therapy for HCC [27]. Two recent prospective studies focusing primarily on the correlation between hepatitis B viral load and recurrence of small HCC after curative resection revealed that HBV DNA level at resection was an important risk factor for recurrence of small HCC after surgery [9,28].

A potential limitation of the present study is that the data were based on a retrospective cohort study. A large-scale prospective trial should be conducted in the future to elucidate the effect of sustained viraemia on survival of HCC patients and the prospective roles of antiviral treatment.

In theory, treating high viral load patients with antiviral drugs both pre- and post-operatively is reasonable. Current treatment in patients with advanced HCC is sorafenib, where median survival can increase by nearly three months [29]. In this study high HBV viral load and hepatic inflammatory activity were both significantly associated with a poor prognosis; median survival was ten months longer in HCC patients with a low viral load [30].

Given the strong association between HBV viral load and overall survival, it is anticipated that the implementation of strategies for the use of antiviral therapy in this setting will result in a durable suppression of HBV replication and ultimately will lead to an increase of survival in HCC patients. We suggest that, for HCC patients with high serum HBV DNA levels, inhibition of viral replication may decrease inflammation and improve survival.

In conclusion, a lower albumin level or a higher serum AST or ALT activity are liver-related factors that are closely associated with a higher hepatitis B viral load. In our dataset as well as in the data of Qu et al. high HBV DNA shortened overall survival [28]. In the current analysis serum AST and viral load independently affected overall survival. This association supports the role for antiviral treatment for patients with a high HBV DNA together with treatment of HCC to increase overall survival. Further clinical trials with this endpoint are required to confirm the beneficial effect of hepatitis B viral suppression after HCC treatment to improve survival.

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PART VI

DISCUSSION, CONCLUSIONS AND FUTURE PERSPECTIVES

Chapter 11

General discussion, conclusions and future perspectives

GENERAL DISCUSSION

PART II - TRENDS IN HEPATOBILIARY CANCER

Hepatocellular carcinoma (HCC) is a disease with a highly uneven geographical distribution due the variation in the main causal factors [1,2]. In industrialised countries HCC is an uncommon type of cancer [1-4]. In the Netherlands the incidence of HCC was stable until 2000, but since then the incidence has increased. This observation is reported by various groups from low-endemic areas [1,2,4].

Chapter 2 describes the increasing incidence of HCC for males, but not for females in the Netherlands between 1989 and 2009. The observed increase in HCC in males might be related to the higher prevalence of non-alcohol fatty liver disease, which can be considered a precursor lesion of non-alcoholic steatohepatitis, as is also suggested by others [5]. Remarkably, the overall 1-year survival rate of patients with HCC receiving surgery, chemotherapy and/or irradiation, has improved. It has been speculated that this outcome may be related to changes in tumour treatment [6]. However, the survival of patients receiving no therapy at all has increased as well suggesting that earlier detection may play a role, perhaps in addition to a better treatment outcome. This lead-time bias may be the result of more effective screening programs. In addition, the improved outcome may also be attributed to an earlier and better treatment of complications of the underlying liver cirrhosis. In the Netherlands over 60% of the patients with HCC has underlying cirrhosis [7].

It has been suggested that the change in incidence of HCC could also be due to improved diagnosis nowadays as compared a decade ago: formerly misdiagnosed liver lesions may nowadays be diagnosed more adequately using computed tomography (CT) and high-resolution magnetic resonance imaging (MRI) of the liver. To date, the differences between HCC and other liver tumours can be demonstrated more clearly and the reliability of diagnosing various types of liver tumours has increased significantly. If changes in incidence are influenced by the quality of imaging technology, tumours previously described as unclassified might be expected to be classified adequately to date [8]. In case of misdiagnosis one would expect the incidence of at least one of the other hepatobiliary tumour to decrease to compensate the rise of HCC. We analyzed epidemiological trends of other liver tumours, including intrahepatic cholangiocarcinoma (ICC), that must be differentiated from HCC (***Chapter 3***). As the incidence rate of ICC has increased for both men and women, it's highly unlikely that misdiagnosis of these liver tumours may account for the increase of HCC. The reason for the rising incidence of ICC is still unknown [9,10]. Several medical conditions have been suggested as cause for ICC, including primary sclerosing cholangitis (PSC) and primary biliary cirrhosis [11,12]. As we didn't analyze data regarding changes in the prevalence of risk factors for ICC, we can not explain the rising incidence of ICC.

As for HCC a survival benefit for ICC patients that have been selected for surgical treatment has been observed. Increased ICC survival might be a reflection of patient selection over time, with an important role for imaging techniques that allow better pre-operative assessment of tumour extension and metastatic disease. Alternatively, increased survival could indicate lead-time bias related to early detection, as patients with associated factors mentioned above are controlled more often, including patients with PSC who have a lifetime risk of ICC ranging from 8-20% [9].

PART III - SCREENING FOR HEPATOCELLULAR CARCINOMA

The epidemiological trend on HCC in the Netherlands might be a reflection of better screening programs for patients at risk, thus leading more frequently to early detection of the disease. For many years alfa-fetoprotein (AFP) was the standard serum marker for the detection of HCC, despite its relatively low sensitivity (60%) [13]. As screening can lead to a better prognosis with increased long-term survival, we reviewed the status of recently reported new biomarkers for HCC screening. In a systemic review (***Chapter 4***) we correlated biomarkers GP73 (Golgi protein 73), IL-6 (interleukin-6) and SCCA (squamous cell carcinoma antigen) for HCC screening as compared to the outcome of screening using AFP. In our review process many papers had to be excluded because of limitations in study design. Our findings eventually were based on 7 manuscripts and suggested an advantage of GP73 over AFP as a serum marker for HCC screening. Although GP73 appears to better than AFP for diagnosing HCC, more research is required on GP73, especially because the assay used in the published clinical studies was less suitable for clinical use as the protein is detected by Western blot analysis.

