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Improved survival of patients with HCC through new therapeutic options and the use of multimodal therapy concepts: Data from a large German university hospital

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List of Abbreviations

AFB1	Aflatoxin B1
AFP	Alphafetoprotein
AIH	Autoimmune hepatitis
BCLC	Barcelona-Clinic Liver Cancer
CI	Confidence interval
CLIP	Cancer of the Liver Italian Program
CR	Complete response (RECIST)
CT	Computed tomography
DFS	Disease-free survival
ECOG	European Cooperative Oncology Group
e.g.	For example
EGFR	Epidermal growth factor receptor
ERBB	ErbB receptor
Fig.	Figure
Gy	Gray
HBV	Hepatitis B virus
HCC	Hepatocellular carcinoma
HCV	Hepatitis C virus
HH	Hereditary hemochromatosis
HR	Hazard ratio
i.e.	*id est* (in other words)
LITT	Laser-induced interstitial thermotherapy
MRI	Magnetic resonance imaging
MTOR	Mammalian target of rapamycin
NAFLD	Non-alcoholic fatty liver disease
NASH	Non-alcoholic steatohepatitis
n.s.	Not significant
OS	Overall survival
PBC	Primary biliary cirrhosis
PD	Progressive disease (RECIST)
PEI	Percutaneous ethanol injection
PET	Positron emission tomography
PR	Partial response (RECIST)
PSC	Primary sclerosing cholangitis

RCT	Randomized controlled trials
RECIST	Response evaluation criteria in solid tumors
RFA	Radiofrequency ablation
SD	Stable disease (RECIST)
SIRT	Selective internal radiation therapy
SLD	Sum of the longest tumor diameters
Tab.	Table
TACE	Transarterial chemoembolization
TAE	Transarterial embolization
TNM	Tumor-Node-Metastasis
VEGFA	Vascular endothelial growth factor A
vs	versus
WHO	World Health Organization

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Zusammenfassung

Verbessertes Überleben von Patienten mit HCC durch neue Therapieoptionen und den multimodalen Einsatz von Therapieverfahren: Erfahrung aus einer großen deutschen Universitätsklinik

Autor: Isaac Myers

Das hepatozelluläre Karzinom (HCC) ist eine bösartige Tumorerkrankung der Leber, die überwiegend bei Patienten mit Leberzirrhose vorkommt, und deren Behandlung seit Jahrzehnten ein Problem für Kliniker darstellt. Da das HCC zur Zeit die dritthäufigste krebsassoziierte Todesursache weltweit ist, sind therapeutische Fortschritte in diesem Bereich von großer medizinischer Bedeutung.

In der vorliegenden Studie wurden 136 HCC-Patienten, die im Zeitraum 2006-2012 unter modernsten therapeutischen Bedingungen in der hepatologischen Ambulanz der Charité Universität Berlin behandelt wurden, retrospektiv ausgewertet. Das Ziel der Arbeit war, prognostische Faktoren für das HCC zu identifizieren, und die Aussagekraft der für das HCC gebräuchlichen Staging-Systeme zu verifizieren. Das Patientenüberleben nach Kaplan-Meier wurde mit dem von ähnlichen Patientenkohorten aus der Literatur verglichen.

Baseline-Faktoren, denen retrospektiv eine signifikante prognostische Bedeutung zugeordnet werden konnte, waren Leberfunktion, Patientenstatus (ECOG score), Tumorgröße, Anzahl der Läsionen, AFP-Spiegel und Gefäßinfiltration. Bei der Auswertung von Staging-Systemen schienen die TNM, Okuda, und CLIP Systeme gleichwertig zu sein. Das BCLC-System zeigte die schlechteste prognostische Wertigkeit, was auf die teilweise subjektive Einschätzung des Patientenstatus zurückzuführen war.

Bei Einsatz der modernsten therapeutischen Ansätze, insbesondere der interstitiellen Brachytherapie mit Afterloading, und des neuen zielgerichteten Therapeutikums Sorafenib, zeigte sich eine deutliche Steigerung im Patientenüberleben verglichen mit Kohorten aus der Vergangenheit (gesamtes Überleben: 42 Monate, krankheitsfreies Überleben: 9 Monate). Der Einsatz von multimodalen therapeutischen Konzepten zeigte sich darüberhinaus als lebensverlängernd sowohl in univariaten als auch in multivariaten Analysen.

Abstract

Improved survival of patients with HCC through new therapeutic options and the use of multimodal therapy concepts: Data from a large German university hospital

Author: Isaac Myers

Hepatocellular carcinoma (HCC) is a malignant tumor disease of the liver that predominantly occurs in patients with liver cirrhosis and whose treatment has posed a problem for clinicians for decades. Given that HCC is currently the third highest cancer-associated cause of death worldwide, therapeutic advances in this area are of the utmost medical significance.

In the current study, 136 HCC patients treated during the time period of 2006-2012 in the hepatological clinic of the Charité University Berlin under the most modern therapeutic conditions were analyzed retrospectively. The aim of this work was to identify prognostic factors for HCC and to verify the validity of the staging systems commonly used for HCC. Kaplan-Meier patient survival was compared to that of similar patient cohorts in the literature.

Baseline factors that could be shown retrospectively to possess a significant prognostic function were liver function, patient status (ECOG score), tumor size, lesion number, AFP level, and vascular invasion. In the analysis of staging systems, the TNM, Okuda, and CLIP systems appeared to be of equal value. The BCLC system showed the poorest prognostic value, which could be attributed to the partially subjective estimation of patient status.

Using the most modern therapeutic strategies, particularly interstitial brachytherapy with afterloading, as well as the new molecular targeted agent, sorafenib, a clear increase in patient survival was shown compared to past cohorts (overall survival: 42 months, disease-free survival: 9 months). Moreover, the use of multimodal therapeutic concepts was shown to improve survival in both univariate and multivariate analyses.

1 Introduction

1.1 General information

Hepatocellular carcinoma (HCC) is an aggressive tumor of the liver that has long proved challenging for clinicians to treat effectively due to its elusive, symptom-free growth in early stages, resistance to classical chemotherapy regimes, and growth in predominantly cirrhotic individuals. Given its high rates of incidence and mortality, particularly in the third world, HCC is also recognized as a cancer of global importance. Hope for the improvement of therapy for HCC has grown out of recent advances in diagnostic tools, locoregional therapy and liver-specific systemic chemotherapy.