Screening programs focus on patients with chronic hepatitis and cirrhosis as these diseases are related to premalignant lesions leading to HCC [13]. HCC occasionally develops in the absence of chronic liver disease or cirrhosis [7]. It is questioned whether both presentations of HCC have a common pathway of carcinogenesis. Some have suggested that HCC in non-hepatitis and non-cirrhotic livers may be the result of a malignant transformation of hepatocellular adenoma (HCA) [14]. If this hypothesis is correct one might assume the co-existence of both HCA and HCC within the same tumour at a certain moment. The exact point at which the switch to malignant transformation occurs remains unknown. Progression from a precursor lesion to cancer might be a multistep process, accompanied by a transition zone from benign hepatocytes towards dysplastic and malignant hepatocytes. However, as described in ***Chapter 5***, in none of the 52 HCCs in non-cirrhotic livers we studied an HCA component could be demonstrated, nor a transition zone displaying HCA changing into HCC. Even after using additional immunohistochemical staining (i.e. β-catenin, glutamine syntetase, CRP, L-FABP, and SAA) no HCA components were found. Ruling out the potential diagnosis of HCA based on the absence of validated markers for HCA is daring, as adenoma can present without

markers being positive. However, the immunohistological staining did not provide any arguments to support the transition from HCA to HCC [15]. In addition, reviewing the radiological images supported our concept as it was confirmed that all lesions were diagnosed as HCC and in no case a lesion was depicted as having characteristics of a liver adenoma.

PART IV - OPTIMIZATION OF STAGING

If patients are diagnosed with HCC it is important to have a prognostic profile to guide treatment decision-making. Nowadays, staging models include performance status, tumour extent and liver function. For those patients included for liver resection, or transplantation, the prognosis can be specified by using histological characteristics of the tumour in the resected specimens. Histological presence of microvascular invasion and a moderate or poor differentiation grade are accepted as independent predictors of poor survival [16-20]. After adjusting for age, gender, level of AFP and tumour size, our results display that the histologically differentiated grade and the presence of microvascular invasion are significantly associated with recurrence of HCC (***Chapter 6***). However, from a clinical point of view it would be preferable if HCC staging could be performed (pre-operatively) by using a non-invasive test. We attempted to correlate features of HCC on MRI with morphological findings of microvascular invasion and the differentiation grade in resection specimens. We found that the presence of wash-out indicates presence of microvascular invasion and/or a moderate or poorly-differentiated tumour. This observation might have clinically relevant implications for the imaging work-up of patients with HCC. Future studies should focus on the feasibility of predicting microvascular invasion and the various differentiation grades by further improving imaging techniques, e.g. diffusion-weighted imaging and hepatobiliary contrast agents to distinguish between the various differentiation grades and the presence of microvascular invasion of HCC. In addition these imaging features may be included in a predictive model defined by radiological criteria.

It has been suggested that a more detailed preoperative evaluation might identify patients with subclinical extra-hepatic disease. For years, bone scintigraphy was routinely used in selecting patients with HCC for liver transplantation or extensive resections. This approach has been subject of debate and for liver transplantation it has been demonstrated that scintigraphy is ineffective. The criteria to select patients for transplantation (Milan criteria) are restrictive and minimize the risk on metastases. Thus a more cost-effective approach is advocated. As described in ***Chapter 7*** many patients underwent a bone scintigraphy to exclude metastases in the workup for resection of HCC. The clinical indication for bone scintigraphy was based on subjective decisions. Our study displayed that at a cost of €53,221 bone scintigraphies (n=134) were performed in asymptomatic patients with HCC without detecting subclinical metastases even in

one patient. We conclude that baseline bone scintigraphy in patients with HCC is not indicated unless specific bone-related complaints are present in patients able to receive treatment with curative intent.

PART V – OUTCOME

Studies analysing treatment decision-making are focused on HCC patients with cirrhosis. A small number of studies has explored clinicopathological characteristics in HCC patients without cirrhosis [13,21,22], although these patients often do have risk factors for cirrhosis and HCC [21]. This selection bias may influence overall survival as it is known that, for example, chronic hepatitis B virus (HBV) influences survival in HCC patients [23]. In ***Chapter 8*** we studied patients with HCC but without any known risk factor for liver cancer or cirrhosis. In a multivariate analysis the presence of an increased AFP serum level pre-operatively was found to be the most important factor associated with a worse outcome and recurrence of HCC in a non-hepatitis and non-cirrhotic liver. Although AFP as a serological marker for HCC screening and surveillance in patients with cirrhosis is still debated [24], AFP seems a useful marker in patients with HCC without risk factors to indicate prognosis.

Apart from the interest in factors associated with outcome and recurrence of HCC in a non-cirrhotic liver, the biological behaviour of HCC in non-diseased liver is also explored [25]. In ***Chapter 9*** we investigated the expression of immunohistochemical markers in HCC in a non-cirrhotic liver. Our immunohistochemical data demonstrate a similarity in immunohistochemical expression of several markers comparing HCC in a non-cirrhotic liver with HCC in a cirrhotic liver. Although no definite conclusions can be drawn from this study, one may speculate that in line with the lack of difference in the immunohistological characteristics between HCC in a non-cirrhotic and in a cirrhotic liver, the oncobiological behaviour of HCC in a non-cirrhotic and in a cirrhotic liver doesn't differ as well. It should be noted that a negative AFP immunohistochemical staining does not exclude HCC and that this marker should be interpreted with care in case of tumour analysis in non-cirrhotic patients.

It has been demonstrated that β-catenin, Glypican-3 (GPC-3) and antigen Ki67 (MIB-1) are significantly more often positive in non-cirrhotic HCC patients with a moderate or poorly differentiated tumour. In addition MIB-1 expression is also increased in HCCs with the presence of vascular invasion. If one needs to distinguish well-differentiated HCC from a HCA on a needle core biopsy, β-catenin, GPC-3 and MIB-1 might be helpful. Also, cytokeratine 19 (CK19) was found to be positive more often in patients who displayed recurrence of disease during their follow-up. The relevance of these findings needs to be validated in a larger cohort in order to establish their use for clinical management.

The Netherlands is a low-endemic area for HCC with 64% of this malignancy occurring in patients with a cirrhotic liver [7]. Because HCC often arises in a cirrhotic liver,

evaluation of the detailed oncogenic process in patients with cirrhosis is an important topic for cancer prediction [13]. It is known that HBV-related HCC is less often associated with the presence of cirrhosis, and this trend becomes more obvious in younger patients (often infected at birth) whose duration of infection is not long enough to develop full-blown cirrhosis [26]. This observation prompted the suggestion that HBV itself has direct carcinogenic potential [26]. Prospective studies have indicated a very strong correlation between the level of the viral replication as measured by HBV-DNA and the risk of developing HCC [27]. In ***Chapter 10*** we studied the relation between the level of hepatocellular carcinogenesis (HBV DNA) and outcome in patients presenting with HCC. HCC patients with a high HBV DNA had a worse outcome. Several factors reported to be associated with a poor survival are included in the staging classification used for prognosis. Until now, however, no quantitative virological data are present in the prognostic models. Therefore, based on our results and reports from high-endemic areas, we suggest including quantitative virological data in these prognostic models, especially because efficient oral treatment of patients with high viral load is available. In our study, both high HBV viral load and hepatic inflammatory activity are significantly associated with a poor prognosis; median survival was 10 months longer in HCC patients with a low viral load. Given the strong association between HBV viral load and overall survival, it is anticipated that the implementation of strategies for the use of antiviral therapy in this setting will result in a durable suppression of HBV replication and lead to an increase of survival in HCC patients. It should be advocated for HCC patients with high serum HBV DNA levels to start antiviral treatment as it will decrease inflammation and may improve survival.

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CONCLUSIONS

Chapter 2: The age-standardized incidence of hepatocellular carcinoma (HCC) among all-male age groups, and among women aged under 60 years, has increased in the Netherlands between 1989 and 2009. Earlier disease detection affected the overall relative survival for HCC patients, while overall treatment-specific survival improved as well.

Chapter 3: The age-standardized incidence of intrahepatic cholangiocarcinoma among both sexes has increased in the Netherlands since 1999. Overall survival improved, suggesting a possible influence of improved imaging techniques, a better patient selection for surgery, and/or improved surgical techniques.

Chapter 4: A review of the literature displayed GP73 to be a valuable serum marker that is superior to AFP and can be useful in the diagnosis and screening for HCC. GP73 may improve detection and treatment of one of the most common malignancies worldwide. More studies are needed to further elucidate the influence of the aetiology of disease on signal strength of GP73.

Chapter 5: Using extensive immunohistochemical staining of non-cirrhotic livers we found no hepatocellular adenoma (HCA) components or a transition zones indicating the transition from HCA into HCC. It is highly unlikely that HCC in a non-cirrhotic liver is a HCA degenerated into a malignancy, and one should be aware of possible overtreatment in patients with HCA.

Chapter 6: Moderate to poorly-differentiated HCC and microvascular invasion are significantly associated with the presence of a capsule and wash-out, as demonstrated on dynamic contrast-enhanced MRI.

Chapter 7: There is no indication for routine use of a bone scintigraphy at baseline to detect metastases in asymptomatic patients included for surgical treatment for resectable HCC.

Chapter 8: If surgical resection is performed in a patient with HCC in a non-cirrhotic liver, good results can be achieved. The higher the preoperative AFP serum level, the worse the outcome.

Chapter 9: The immunohistochemical profile of HCC in a non-cirrhotic liver is comparable to the immunohistochemical profile of HCC in a cirrhotic liver. A negative immunohistochemical AFP marker does not exclude the presence of HCC in cirrhotic as well

as non-cirrhotic lesions. Immunohistochemical markers β-catenin, GPC-3 and MIB-1 may be helpful in establishing HCC differentiation grade. However, these markers cannot be used as a predictor for prognosis and survival.

Chapter 10: In HBV patients enhanced viral load and enhanced AST independently affect overall survival in patients with HCC. This correlation supports the role for antiviral treatment in HCC patients with a high HBV DNA, together with treatment of HCC to increase overall survival.

FUTURE PERSPECTIVES

The new insights in hepatocellular carcinoma, as discussed in this thesis, indicate that future research should focus on three main topics.