1.2 Epidemiology

According to recent cancer statistics, cancer of the liver currently represents the sixth most frequently diagnosed cancer worldwide (fifth in men, seventh in women) but due to its high fatality, it is the third most frequent cause of cancer death (second in men, sixth in women).[1] Of the 696,000 deaths attributed worldwide to liver cancer in 2008, HCC accounted for an estimated 85-90% of cases.[2] Interestingly, as suggested by the incidence and mortality rankings given above, HCC shows a strong male predominance, with a worldwide male to female ratio of 2.4.[3] However, this pattern of distribution differs greatly depending on the region in question, for instance, medium-risk European nations such as Italy and France have reported ratios greater than 5:1, whereas lower-risk areas in South America such as Colombia and Costa Rica have an almost equal distribution (1.2:1 and 1.6:1 respectively).[4]

Levels of incidence are also strongly dependent on geographic context, with the distribution of disease clearly to the burden of developing countries. Areas particularly affected are south-eastern Asia, eastern Asia, sub-Saharan Africa and Melanesia, which together account for 85% of all cases.[3,5] China alone has been estimated as the source of 50% of all HCC cases worldwide and has an incidence of 37/100,000 in men and 14/100,000 in women.[3,4,6] In developed countries the incidence of HCC is significantly lower, with North America and most western European nations displaying incidence levels of 4-8/100,000 in men and approximately 2/100,000 for woman. An exception is seen in southern European regions (i.e. Italy and Greece), which have levels reaching 13/100,000 for men. The incidence in Germany, where this study took place, is approximately 6/100,000 for men and 2/100,000 for women.[3]

Independent of gender and regional differences, incidence of HCC also increases progressively with advancing age in all populations. A peak in incidence is reached at 70 years in most regions, with the exception of Chinese and black African populations, where the mean age of patients is appreciably lower due to the prevalence of endemic risk factors, and Japan, where

the peak is higher, due to risk factor exposure in certain age cohorts (see below).[7,8] The peak age for women is generally about five years older in all populations.[4]

Although some key regions (i.e. China, Taiwan, and Japan) have reported a decline in HCC incidence in recent decades,[7,9,10] the worldwide incidence appears to be growing.[8] While many developing countries are experiencing downward trends in HCC incidence, incidence is increasing in low-risk areas, such as North America and many European nations (i.e. Denmark, Germany, Spain, and United Kingdom). Conversely, European countries that have hitherto had higher levels of incidence (i.e. France and Italy) have experienced decreases in incidence within the last two decades.[11] A worrying trend can be seen in North America, where HCC incidence tripled in the period 1975 - 2005[12] and HCC mortality increased by 40% in the time from 1990 – 2004, in spite of an 18% overall decrease in cancer mortality during the same period.[1] It is generally accepted that these regional variations in HCC incidence are directly linked to differences in risk factor exposure amongst populations. Thus, one cannot fully appreciate the reasons for these upward or downward trends in incidence without exploring the factors that facilitate the development of HCC.

1.3 Etiology and risk factors

HCC is unique as a malignant tumor in that – rather than showing familial patterns of development, as is often the case in tumor disease – it arises in the context of chronic liver disease and cirrhosis in up to 90% of cases.[13,14] The 5-year cumulative risk of acquiring HCC in the presence of liver cirrhosis ranges from 5% to 30%, depending on the cause of cirrhosis, the region, ethnicity, and the stage of cirrhosis.[14,15] Hence, liver cirrhosis can be regarded as a premalignant condition, and the key risk factors for HCC are therefore conditions that lead to cirrhotic transformation of the liver. However, given that cirrhosis is a process that occurs over many years, there is often a significant time lapse between risk factor exposure and occurrence of HCC, as will become clear in the following. The risk factors responsible for HCC development differ vastly depending on the region in question (i.e. third-world versus first-world). Hence, it is easiest to outline the most relevant risk factors in terms of global region although it must be noted that some overlap between regions does occur. The following table illustrates the main differences in risk factor distribution:[16,17]

Table 1: Distribution of the main risk factors for HCC in Asia and Africa versus Europe

Risk factor	Asia and Africa (%)	Europe (%)
HBV	70	10
HCV	20	60
Alcohol	10	20
Others	<10	10

1.3.1 Risk factors in the developing world (hepatitis B and aflatoxin B1)

On a worldwide scale, chronic infection with the hepatitis B virus (HBV) is by far the most important risk factor (400 million people are infected with HBV globally), due to its endemic nature in Asian and African regions. In these areas, HBV is transmitted at childbirth from mothers to newborns and thus the peak age in incidence for HCC is much younger than in developed countries, where new infections generally occur in adults. HBV is thus considered to virtually be the sole cause of HCC in children, and in all age groups it accounts for more than 50% of cases worldwide.[15,18] While the development of HCC usually occurs via HBV-induced cirrhotic transformation of the liver, it can also arise in non-cirrhotic livers, as HBV DNA is integrated into the host's genome, causing mutations in growth factors and tumor suppressor genes.[19]

An important co-factor for HCC in developing regions is dietary exposure to aflatoxin B1 (AFB1), a fungus that grows on food products stored in damp, warm conditions. If ingested, AFB1 acts as a powerful carcinogen in the liver and causes a characteristic mutation in the p53 tumor-suppressor gene.[4,20,21] Studies based in China, where HBV infection and AFB1 exposure are highly prevalent, have shown increased HCC risk ranging from 3-fold[22] to 60-fold[23] for individuals exposed to both risk factors.

In an effort to prevent exposure to risk factors, immunization programs have been introduced by the World Health Organization (WHO) with the aim of universally vaccinating infants against HBV. As of 2011, this had been achieved in 93% of countries.[24] The benefit of vaccination can be demonstrated by studies performed in Taiwan where, following the introduction of a universal infant vaccination program against HBV, incidence of HCC in children and young adults decreased by approximately 70% within 20 years. The remaining cases of HCC could be attributed to a lack of, or incomplete HBV vaccination.[10,25] Less successful examples can be found in other low-income countries, particularly in sub-Saharan Africa, where as few as 10% of infants (in Chad) receive the full course of vaccination, despite the implementation of a universal vaccination program.[26]

A further strategy aimed at reducing HCC incidence in the developing world is the reduction of AFB1 ingestion. This has proven to be achievable through improved grain storage practices, crop protection with fungicides, and substitution of crops susceptible to AFB1 contamination by ones that are not (i.e. substituting maize for rice).[27,28]

1.3.2 Risk factors in the developed world (hepatitis C and alcohol)

In contrast, in developed areas, such as Europe, North America and Japan, the main risk factors are chronic infection with the hepatitis C virus (HCV) followed by excessive alcohol intake.[1,15] Interestingly, it has been noted that time trends in HCC incidence in developed nations run parallel to the spread of HCV and the consequent development of cirrhosis, however at a time lag of several decades.[19,29,30] For instance, HCV infection spread amongst large numbers of young adults in Japan in the 1920s (due to the practice of injection treatment for schistosomiasis)[31] and in southern Europe in the 1940s (resulting largely from needle-sharing for penicillin treatment and unscreened blood products) following the Spanish Civil War and the Second World War.[15,29] In these areas, incidence has now slowed almost to a plateau, and is declining in some regions.[7] This effect also explains the higher incidence age peak in Japan, where old HCV infections are causing a larger portion of older patients to develop HCC. In North America on the other hand, where HCV infection spread most severely in the 1960s and 1970s (due to needle-sharing in intravenous drug users and contaminated blood transfusions), one can still see an upward trend in HCC incidence[12] and predictions are for this to remain so for the next two decades.[7,32]

Prolonged and excessive alcohol intake is also a well-established risk factor and the second most common cause of HCC in developed countries. Excessive intake of alcohol is defined as a daily ingestion of at least 40-60 g of alcohol (one standard drink contains 13.7 g).[15] At these levels of daily ingestion, alcohol abuse often leads to the development of liver cirrhosis. In the setting of the cirrhotic liver there is a predisposition for HCC to occur (the risk of developing HCC among alcoholics appears to increase 10-fold in the presence of cirrhosis).[14,33,34] Although there is a strong association between alcohol abuse and liver cirrhosis, there is otherwise little evidence of a direct carcinogenic effect of alcohol.[4,14] A synergistic effect of alcohol abuse with HCV (and, to a lesser extent, HBV) infection in promoting HCC has also been described, with the risk of developing HCC doubling in HCV-infected individuals who also drink alcohol excessively.[4,35]

1.3.3 Other risk factors in the developed world (metabolic syndrome)

The upward trend in HCC incidence in North America during recent decades has sparked research into other possible causes for the development of HCC, particularly as some studies

based in the United States failed to identify the main risk factors of HCV, HBV, or alcohol abuse in a large proportion (30-40%) of patients.[4,36] An increasing amount of evidence has been gathered that suggests that the epidemic of metabolic syndrome in the developed world plays a significant role in this discrepancy. The pathogenicity of metabolic syndrome in terms of HCC can be further broken down into the three interrelated conditions of diabetes mellitus, obesity, and non-alcoholic steatohepatitis (NASH).