First of all, early tumour detection. Benefit can be gained from a proper surveillance program, as HCC is a tumour with a well-defined population at risk. Patients at risk should be registered and closely monitored by a social healthcare program. Primary and secondary surveillance remains important. The second topic should be the development of imaging techniques. This will continue to be important as non-invasive diagnostic test are preferred. Future research should focus on the feasibility of predicting prognosis by imaging, by developing new diffusion-weighted imaging techniques, or producing new targeted hepatobiliary contrast agents. However, we have to bear in mind the cost of staging investigations, as healthcare costs and (in particular) cancer costs are rising. Unnecessary staging investigations should be avoided; therefore, patients with a HCC should be discussed in a multidisciplinary team. Besides developing imaging techniques, research in biomarkers should be continued. With a non-invasive test serum, saliva or urine can be analyzed to see whether they contain biomarkers superior to GP73.

Finally, future studies should examine whether HCC in a non-cirrhotic liver has a different entity compared to HCC in a cirrhotic liver and compared to hepatocellular adenoma. In order to answer these questions, research on the oncogenic process of HCC in a non-cirrhotic liver is needed. Molecular genetic studies will be of importance to support or to reject the hypothesis of an adenoma-carcinoma sequence in HCC, and to confirm the hypothesis that the carcinogenesis of HCC in a cirrhotic and a non-cirrhotic liver is comparable using common pathways.

Chapter 12

Nederlandse samenvatting

DISCUSSIE

Deel II – TRENDS IN HEPATOBILIAIRE KANKER

Het voorkomen van het hepatocellulair carcinoom (HCC) kent wereldwijd een sterk variatie [1,2]. Dit verschil is een uiting van de verscheidenheid aan oorzaken [1,2]. In geïndustrialiseerde landen is HCC een weinig voorkomende vorm van kanker [1-4]. In Nederland was de incidentie met 1,7 en 0,5 per 100.000 personen per jaar voor respectievelijk mannen en vrouwen tot het jaar 2000 stabiel [4]. Daarna is er een toename te zien die overeenkomt met de toename in andere laag endemische landen [1,2,4].

In ***hoofdstuk 2*** wordt de stijgende incidentie voor mannelijke HCC patiënten in Nederland in de periode 1989 tot 2009 beschreven. De stijgende incidentie bij mannen kan gerelateerd zijn aan een hogere prevalentie van niet-alcoholische leververvetting (NAFLD). Onderzoekers suggereren dat NAFLD gezien kan worden als een voorstadium van niet-alcoholische steatohepatitis, een indirecte risicofactor voor HCC [5].

De 1-jaars overleving van HCC patiënten is verbeterd; dit geldt niet alleen voor patienten die behandeld zijn met chirurgie, chemotherapie en/of radiotherapie, maar ook voor onbehandelde patiënten. Een verklaring zou kunnen zijn dat naast een verbeterd behandelingsresultaat, vroegdetectie een rol speelt [6]. Deze 'lead-time bias' kan het gevolg zijn van de introductie van een echografisch screeningsprogramma voor patiënten die een verhoogd risico lopen op HCC door een onderliggende leverziekte. Bovendien kan de verbeterde overleving een gevolg zijn van een snellere en betere behandeling van de complicaties van levercirrose [7].

Met behulp van computertomografie (CT) en hoogresolutie magnetische resonantie beeldvorming (MRI) kan op een adequatere manier de diagnose HCC gesteld worden. De verschillen tussen HCC en andere levertumoren zijn duidelijker te definiëren zonder dat een biopsie nodig is voor de uitvoering van weefselonderzoek. Men kan de vraag stellen of de geconstateerde veranderingen in incidentie beïnvloed zijn door de verbetering in de kwaliteit van de beeldvormende technologie; immers, tumoren die eerder beschreven werden als "niet-geclassificeerd" zouden met de huidige, verbeterde technieken een diagnose kunnen krijgen [8]. Bij een onjuiste of onvolledige diagnose in het verleden is te verwachten dat percentueel tenminste een van de andere hepatobiliaire tumoren afneemt om de toename van HCC te verklaren. Wij analyseerden de epidemiologische trends van de andere levertumoren, waaronder het intrahepatische cholangiocarcinoom (ICC), om te bestuderen of dit effect bij deze tumor zichtbaar was (***hoofdstuk 3***). Het is zeer onwaarschijnlijk dat een verbeterde classificatie verantwoordelijk is voor de toename van HCC omdat ook de incidentie van ICC is toegenomen voor zowel mannen als vrouwen. De reden voor de stijgende incidentie van ICC is onduidelijk [9,10]. Verschillende aandoeningen zijn in verband gebracht met ICC waaronder primaire scleroserende cholangitis (PSC) en primaire biliare cirrose [11,12]. Omdat we de

prevalentie van de risicofactoren voor ICC niet konden analyseren met het beschikbare databestand, kunnen we de stijgende incidentie van ICC niet verklaren.

Een overlevingsvoordeel dat bij HCC patiënten gezien werd, is ook waargenomen bij ICC patiënten die chirurgische behandeld werden. Een verbetering van de overleving van ICC patiënten kan een gevolg zijn van een verbeterde patiëntenselectie. Hierin spelen de beeldvormende technieken een belangrijke rol, omdat de preoperatieve beoordeling van tumoruitbreiding en metastasering sterk verbeterd is. Een andere mogelijkheid is ook hier dat de toename van de overleving een gevolg is van 'lead-time bias', omdat patiënten met risicofactoren vaker gecontroleerd worden. Patiënten met PSC hebben gedurende hun leven bijvoorbeeld een life-time risico op ICC, dat geschat wordt, tussen de 8-20% [9].

DEEL III- SCREENEN VOOR HET HEPATOCELLULAIR CARCINOOM

De epidemiologische trend van HCC in Nederland kan een afspiegeling zijn van een screeningsprogramma bij risicopatiënten. Vele jaren was het alfa-fetoproteïne (AFP) de standaard serummarker voor het opsporen van HCC, ondanks de relatief lage sensitiviteit (60%) [13]. Omdat screening kan leiden tot een betere prognose, hebben we de status van de recent gerapporteerde nieuwe biomarkers voor HCC screening geanalyseerd. In een systematische review (***hoofdstuk 4***) hebben we de biomarkers GP73 (Golgi-eiwit 73), IL-6 (interleukine-6) en SCCA (plaveiselcelcarcinoom antigeen) vergeleken met AFP als detectiemarker. Onze bevinding, gebaseerd op 7 goed opgezette publicaties, stelt een voordeel van GP73 boven AFP als serummarker voor HCC screening. Hoewel GP73 superieur aan AFP lijkt, is meer onderzoek nodig. Ook zal de klinische bruikbaarheid van de test moeten worden aangepast naar een ELISA formaat omdat de test die in de gebruikte publicaties gebruikt wordt, een 'Western blot' analyse, zeer bewerkelijk is voor de routine diagnostiek.

Screeningsprogramma's richten zich op patiënten met chronische hepatitis en levercirrose omdat deze patiënten een risicogroep vormen voor het ontwikkelen van HCC [13]. HCC kan zich echter ook ontwikkelen in afwezigheid van chronische leverziekten of levercirrose [7]. Het is de vraag of beide vormen van HCC een vergelijkbare carcinogenese kennen. Door sommige auteurs wordt gesuggereerd dat HCC in een niet-hepatitis en niet-cirrose lever ontstaat uit een maligne getransformeerd hepatocellulair adenoom (HCA) [14]. Als deze veronderstelde hypothese juist is, kan het naast elkaar bestaan van HCA en HCC binnen dezelfde tumor verwacht worden. Een premaligne laesie zal een overgangszone tonen van goedaardige hepatocyten naar dysplatische (en kwaadaardige) hepatocyten. Wij onderzochten deze hypothese van benigne naar maligne transformatie en beschrijven in ***hoofdstuk 5*** dat wij geen aanwijzingen vinden die het concept bevestigen. Er zijn geen HCC- delen in niet-cirrotische levers waarin een HCA-component kan worden aangetoond. Ook na gebruik van aanvullende im-

munohistochemische kleuringen (o.a. β-catenine, glutamine syntetase, CRP, L-FABP en SAA) worden geen HCA componenten teruggevonden in HCC-haarden. Het uitsluiten van de mogelijke diagnose HCA op basis van het ontbreken van gevalideerde immunohistochemische markers voor HCA is gedurfd, adenomen kunnen zich namelijk presenteren zonder aanwezigheid van een positieve immunohistochemische marker. Echter, de immunohistochemische kleuringen geven geen enkel argument om de overgang van HCA naar HCC te steunen [15]. Ter ondersteuning van ons concept hebben we de radiologische beelden herbeoordeeld. Bij de herbeoordeling zijn alle tumoren geduid als HCC, geen van de laesies werd geduid als HCA.

DEEL IV – HET OPTIMALISEREN VAN DE STADIERING

Voor patiënten met een HCC is het maken van een prognostisch profiel belangrijk omdat dit kan dienen als leidraad bij de besluitvorming over de behandeling. Modellen die gebruikt worden richten zich op de vitaliteit van de patiënt, de grootte van de tumor en de functionaliteit van de lever. Voor patiënten die geopereerd of getransplanteerd zijn, geven de histologische kenmerken postoperatief een goed beeld van de prognose. Aanwezigheid van vasculaire invasie en een matige of slechte differentiatiegraad vormen geaccepteerde voorspellers van een slechte overleving [16-20]. Na correctie voor leeftijd, geslacht, hoogte van het serum AFP en grootte van de tumor zijn in onze resultaten histologische differentiatiegraad en aanwezigheid van vasculaire invasie significant geassocieerd met het optreden van recidief HCC (***hoofdstuk 6***). Echter, uit klinisch oogpunt verdient het de voorkeur dat de prognose pre-operatief bepaald kan worden, bijvoorkeur met een niet-invasieve methoden. Wij probeerden om histologische eigenschappen van HCC, zoals vasculaire invasie en differentiatiegraad, te correleren aan radiologische kenmerken bij MRI onderzoek en stellen vast dat een typische opname en uitscheiding patroon van contrast ('washout') vaker aanwezig was bij tumoren met vasculaire invasie en/of een matige of slechte differentiatiegraad. Deze observatie kan gevolgen hebben voor de waarde van beeldvorming tijdens de preoperatieve analyse van patiënten met een HCC en vervolgonderzoek zal dit moeten bevestigen. Dit kan gedaan worden door diffusie-gewogen beeldvorming te gebruiken of door gebruik te maken van andere hepatobilliare contrastmiddelen. Gedetailleerde pre-operatieve evaluatie draagt bij aan het identificeren van patiënten met subklinische extra-hepatische ziekten. Jarenlang was botscintigrafie een routine onderzoek bij HCC patiënten die in aanmerking kwamen voor een uitgebreide leverresectie of -transplantatie. Deze aanpak is echter onderwerp van debat nu aangetoond is dat het geen zin heeft om routinematig een botscintigrafie te maken bij patiënten die getransplanteerd worden voor HCC. De criteria om patiënten te selecteren voor transplantatie (Milaan criteria) zijn restrictief en minimaliseren het risico op uitzaaiingen. De patiënten beschreven in ***hoofdstuk 7*** ondergingen een botscintigrafie om te beoordelen of er sprake was van gemetasta-