Large epidemiologic studies performed across several developed countries have observed a positive significant association between diabetes and HCC,[4,37-39] with a risk of HCC increasing approximately 2-fold in men with diabetes, independent of alcoholic liver disease, viral hepatitis or demographic characteristics.[38] In a complex interplay of pathogenicity, diabetes is strongly associated with obesity, and together these two factors are known to contribute to the development of NASH via its precursor, non-alcoholic fatty liver disease (NAFLD).[36,40,41]
Although evidence linking obesity to HCC is relatively scant,[4] studies in the United States, Denmark and Sweden have shown up to a 5-fold increase in liver cancer mortality in men in the highest body mass index range (35-40) compared with a normal body mass index, and a less pronounced increase for women (less than 2-fold).[42-44]
As mentioned above, the presence of both obesity and diabetes are known to contribute substantially to the development of NAFLD. This in turn can progress to the more severe form of the disease, NASH, which may lead to cirrhotic transformation of the liver and HCC. However, once cirrhosis and HCC arise, it can be difficult to identify pathologic features of NASH.[4,45,46] In cases where the cause of cirrhosis was considered cryptogenic, studies have found a comparative dominance of demographic characteristics suggestive of NASH (i.e. predominance of women, diabetes, and obesity) compared to other HCC patients with a clear cirrhotic etiology.[4,36,40,41] There is, however, limited evidence of a direct progression from NASH to HCC.

1.3.4 Congenital risk factors

For a small proportion of patients, congenital metabolic disorders that cause liver cirrhosis can increase the risk of HCC. The most important example of such a disorder is hereditary hemochromatosis (HH), a disease that causes excess iron to be absorbed from the digestive tract and stored in the body. Studies have suggested that HCC is due to underlying HH in 3-5% of cases.[47,48] Moreover, an increased relative risk of developing HCC has been observed in HH patients, ranging from 1.7[49] to 20[48,50] compared to the normal population, although the 20-fold increase has only been associated with one genetic subtype of HH (genotype HFE C282Y).[48]
For patients with HH and liver cirrhosis there is a 20% 5-year cumulative risk of developing HCC.[49]

In contrast to HH, other congenital diseases, such as Wilson's disease and α1-antitrypsin deficiency can lead to HCC by inducing cirrhotic transformation of the liver, however the diseases themselves are not associated with a significantly increased risk of developing HCC compared to the normal population.[51,52]

In theory, any other cause of liver cirrhosis can likewise lead to HCC via a pathway of chronic inflammation, tissue damage, and regeneration. Therefore, autoimmune diseases that lead to liver cirrhosis, such as autoimmune hepatitis (AIH), primary biliary cirrhosis (PBC), and primary sclerosing cholangitis (PSC) can also be considered risk factors for HCC. In the context of these diseases, HCC arises infrequently and usually in patients with more advanced disease.[14] In the context of AIH it has recently been speculated that therapeutic improvement in managing the disease may prolong patient survival in the future but potentially increase the risk of HCC through a longer period of liver damage.[53]

1.4 Pathologic characteristics

1.4.1 Cellular pathology

The pathologic subtype of HCC plays an important role in the growth pattern of the tumor, which in turn influences the treatment options and prognosis of disease. In gross terms, tumors are firstly classified as to presence of tumor capsule, presence of underlying liver cirrhosis, and portal vein invasion (tumor thrombus of the portal vein). Encapsulated tumors lend themselves well to surgical resection, the extent of underlying liver cirrhosis determines the applicability of various therapies, and portal vein thrombus is an important negative prognostic factor.[8,54] The patterns of tumor growth at a gross level are classified as infiltrative, expansive, diffuse, or mixed type. Infiltrative tumor growth is found in approx. one third of cases and is characterized by irregular tumor-liver boundaries and unclear tumor demarcation. Expansive growth occurs when adjacent intact tissues are pushed aside by the growing HCC. It is sub-classified into single nodular and multinodular types and is found in approx. one fifth of cases. Diffuse growth is generally associated with liver cirrhosis and occurs as multiple small (< 1 cm) nodules, scattered throughout the liver. Diffuse growth accounts for approx. 5% of cases. The remaining portion of patients is diagnosed with mixed growth types (usually infiltrative-expansive).[54]

At a microscopic level, HCC is classified based on tumor tissue differentiation according to Edmondson and Steiner. This classification system takes into account the degree of resemblance that tumor tissue has with normal liver tissue, for instance, in terms of cellular growth patterns, nucleus-cytoplasm ratio, staining characteristics, and degree of single cell

growth in vascular channels.[54,55] A further characteristic that has become a pathological hallmark of HCC is stromal invasion, whereby the tumor shows destructive invasion of the stroma of the portal tracts.[56] The possible grades of differentiation are given below:

Table 2: Pathological grading scores

Grade	Level of tumor cell differentiation (grade of malignancy)
1	Well differentiated (low grade)
2	Moderately differentiated (intermediate grade)
3	Poorly differentiated (high grade)
4	Undifferentiated/anaplastic (high grade)

In very early stages, when tumor diameter measures < 2 cm, HCC is considered similar to carcinoma *in situ*. At this stage, tumor tissue is generally very well differentiated and, by definition, no invasion of surrounding structures has yet occurred, meaning that these patients are particularly suitable for liver resection.[57,58] Although HCC is not highly vascularized at first, once it progresses and reaches a size of > 2 cm, it begins to display intense neoangiogenesis, deriving its blood supply predominantly from the hepatic artery.[54,59] This characteristic is exploited in certain locoregional therapeutic approaches described further below (section 1.6.3).

1.4.2 Molecular pathology

Given the varied etiologies of HCC (i.e. HBV, HCV, alcohol abuse, metabolic syndrome), it is clear that the development of this cancer is a complex process at a molecular level. While a thorough description of the various molecular mechanisms in terms of disease etiology would be outside the scope of this work, it is important to mention some of the typical molecular pathways affected, as these are important targets for novel therapies. Regardless of the cause of disease, the genetic changes mostly result from direct p53 mutation (as seen in AFB1 intoxication), chronic inflammation (i.e. recurring necrosis and regeneration of liver tissue), or oxidative stress (which modulates certain cancer-relevant signaling pathways).[60] Mutation in the tumor-suppressor gene p53 is present in 25-40% of cases and results in uncontrolled proliferation of the affected cells and, eventually, genomic instability.[17,60] A further gene that is commonly affected (25% of cases) is β-catenin, a component of the Wnt signaling pathway. When mutated, β-catenin associates with transcription factors that influence numerous cancer-relevant genes, including MYC (an oncogene), cyclin D1 (a cell cycle regulator), and matrix metallopeptidase 7 (MMP7, a gene involved in metastasis).[60] Chromosomal amplifications and deletions that affect important oncogenes and tumor suppressor genes are also common in HCC. In 5-10% of patients, high-level amplifications have been reported in genomic areas representing vascular endothelial growth factor A (VEGFA), an important signaling molecule in

vasculogenesis, and cyclin D.[17,60,61] The collective result of these genetic alterations is that entire signaling cascades are then affected, the most prominent example being the ErbB receptor family (ERBB1-ERBB4), four receptor tyrosine kinases that are implicated in numerous types of cancer. Two of these receptors play a particularly important role in HCC progression, namely ERBB1 (commonly known as epidermal growth factor receptor or EGFR), which is expressed in approx. 70% of HCC cases, and ERBB3, which has been observed in 85% of HCC patients. Excessive activation of these receptors correlates with a more aggressive disease presentation (i.e. large tumor size, poor differentiation, intrahepatic metastasis).[60,62] The mammalian target of rapamycin (MTOR) pathway is also disrupted in up to 50% of patients due to upstream mutations in several different genes, and is currently being studied as the target of new therapies. A further signaling molecule that is known to play a carcinogenic role is insulin-like growth factor (IGF), which is active in 20% of patients.[63] Epigenetic alterations and mircoRNA have also been shown to affect the silencing of various tumor suppressor genes or activation of oncogenes, however to a less uniform extent.[60,61,64]

1.5 Diagnosis

The diagnosis of HCC is made based on the findings of radiologic examination, biopsy and, to a lesser extent, alphafetoprotein (AFP) serology. Given that liver cirrhosis is usually the premalignant setting in which HCC occurs, European and American guidelines both recommend regular sonographic monitoring of cirrhotic patients at 6-month intervals.[8,65] Dysplastic nodules commonly develop in the cirrhotic liver and, of these nodules, at least one third become malignant.[66,67] If a lesion is detected during routine monitoring, the subsequent diagnostic algorithm depends on its size. Lesions measuring less than 1 cm in diameter are sonographically examined again four months later. This process is continued every four months until the lesion appears unstable or grows in size.[8] Both lesions greater than 1 cm, and the aforementioned smaller lesions that display progress, should be examined using latest generation 4-phase CT or dynamic contrast enhanced MRI.[68] For smaller lesions, or in clinics without high-end radiological equipment, both CT and MRI are recommended.[8,65] In these imaging techniques, many HCC lesions display a radiological hallmark of arterial phase hypervascularity and portal venous phase washout and the identification of this vascular behavior is sufficient for the diagnosis of HCC.[69] However, this radiological hallmark has been shown to be absent in a large proportion (approx. 40%) of patients with tumors measuring 1-2 cm.[70] Thus, in the absence of HCC's radiological hallmark, liver biopsy is recommended. In cases where the results of biopsy are inconclusive it is recommended to perform a repeat biopsy. If this fails to provide a solid diagnosis, the patient should undergo 4-monthly ultrasound monitoring until further evidence of tumor progress is seen, followed by reevaluation with

imaging/biopsy.[8] For the biopsy-based diagnosis of HCC, alongside classical morphologic criteria (see section 1.4 above), the pathological hallmark of HCC is stromal invasion. However, depending on the quality of the biopsy, this is often difficult to identify, and false negative findings do not indicate an absence of HCC.[17,56] Several tissue markers have thus been studied in order to ameliorate the biopsy-based diagnosis of HCC. The markers that have shown the most usefulness are Glypican-3 (GPC3), heat shock protein 70 (HSP70), glutamine synthetase (GS), and clathrin heavy chain (CHC).[56,71,72] Using a 4-marker panel, where at least two markers, regardless which, were positive, this showed a sensitivity and specificity for early, well-differentiated HCC of 64% and 100% respectively. A positive result for two of these four markers is therefore considered sufficient for the diagnosis of HCC.[71]

Although AFP is still often routinely determined in patients under surveillance for HCC, its role in the diagnosis of HCC has diminished in importance in recent years, given its inadequate sensitivity and specificity, and it is no longer recommended as a surveillance test for patients at risk[8,65,73,74] It is, however, still considered useful as a tracking parameter for disease activity in diagnosed individuals.

As in any other malignant cancer, diagnosis of HCC is followed by an assessment of the disease extension. This generally involves CT or MRI imaging, and in the event of suspicion of bone metastasis, PET scintigraphy.[8,65]

1.6 Treatment

The preferred treatment of HCC depends on numerous factors including extent and location of tumor growth, metastasis, liver function, and patient performance status. The various treatments can be categorized into one of four groups: Surgical therapy (liver resection and liver transplantation), local ablation, transcatheter therapies, and systemic therapy.

1.6.1 Surgical therapy (liver resection and liver transplantation)

Surgical therapy, when performed in well-selected cases, achieves the best treatment outcome with a 5-year survival rate of 60-80%.[8]

1.6.1.1 Liver resection

Liver resection is the treatment of choice for patients with solitary tumors and very well-preserved liver function (defined in terms of bilirubin level, hepatic venous pressure and platelet count) without portal hypertension. It is likewise the treatment of choice for HCC in non-cirrhotic patients.[8] In spite of the good survival rates mentioned above, tumor recurrence complicates approximately 70% of cases at five years.[75] Unfortunately, of the numerous studies carried out

testing the efficacy of adjuvant and neo-adjuvant therapy in reducing tumor recurrence, so far no treatment has shown a convincing therapeutic benefit.[76,77] In 70-80% of all HCC patients, the disease is too advanced for resection upon diagnosis. This has led to the use of multimodal therapeutic concepts to 'downstage' patients (therapeutically reducing tumor disease to a less advanced stage), in order to make resection possible. An example of this would be treating tumors too large for resection with locoregional therapies (i.e. TACE, SIRT – see section 1.6.3) in order to consolidate and reduce tumor disease prior to resection.[78] There is, however, insufficient evidence that downstaging increases overall survival and no well-defined downstaging strategy has officially been endorsed by expert panels.[8,77] In properly selected patients, the main predictors of postoperative survival following resection are tumor size (66% 5-year survival rate for ≤ 2 cm, 37% for > 5 cm), tumor number (57% 5-year survival rate for single tumors, 26% for ≥ 3 nodules), the presence of microsatellites and vascular invasion.[77,79]

1.6.1.2 Liver transplantation

In accordance with the so-called 'Milan criteria' first published in 1996, liver transplantation is recommended as the first-line treatment option for patients without metastasis or vascular invasion who have advanced liver dysfunction and single tumors measuring ≤ 5 cm, or those with up to three tumors, where each measures ≤ 3 cm.[80] Due to the good quality of patient selection that the Milan criteria deliver, in recent years they have also often been applied to patients considered for surgical resection.[81,82] In theory, transplantation may cure both the tumor disease and the underlying cirrhosis and the success of the procedure is not limited by the degree of liver function impairment.[17] Providing that the Milan criteria have been fulfilled, other prognostic factors for postoperative survival are the presence of microvascular invasion and the tumor's histopathologic grading.[83] The rate of recurrence appears to be much lower than resection (less than 15%).[17,84] In spite of these benefits, due to the scarcity of organ donors, not all patients fulfilling the Milan criteria undergo liver transplantation. In the past, some studies showed that well-selected resection patients survived longer than transplantation patients, since the potential success of transplantation was hampered by long waiting times.[85] If, during this waiting period, tumors progress beyond the Milan criteria, develop macrovascular invasion, or metastasize, patient prognosis worsens dramatically. Several strategies have been explored to address this issue, including the development of a priority system for HCC patients awaiting transplantation, bridging therapies during the waiting period to prevent tumor progression, and living donor liver transplantation.[8,86] However, the key to optimizing the potential of liver transplantation in the treatment of HCC is to increase the number of organ donors.