seerde ziekten bij patiënten die in aanmerking kwamen voor een leverresectie. De klinische indicatie voor de botscintigrafie was gebaseerd op subjectieve bevindingen. Onze studie geeft weer dat een bedrag van €53.221,- wordt besteed aan botscintigrafiën bij asymptomatische patiënten (n=134) zonder dat er één metastase ontdekt wordt. Wij concluderen dat preoperatieve botscintigrafie bij patiënten die in aanmerking komen voor een in opzet curatieve behandeling van het HCC niet geïndiceerd is tenzij er specifieke botgerelateerde klachten zijn.

DEEL V – DE UITKOMST

Veel studies richten zich op patiënten met HCC in aanwezigheid van levercirrose. In een enkele studie onderzocht men klinisch-pathologische kenmerken van HCC patiënten zonder levercirrose [13,21,22]. Deze patiënten hebben echter vaak risicofactoren voor het ontwikkelen van levercirrose en een HCC [21]. Deze selectie bias kan van invloed zijn op de uitkomst, vooral op de overleving aangezien bekend is dat bijvoorbeeld chronische hepatitis B (HBV) de overleving van HCC patiënten beïnvloed [23].

In ***hoofdstuk 8*** analyseerden we patiënten met HCC in afwezigheid van risicofactoren voor levercirrose of HCC. In de multivariabele analyse bleek de aanwezigheid van een preoperatief verhoogd serum AFP als belangrijkste factor geassocieerd te zijn met een slechtere uitkomst en een grotere kans op HCC recidieven in een niet-hepatitis en niet-cirrotische lever. Hoewel AFP als een serologische marker voor HCC screening en surveillance bij patiënten met levercirrose nog steeds ter discussie staat [24], lijkt het een nuttige prognostische marker bij patiënten met een HCC zonder risicofactoren. Naast de belangstelling voor factoren die samenhangen met recidief HCC in een niet-cirrotische lever, werd het biologische gedrag van HCC in een niet-hepatitis en niet-cirrotische lever onderzocht [25]. In ***hoofdstuk 9*** analyseerden we de kenmerken van immunohistochemische markers bij HCC in een niet-cirrotische lever. Als we HCC in een niet-cirrotische en HCC in een cirrotische lever vergelijken tonen onze resultaten veel overeenkomsten tussen de tumor markers in de verschillende groepen. Dit gegeven, gecombineerd met het oncobiologisch gedrag van HCC in een niet-cirrotische en cirrotische lever, lijkt de conclusie te rechtvaardigen dat de onderliggende cirrose niet van invloed is op de oncologische genese van het HCC. Opgemerkt moet worden dat een negatieve AFP immunohistochemische kleuring een HCC niet uitsluit en dat deze marker dan ook met zorg geïnterpreteerd dient te worden bij het analyseren van tumoren in niet-cirrose patiënten. Aangetoond is dat β-catenine, Glypican-3 (GPC-3) en antigen Ki67 (MIB-1) significant vaker positief zijn bij patiënten met een matig of slecht gedifferentieerd HCC in een niet-cirrotische lever. Bovendien is de MIB-1 expressie verhoogd bij HCC met vasculaire invasie. Het gebruik van β-catenine, GPC-3 en MIB-1 kan misschien nuttig zijn als het onderscheid tussen een goed gedifferentieerd HCC en HCA gemaakt moet worden op bijvoorbeeld weefsel afkomstig van een naaldbiopsie.

Cytokeratine 19 (CK19) is vaker positief bij patiënten met een recidief. De relevantie van deze bevindingen moet worden gevalideerd in een groter cohort om de voorspellende waarde klinisch te toetsen.

Nederland is een laagendemisch gebied voor HCC en HCC presenteert zich in 64% bij patiënten met levercirrose [7]. Omdat HCC zich vaak in een cirrotische lever ontwikkelt, is studie naar de carcinogenese bij levercirrose belangrijk [13]. Het is bekend dat een HBV-gerelateerd HCC minder vaak geassocieerd is met de aanwezigheid van levercirrose. Dit is meer voor de handliggend bij jonge patiënten (vaak besmet bij de geboorte), waarbij de duur van de infectie niet lang genoeg is om een volledige levercirrose te ontwikkelen [26]. Dit geeft aan dat HBV zelf carcinogenese kan induceren zonder de tussenstap van cirrose [26]. Recente studies hebben aangetoond dat er een sterke correlatie is tussen het niveau van de virale replicatie gemeten als kwantitatief HBV-DNA en de kans op het ontwikkelen van HCC [27]. In ***hoofdstuk 10*** hebben we de relatie tussen de hoogte van het HBV-DNA en de uitkomst bij patiënten met een geopereerd HCC geanalyseerd. HCC patiënten met een hoog HBV-DNA hadden een slechtere uitkomst. Verschillende factoren zijn in verband gebracht met een slechtere overleving en daarom opgenomen in classificatie systemen waarmee de prognose van HCC patiënten voorspeld wordt. Echter, tot nu toe zijn kwantitatieve virologische gegevens niet geïmplementeerd in deze prognostische modellen. Op basis van onze resultaten en verslagen uit hoogendemische gebieden, stellen we voor om de kwantitatieve virologische gegevens in de prognostische modellen te implementeren. Vooral ook omdat een efficiënte behandeling van patiënten met een hoog HBV-DNA beschikbaar is. In onze studie zijn zowel een hoog HBV-DNA als leverinflammatie significant geassocieerd met een slechtere prognose; de mediane overleving was 10 maanden langer in HCC patiënten met een laag HBV-DNA. Gezien de sterke associatie tussen het HBV-DNA en de totale overleving, is de verwachting dat het gebruik van antivirale therapie zal resulteren in een onderdrukking van HBV replicatie, wat zal leiden tot een toename van de overleving van HCC patiënten. Wij pleiten ervoor om bij HCC patiënten met een hoog serum HBV-DNA te starten met antivirale therapie om zo de ontsteking te verminderen en de overleving te verbeteren.