1.6.2 Local ablation

Local ablation of HCC refers to the image-guided percutaneous injection of probes into tumor lesions that apply chemicals, thermal energy, or radiation energy to the surrounding tissue. It is indicated in early-stage HCC in patients not suited for surgery and is a potentially curative therapy. The two most common methods of local ablation are percutaneous ethanol injection (PEI) and radiofrequency ablation (RFA).

1.6.2.1 Percutaneuous ethanol injection (PEI)

PEI is the seminal technique of local ablation, involving the local application of ethanol into tumor lesions, which causes cellular dehydration, protein denaturation, and chemical occlusion of smaller blood vessels, resulting in coagulative necrosis of the lesion. Its success is however limited to early-stage nodular tumor lesions (as opposed to diffuse tumor manifestation), tumors without septa/capsules, and small tumor size.[8,87] Complete necrosis is achieved in 90% of cases where tumors measure < 2 cm but only 50% in lesions measuring 3-5 cm.[75,88] In lesions exceeding 3 cm, there is a 2-year local recurrence rate greater than 40%. The presence of a tumor capsule or intratumoral septa inhibits the therapeutic penetration of ethanol, and is also associated with high 2-year local recurrence (greater than 60%).[87] Given this limited therapeutic spectrum, PEI is no longer considered an appropriate treatment modality in the developed world, and it has largely been replaced by radiofrequency ablation.

1.6.2.2 Radiofrequency ablation (RFA)

Radiofrequency ablation utilizes thermal energy (50-100°C) to induce local coagulative necrosis of tumor tissue. The benefit of RFA appears to be derived from the homogenous fashion in which heat is applied to the tumor tissue, thereby eliminating small, undetected satellite lesions more effectively than ethanol injection.[89,90] Studies have shown that, in early-stage HCC, RFA demonstrates much better local control (2-year local recurrence 2-5 times less) than PEI in fewer treatment sessions.[91-93] While both techniques display comparable survival in early-stage tumors < 2 cm, RFA also offers survival benefit in tumors measuring from 2-5 cm.[94,95] The 5-year survival rate of RFA in Child A patients is even comparable to surgical therapies at 50-75%[94-96] and alongside liver resection and transplantation, RFA is also recognized as a curative treatment, providing that patients are well selected.[97] However, the application and success of RFA are limited by tumor location (subcapsular location and adjacency to the gallbladder have a higher risk of complications and incomplete ablation), tumor size (< 5 cm), and proximity to blood vessels (heat loss through the perfused vessels results in tissue cooling and a minimalized therapeutic effect).[98,99] In all percutaneous procedures there is also a small risk of malignant needle track seeding (the accidental dragging of tumor cells into the surrounding

tissue following percutaneous injection). For RFA and PEI this is a complication that arises in approximately 2-3% of patients.[100,101]

1.6.2.3 Interstitial brachytherapy

A further potentially curative ablative approach that has shown promise in recent years is CT-guided interstitial brachytherapy in the afterloading technique.[102] This involves the CT-guided positioning of percutaneous catheters into the tumor, followed by delivery of a source of radiation (typically Iridium-192) through the catheters and into the vicinity of tumor tissue. The radioactive material remains in the catheter for a duration of 20-40 minutes before being removed.[103] This technique allows for the application of high local doses of radiation to precisely defined regions of the liver and has shown 1-year tumor control rates exceeding 90%.[102,104] Interstitial brachytherapy with afterloading also fills a valuable therapeutic niche because it overcomes several of the shortcomings of other local ablative techniques. The above-mentioned weakness of RFA (maximum size that can be treated successfully < 5 cm, cooling effects from adjacent blood vessels, damage to adjacent structures such as the gall bladder) do not limit interstitial brachytherapy, which can be used to treat very large tumors (> 10 cm, albeit in several sittings when tumor size > 8 cm), does not have a therapeutic mechanism limited by cooling through blood perfusion, and is comparatively tolerable for the surrounding structures. Thus interstitial brachytherapy can be employed even for tumors that have invaded the portal vein or large hepatic veins, or that are located in the liver hilus, close to the bile duct and gallbladder.[103,104] The main risk associated with interstitial brachytherapy is excessive irradiation to healthy tissues (i.e. liver, stomach, duodenum, biliary tree bifurcation). This is overcome by not exceeding defined maximum doses in given regions (e.g. a maximum allowable dose exposure of 5 Gy to no more than 2/3 of the liver), and, if applicable, employing suitable prophylaxis (i.e. proton-pump inhibitors for prophylaxis against gastric or duodenal toxicity, steroids to prevent radiation-induced edema and subsequent bile duct obstruction for tumors with direct contact to the biliary tree bifurcation).[103] A limitation of interstitial brachytherapy is the need for trained interventional radiologists and special equipment.

1.6.3 Transcatheter therapies

Transcatheter therapy exploits HCC's tendency to promote intense neoangiogenesis, using the tumor-feeding blood vessels for direct application of chemotherapeutic agents, embolic particles, or radioactive substances via catheter. Early HCCs (< 2 cm) receive their blood supply from the portal vein, along with most of the healthy liver tissue. As HCC progresses, neoangiogenesis promotes tumor-feeding arteries to sprout from the hepatic artery and this subsequently makes up the vast majority of the tumor's blood supply.[54] This 'splitting' of blood

supply between the tumor and the rest of the liver is the conceptual basis for transcatheter therapies.

1.6.3.1 Transarterial chemoembolization (TACE)

The transcatheter technique most widely used is transarterial chemoembolization (TACE), which involves image-guided delivery of cytotoxic agents (generally doxorubicin or cisplatin) to the arteries nourishing the tumor, followed by embolization of these blood vessels. This results in both a high local concentration of the cytotoxic drugs and a strong ischemic effect.[8,17] The effect is not permanent however, and tumors become revascularized, with 1-year local recurrence rates up to 65%.[105] Thus it is recommended to repeat the TACE procedure 3-4 times per year in order to delay tumor progression and vascular invasion. TACE is recommended for patients with intermediate tumor disease, meaning asymptomatic multinodular / large unresectable tumors without vascular invasion or extra-hepatic spread. In such cases a survival benefit has been shown in up to 60 % of patients,[106,107] with a median survival of over 3 years (untreated, the median survival for this patient group is 16 months).[8,105] A limitation of TACE is the risk of treatment-induced liver failure (irreversible hepatic decompensation in 3% of cases)[108] resulting from the cytotoxic and ischemic insult to viable liver tissue in individuals with already decompensated liver disease. A recent advance in the TACE technique that minimizes this risk is the development of embolic microspheres (or drug-eluting beads) that provide both a calibrated vessel obstruction and a controlled, slow release of chemotherapeutic agents over a 1-week period. This has been shown to increase the local concentration of the drug and reduce the drug-related systemic toxicity.[109] These characteristics are of particular benefit in the treatment of patients with more advanced disease.[110]

1.6.3.2 Selective internal radiation therapy (SIRT)

Selective internal radiation therapy (SIRT) is a modern therapeutic technique that employs microscopic radioactive spheres to embolize tumor-feeding arteries and at the same time locally irradiate tumor tissue. The radioactive isotope most commonly used is Yttrium-90, a high-energy, low-penetration beta emitter with a half-life of approx. 2.5 days. In a technique akin to TACE, the radioactive spheres are delivered via arterial catheter to the hepatic artery and accumulate within the tumor-feeding arterial bed. The high level of local irradiation destroys tumor tissue while the low-penetration spares adjacent healthy liver tissue.[8,78,111] SIRT can be employed in intermediate cases of disease (multifocal or diffuse HCC) where ablative and other transcatheter therapies are not indicated.[78] It is also considered to have potential in multimodal therapeutic concepts, either to downstage tumors prior to resection/transplantation (see above – section 1.6.1.1) or to be used in addition to systemic therapy in more advanced cases.[78,112] Exclusion criteria for using SIRT include poor liver function (> 6-7 points in the Child-Pugh

classification system for cirrhosis, see section 2.1 below), extrahepatic metastasis, and arterial shunts to the lungs or gastrointestinal tract. Therefore, prior to undergoing SIRT, patients' liver function is tested, a staging CT is carried out and existing shunts are examined angiographically and, where possible, embolized to render the SIRT procedure possible.[78] As SIRT is a relatively new therapy, it has not yet been tested against established therapies in RCTs.[8] Cohort studies reporting long-term outcomes have however suggested a survival benefit for selected patient groups, particularly patients with locally advanced disease and good liver function (both with and without portal vein invasion).[113-115] The potential role of SIRT in multimodal therapeutic concepts remains to be validated in RCTs. Similar to interstitial brachytherapy, a limitation of SIRT is the need for sophisticated equipment and trained interventional radiologists, as well as handling authorization for Yttrium-90.[8,78]

Other transcatheter therapies that have largely been replaced by the above-mentioned therapy forms are transarterial embolization (TAE), where embolization alone is performed, and intra-arterial chemotherapy, where a cytotoxic drug is administered without embolization.[8,59]

1.6.4 Systemic therapy

Until recent years, patients diagnosed with advanced stage HCC, or whose HCC had progressed following locoregional treatment had no therapeutic option beyond best supportive care. This was due to a lack of an effective systemic therapy for HCC.[116] The development of sorafenib (trade name, Nexavar®), a multi-tyrosine kinase inhibitor, and its approval for use in HCC by European and American regulatory bodies in Oct/Nov 2007 was a long-awaited breakthrough for these patients. Sorafenib is a small molecule that inhibits both tumor growth and angiogenesis by disrupting signaling pathways that are activated in HCC. The targeted areas are the Raf-1 pathway, which can be found downstream from the ERBB receptors EGFR and ERBB3, and the neoangiogenesis regulator, VEGF (see section 1.4.2 above).[57,61,117] A large, double-blinded, placebo-controlled investigation conducted in 2005-2006 showed a median survival benefit for sorafenib verses placebo of 3 months (10.7 vs 7.9 months). Sorafenib likewise increased the time to disease progression by 2.5 months verses the placebo group (5.5 vs 2.8 months). A benefit from treatment with sorafenib was consistently identified regardless of individual prognostic factors, such as poor patient ECOG score (see section 2.1 below), macrovascular invasion, or metastasis.[117] Since its approval, sorafenib has become the standard of care for patients with advanced disease. Its use is, however, limited to patients with well-preserved liver function (Child A and, to a lesser extent, Child B).[8,65] Sorafenib has shown good tolerability with no drug-related death described in RCTs.[117,118] The most common side effects are hand-foot skin reaction and diarrhea in less than 10% of cases.[117]

Apart from sorafenib, other systemic therapies are currently being investigated that block either signaling pathways (e.g. the MTOR-inhibitor, everolimus), or receptors (e.g. the EGFR antagonist, erlotinib) involved in HCC. Sorafenib is also being investigated as an adjuvant therapy after resection, ablation, or in combination with TACE.[8,17]

1.7 Prognostic parameters and staging systems

An accurate staging of HCC is essential, both for the correct evaluation of patients at baseline, and allocation of appropriate treatment. A hindrance to assessing the disease accurately is the almost obligatory co-presence of liver cirrhosis (80-90% of cases), a condition considered more life-threatening than HCC itself.[119] Staging systems for HCC have evolved as understanding of the disease has grown and today they endeavor to incorporate underlying cirrhosis and other prognostic factors, in addition to the tumor-relevant parameters. The four systems explained below are those most commonly used in Europe and the United States.

1.7.1 Prognostic factors of survival

There are several known factors that help to predict patient survival and should therefore be incorporated into staging systems. Alongside tumor status (i.e. number and size of nodules, vascular invasion, metastasis), liver function plays an important prognostic role (i.e. Child-Pugh stage, portal hypertension), as does the patient's general health status (ECOG performance status, see section 2.1).

1.7.2 Okuda classification

The Okuda staging system was introduced in 1985 as the result of retrospective analysis of 850 HCC patients.[120] The factors considered are tumor size, ascites, bilirubin level, and albumin level. Although employed for many years, this system is now criticized for being one-dimensional in its representation of disease (i.e. it does not consider important prognostic factors such as vascular invasion, metastasis, and patient's health status), and for not being representative of current patients, who are generally diagnosed at a much earlier stage than when the Okuda system was first conceived.[121] It has also been criticized for not adequately stratifying patients with better prognosis, and for the fact that it has never been validated prospectively.[122]

Table 3: Okuda staging system[120]

Okuda score	0	1
Tumor > 50 % liver volume	N	Y
Ascites present	N	Y
Albumin < 3 g/dl	N	Y
Bilirubin > 3 mg/dl	N	Y

Total score: 0 = Okuda stage 1, 1-2 = Okuda stage 2, 3-4 = Okuda stage 3

1.7.3 TNM staging system

The Tumor-Node-Metastasis (TNM) system of cancer classification is the most well-known staging system in oncology. For HCC, this system takes many important disease-related prognostic factors into account (e.g. vascular invasion, tumor size, metastasis), however it has been criticized for not including information regarding liver functional status, thereby disregarding an important comorbidity (i.e. liver cirrhosis) found in most patients. Further points of criticism are that it does not include information concerning patients' general health status, nor does it allow for proper classification in patients where no biopsy was performed, due to microvascular invasion being a primary factor differentiating the lower stages of T1 and T2.[121]

Table 4: TNM staging system[123]

Primary tumor (T)	
Tx	Primary tumor cannot be assessed
T0	No evidence of primary tumor
T1	Solitary tumor without vascular invasion
T2	Solitary tumor with vascular invasion or multiple tumors, none > 5 cm
T3a	Multiple tumors, at least one > 5 cm
T3b	Single tumor or multiple tumors of any size involving a major branch of the portal or hepatic vein
T4	Tumor(s) with direct invasion of adjacent organs other than gallbladder or with visceral peritoneum
Regional lymph nodes (N)	
Nx	Regional lymph nodes cannot be assessed
N0	No regional lymph node metastasis
N1	Regional lymph node metastasis
Distant metastasis (M)	
M0	No distant metastasis
M1	Distant metastasis