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CONCLUSIES

Hoofdstuk 2: Tussen 1989 en 2009 is in Nederland de voor leeftijd gecorrigeerde incidentie van het hepatocellulair carcinoom voor mannen in alle leeftijdsgroepen en voor vrouwen tot 60 jaar gestegen. Het vroeg vaststellen van de diagnose heeft geresulteerd in een stijging van de relatieve algehele overleving voor patiënten met een hepatocellulair carcinoom.

Hoofdstuk 3: De voor leeftijd gecorrigeerde incidentie van het intrahepatische cholangiocarcinoom bij zowel mannen als vrouwen is toegenomen in Nederland sinds 1999. De overleving verbeterde, hetgeen duidt op een mogelijke invloed van verbeterde beeldvormende technieken, een betere patiëntselectie voor operatie en een verbetering van de chirurgische technieken.

Hoofdstuk 4: Een meta-analyse van de literatuur geeft aan dat Golgi-eiwit 73 een waardevolle serummarker is, superieur aan alfa-fetoproteïne en nuttig bij de diagnostiek en het screenen voor hepatocellulair carcinoom. Golgi-eiwit 73 kan het diagnosticeren en behandelen verbeteren en aanvullende studies zijn nodig om de plaatsbepaling van deze tumormarker te bevestigen.

Hoofdstuk 5: Wij vinden het hoogst onwaarschijnlijk dat hepatocellulair carcinoom in een niet-cirrotische lever ontstaat uit een hepatocellulair adenoom. Gebruik makend van immunohistochemische kleuringen vonden we geen hepatocellulair adenoom componenten of een overgangszone van hepatocellulair adenoom naar hepatocellulair carcinoom. Deze bevinding kan erop wijzen dat het risico op maligne degeneratie van een leveradenoom zeer klein is en dat overbehandeling uit angst voor maligniteit voorkomen moet worden.

Hoofdstuk 6: Een matig tot slecht gedifferentieerd hepatocellulair carcinoom en/ of een hepatocellulair carcinoom met vasculaire invasie is geassocieerd met de aanwezigheid van een kapsel en 'washout' zoals zichtbaar is op een dynamische MRI met contrast.

Hoofdstuk 7: Er is geen indicatie voor het routinematig verrichten van een preoperatieve botscintigrafie ter opsporing van metastasen bij asymptomatische patiënten die in aanmerking komen voor een in opzet curatieve behandeling van het hepatocellulair carcinoom.

Hoofdstuk 8: Goede resultaten kunnen bereikt worden bij een chirurgische resectie van een hepatocellulair carcinoom bij patiënten met een niet-cirrotische lever. Echter, hoe hoger het preoperatieve serum alfa-fetoproteïne, hoe slechter de uitkomst.

Hoofdstuk 9: Het immunohistochemische profiel van een hepatocellulair carcinoom in een niet-cirrotische lever is vergelijkbaar met het immunohistochemische profiel van een HCC in een cirrotische lever. Een negatieve alfa-fetoproteïne marker sluit de aanwezigheid van een hepatocellulair carcinoom niet uit. De markers β-catenine, Glypican-3 en antigen Ki67 kunnen nuttig zijn bij het vaststellen van de differentiatiegraad. Deze markers zijn echter geen prognostische voorspellers, noch van invloed op de overleving.

Hoofdstuk 10: De hoogte van het HBV-DNA en het aspartaat aminotransferase beïnvloeden onafhankelijk van elkaar de overleving van hepatocellulair carcinoom patiënten. Deze correlatie ondersteunt de rol van antivirale therapie bij hepatocellulair carcinoom patiënten met een hoog HBV-DNA, dat deze tezamen met de behandeling van het hepatocellulair carcinoom de totale overleving zal verbeteren.

TOEKOMST VISIE

De nieuwe inzichten in het hepatocellulair carcinoom, zoals besproken is in dit proefschrift, maken dat toekomstig onderzoek zich moet richten op drie belangrijke onderwerpen.

Allereerst vroegdetectie. Voordeel kan behaald worden uit een goed georganiseerd screeningsprogramma, aangezien hepatocellulair carcinoom voornamelijk voorkomt in een goed definieerde risicopopulatie. Patiënten die een verhoogd risico lopen moeten geregistreerd worden en primaire en secundaire preventie moet worden aangeboden.