1.7.4 Cancer of the liver Italian Program (CLIP)

The CLIP staging system was first presented in 1998 based on retrospective analysis of 435 patients. It was designed to address the abovementioned shortcomings of the existing staging systems by including information concerning liver cirrhosis, serum AFP, and vascular invasion.[124] Prospective studies comparing the CLIP system to the Okuda and TNM systems showed that it provided a more precise prognosis of patient survival through accurate stratification of patient groups (i.e. both patients with a good, and with a very poor prognosis were more accurately identified using the CLIP criteria).[125-127] The CLIP system is composed of stages 0-6, where the stage depends on the total number of points scored using the system below. A shortcoming of the CLIP system is that it does not consider patients' general state of health, which has been shown to be a robust independent prognostic variable (e.g. ECOG performance status, see section 2.1).[128] However, a large, recent study found CLIP to be the most accurate staging system for all patients.[129]

Table 5: CLIP staging system[124]

	Score
Child-Pugh stage	
A	0
B	1
C	2
Tumor morphology	
Uninodular and extension ≤ 50%	0
Multinodular and extension ≤ 50%	1
Massive or extension > 50%	2
AFP level (ng/dl)	
< 400	0
≥ 400	1
Portal vein thrombosis	
No	0
Yes	1

1.7.5 Barcelona-Clinic Liver Cancer (BCLC) staging system

Of the four staging systems given here, the Barcelona-Clinic Liver Cancer (BCLC) staging system is the recommended system in both Europe and the United States.[8,69] The advantage of the BCLC system is that it not only includes several types of prognostic variables (i.e. concerning HCC, underlying cirrhosis, and patient health), but it links these prognostic

subclasses (categorized as 0, A, B, C, D) to specific treatments.[17,130] Since its introduction in 1999, the BCLC system has been refined regularly to incorporate new developments (e.g. incorporating sorafenib as the treatment for advanced patients).[131] Like CLIP, the BCLC system has been both retrospectively and prospectively validated.[132]

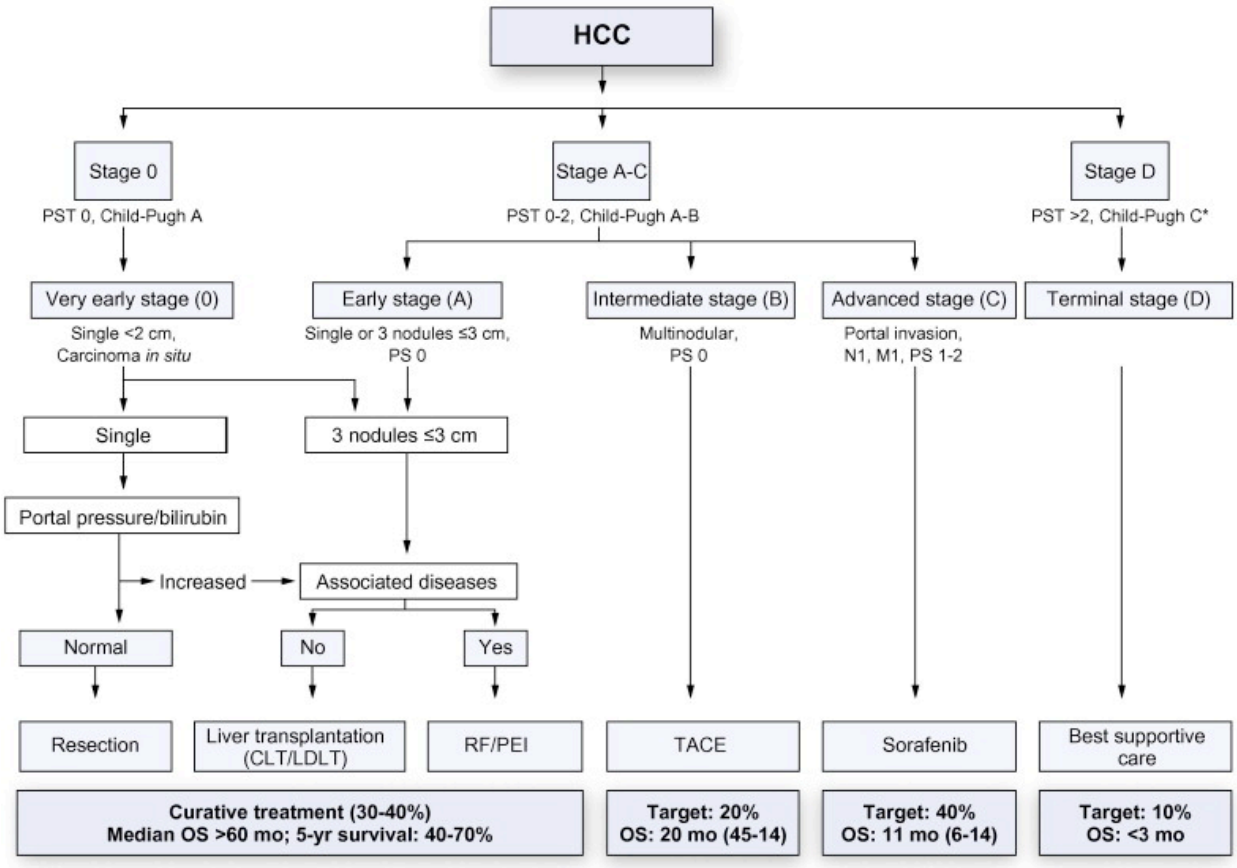

Figure 1: BCLC staging system[8]

1.8 Formulation of hypothesis

The purpose of this work was to retrospectively study overall and disease-free survival in a recent cohort of HCC patients and identify factors that influenced the course of disease. Given the rapid evolution of HCC treatment in recent years, with novel therapies such as interstitial brachytherapy with afterloading, sorafenib, and SIRT being ascribed very good therapeutic effects, new research into this area seemed warranted. A comparison of survival in this patient cohort with older studies found in the literature on HCC was therefore also required in order to validate the findings of this study. A second aim of this study was to reevaluate the four above-mentioned staging systems in order to assess their accuracy in a modern clinical setting.

2 Methods

2.1 Collection of data

Retrospective clinical data for this study was collected in the hepatological outpatient clinic of the Charité Virchow Klinikum in Berlin, Germany during the period of January 2011 until June 2012. Patient charts and the hospital's computer-based archiving system were the key sources of information and patients' primary care physicians were contacted if further data was required (this was particularly necessary when following up on patient survival). All data was collected and analyzed using the Statistical Package for the Social Sciences (SPSS version 20; SPSS Inc., Chicago Ill., USA).

Included in the data set were patients who received diagnosis, treatment and follow-up for HCC in the abovementioned clinic in the timeframe from 01 January 2006 – 30 April 2012. Patients diagnosed earlier than 2006 but still receiving ongoing therapy and follow-up within this timeframe were included in the study and their pre-2006 data was entered for the baseline comparison. Patients diagnosed after 31 December 2011 were excluded since it was likely that follow-up data would be insufficient by the end of the data collection period. Further patients were excluded if the initial diagnosis of HCC later proved incorrect (for instance, upon histologic examination of a resected tumor), if the main therapy was conducted in the setting of a clinical trial, or if records were incomplete due to diagnosis, treatment and follow-up primarily taking place at another medical facility. This resulted in an inclusion of 136 patients into the baseline study data. Of these, a further six patients were excluded from the analysis of survival data as they were lost to follow-up shortly following diagnosis.