Het tweede onderwerp is de verdere perfectionering van beeldvormende technieken. Dit is belangrijk omdat er vraag is naar niet-invasieve diagnostische testen, voornamelijk voor de kleinere afwijkingen. Toekomstig onderzoek moet zich richten op de haalbaarheid van het voorspellen van de aard van voornamelijk de kleine afwijkingen met behulp van beeldvormend onderzoek. Het ontwikkelen van nieuwe diffusie-gewogen beeldvormende technieken, of het produceren van nieuwe hepatobiliair specifieke contrastmiddelen kan bijdragen aan de bepaling van de mate van agressiviteit van de tumor. We moeten echter rekening houden met de kosten van deze onderzoeken. Naast het ontwikkelen van beeldvormende technieken moet onderzoek naar biomarkers voortgezet worden. Met niet-invasieve testen kan serum, speeksel of urine geanalyseerd worden om te zien of ze biomarkers bevatten die superieur zijn aan GP73.

Het derde onderwerp van onderzoek moet gericht zijn op de analyse van hepatocellulair carcinoom in cirrotische en niet-cirrotische levers en het proces van carcinogenese. Om deze vragen te beantwoorden, is moleculair genetische onderzoek naar het oncogenetisch profiel van hepatocellulair carcinoom in verschillende situaties nodig met als controlemateriaal leverweefsel met chronische hepatitis en leverweefsel met cirrose. Beter inzicht in het mechanisme van tumorontwikkeling en groei in diverse parenchymale condities kan de behandelingsmogelijkheden verder verbeteren.

Chapter 13

Dankwoord, List of publications, Curriculum Vitae, PhD Portfolio

DANKWOORD

Het is af, met opluchting begin ik aan het dankwoord van mijn proefschrift! Vele hebben een bijgedrage geleverd aan de totstandkoming van dit proefschrift. Zonder hun hulp zou het niet gelukt zijn en daarom is mijn dank aan hen groot. Een aantal personen wil ik in het bijzonder bedanken.

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Mijn copromotor, dr. R.A. de Man, beste Rob, pas later realiseerde ik me dat ik tijdens onze eerste ontmoeting al kennis maakte met je alles overziende blik. Je hebt de kwaliteit om haarfijn de kern van een onderwerp bloot te kunnen leggen. Ik waardeer je om je scherpe visie, het meedenken en je opbeurende woorden. Ik ben blij dat je mijn copromotor bent.

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De medeauteurs van de gebruikte artikelen zijn van grote waarde geweest. Hoofdstuk 2, 3 en 4 zouden niet gelukt zijn zonder de hulp van de Intergrale Kanker Centra. Speciale dank gaat uit naar professor Coebergh, Henrike Karim-Kos, Esther de Vries en Otto Visser.

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LIST OF PUBLICATIONS

Publications (this thesis):

Witjes CD, Karim-Kos HE, Visser O, van den Akker SA, de Vries E, IJzermans JN, de Man RA, Coebergh JW, Verhoef C. Hepatocellular carcinoma in a low-endemic area: rising incidence and improved survival. Eur J Gastroenterol Hepatol 2012;24:450-457.

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Is bone scintigraphy indicated in surgical work-up in HCC patients? Accepted by J Surg Res

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CURRICULUM VITAE

Caroline Dorothe Marjoleine (Carlijn) Witjes was born on March 3rd 1982 in Bergen, Limburg, the Netherlands. After graduation from high school at the Elzendaalcollege in Boxmeer in 2001, she started her medical studies at Erasmus University Rotterdam. During this period she did an internship at the Department of Surgery at the St. Elisabeth Hospital in Curaçao, the Netherlands Antilles. Her medical degree was obtained in October 2008, after which she took up a surgical residency at the Erasmus Medical Centre in Rotterdam (prof.dr. J.J.B. van Lanschot). After almost one year she started her PhD project (in September 2009) at the Department of Surgery at the Erasmus University Medical Centre in Rotterdam, under the supervision of prof.dr. J.N.M. IJzermans, dr. R.A. de Man and dr. C. Verhoef, which has resulted in this thesis.

In July 2012 she started her general surgical training at the Amphia Hospital in Breda, the Netherlands (supervisors: dr. L. van der Laan and prof.dr. J.N.M. IJzermans).

PHD PORTFOLIO

Summary of PhD training and teaching

Name PhD Student:
Caroline Dorothe Marjoleine Witjes, MD

Erasmus MC Department:
Surgery

PhD period:
September 2009- June 2012

Promotor:
professor J.N.M. IJzermans, MD PhD

Supervisors:
R.A. de Man, MD PhD and C. Verhoef, MD PhD

1. PhD training

	Year	Workload (ECTS)
General courses		
Introduction to clinical research	2010	0.9
Biostatistics for clinician	2010	1.0
BROK *('Basiscursus Regelgeving Klinisch Onderzoek')*	2010	1.0
Seminars and workshops		
Journal club	2009-2011	3.0
Schrijven van een wetenschappelijke publicatie	2009	0.3
CPO Minicursus voor Methodologie van Patiëntgebonden Onderzoek en Voorbereiding van Subsidieaanvragen	2010	0.3
Presentations		
National conferences	2009	3.0
International conferences	2009	1.0
National conferences	2010	3.0
International conferences	2010	6.0
National conferences	2011	5.0
International conferences	2011	7.0

2. Teaching

	Year	Workload (ECTS)
Lecturing		
Teaching (nurses in training)	2009	0.5
Supervising practicals and excursions, Tutoring	2009-2010	1.0
Examination of Basic Life Support (EHBO) of medical students		