There were four kinds of data collected: Baseline patient information, baseline disease status, course of therapy, and disease activity at follow-up. The baseline patient information collected included name, date of birth, gender, ethnic origin, date of diagnosis of liver cirrhosis (if applicable), date of diagnosis of HCC, and age at diagnosis of HCC (which was subsequently assigned to a categorized age group of <50 years, 50-59 years, 60-69 years and so on). Once records were complete, the data was made anonymous prior to further analysis by issuing an ID number in the place of patient name.
Data concerning disease status included information on both HCC and liver cirrhosis, the latter as it is generally considered a premalignant condition for HCC and a factor that limits patient survival.[14,119] As the underlying cause of liver cirrhosis has been speculated as having an effect on the progression of HCC,[133] the corresponding cause(s) of cirrhosis was also noted, and the

degree of liver cirrhosis was ascertained by collecting the parameters used in the Child-Pugh scoring system (see table below).

Table 6: Child-Pugh classification system of cirrhosis (simplified)

Parameter	1 point	2 points	3 points
Quick (%)	>70	70-40	<40
Albumin (g/dl)	>3.5	3.5-2.8	<2.8
Bilirubin (mg/dl)	<2	2-3	>3
Ascites	Absent or very mild	Moderate, suppressed with medication	Severe, therapy resistant
Encephalopathy	None	Moderate, suppressed with medication	Severe, therapy resistant

≤ 7 points: Child A (good liver function)
8-10 points: Child B (moderate liver function)
≥ 11 points: Child C (poor liver function)

In patients in whom no cirrhosis was present upon diagnosis of HCC, risk factors for the development of cirrhosis/HCC (i.e. HBV, HCV, alcohol abuse, diabetes) were also noted. Aside from liver function, other independent prognostic factors of HCC survival, namely portal hypertension and patient performance status were likewise collected. For the latter, the Eastern Cooperative Oncology Group (ECOG) Performance Status score (see table below) was used to assess patients' general health, as it has been shown to accurately predict long-term survival in HCC patients.[128]

Table 7: ECOG Performance Status[134]

Grade	ECOG
0	Fully active, able to carry on all pre-disease performance without restriction
1	Restricted in physically strenuous activity but ambulatory and able to carry out work of a light or sedentary nature, e.g. light house work, office work
2	Ambulatory and capable of all self-care but unable to carry out any work activities. Up and about more than 50% of waking hours
3	Capable of only limited self-care, confined to bed or chair more than 50% of waking hours
4	Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair
5	Dead

The tumor characteristics of interest were: Number of lesions, size of largest lesion, infestation of liver volume < or > 50%, vascular invasion (absent, present without portal/hepatic vein involvement, present with portal/hepatic vein involvement), metastasis (absent, regional, distant), organ(s) of metastasis, alpha-fetoprotein level upon initial diagnosis (< or > 400 ng/ml, as per the CLIP staging system, as well as < or > the normal limit of 13.4 ng/ml), and Edmondson & Steiner pathological grading score (G score) of tumor (see section 1.4). Number of lesions and lesion size were both categorized further into subgroups, with number of lesions being first assigned to the groups uninodular versus multinodular, and then further categorized as 1-3, 4-5, or > 5 lesions. Lesion size referred to the diameter of the largest lesion and was grouped as being ≤ 2 cm, > 2-6 cm, or > 6 cm. These lesion number and lesion size categories were selected in order to approximately group patients according to important principles in HCC disease management, such as the number of lesions commonly considered treatable by transplantation or resection (up to 3 lesions, each under 3 cm in diameter, see 'Milan criteria' in section 1.6.1.2), and the threshold lesion sizes in terms of treatability and invasive tumor behavior (e.g. ≤ 2 cm). The above parameters were then used (where applicable) to generate scores for the four most common scoring systems used in HCC staging; Okuda, TNM, CLIP, and BCLC (see section 1.7).

The course of treatment that patients underwent was recorded using the parameters type of treatment (see table below), date of treatment, imaging performed at follow-up, date of best response to treatment, and tumor response to treatment in terms of the RECIST criteria (Response Evaluation Criteria in Solid Tumors, see below). Only the first three rounds of therapy were used for further analyses, due to a limited number of patients in subsequent rounds. Patients were then further categorized into subgroups based on the number of different therapeutic modalities they received (i.e. no therapy/one type of therapy/multiple types of therapy). Three patients were excluded from all analyses of therapy, namely those who had received conventional chemotherapy, as it is now widely held to be an ineffective treatment for HCC, and given that there were not enough patients to construct a control group.

Table 8: Types of treatment

Curative	Liver resection
	Liver transplantation
	Interstitial brachytherapy with afterloading
	Radiofrequency ablation (RFA)
	Percutaneous ethanol injection (PEI)
Non-curative, locoregional	Transarterial chemoembolization (TACE)
	Transarterial embolization (TAE)
	Selective internal radiotherapy (SIRT)
	Laser-induced interstitial thermotherapy (LITT)
Systemic	Conventional chemotherapy
	Chemotherapy with sorafenib (Nexavar®)
Metastatic	Metastasis excision
	Metastasis radiation therapy
Best supportive care	No treatment possible, observation

The aforementioned RECIST criteria are commonly used for evaluating therapeutic success in the treatment of solid cancers.[135] Tumor disease is examined at the baseline visit and "target lesions" (measurable lesions representative of tumor disease) are distinguished from "non-target lesions" (qualitatively assessed lesions that are not measured and not representative of disease). Target lesions must measure at least ≥ 10 mm in spiral CT (or ≥ 20 mm in other imaging techniques), and a maximum of five target lesions per organ and 10 lesions in total are used to define the disease at the baseline visit.[135] The sum of the longest tumor diameters (SLD) is recorded at baseline and used for comparison at further follow-ups. Depending on the cancer's response to treatment, the disease activity is classified as one of the following upon follow-up:

Table 9: RECIST criteria[136]

Complete Response (CR):	Disappearance of all target lesions
Partial Response (PR):	At least a 30% decrease in the SLD of target lesions compared to baseline/pretreatment SLD
Progressive Disease (PD):	At least a 20% increase in the SLD of target lesions compared to baseline/pretreatment SLD
Stable Disease (SD):	Neither sufficient decrease in SLD to qualify for PR, nor sufficient increase in SLD to qualify for PD

The appearance of new malignant lesions also indicates PD, regardless of the response of other lesions. Likewise, unequivocal progression in non-measurable lesions also constitutes PD.[135,136]

The final type of data that was collected concerned patient follow-up and survival. The RECIST criteria were also employed here to rate disease activity at regular intervals. These intervals were structured as follows:

Every three months for the first two years
Every six months for the next three years
Every 12 months for each following year

2.2 Statistical analysis

Frequencies in patient data were generated for the descriptive statistical analysis and included median, average, minimum and maximum values. This served to group and analyze data concerning patients (e.g. median age upon HCC diagnosis, gender distribution), tumors (e.g. median size of largest lesion) and treatment (e.g. frequencies in type of treatment).

In order to ascertain if parameters showed interdependence, Pearson's chi-squared test was employed. In cases where only two possible outcomes were tested for both parameters, Fisher's exact test was used. These tests assisted in the detection of variables that appeared to influence one another, for instance the dependence of the treatment chosen on the disease characteristics.

Overall survival and disease-free survival in terms of single variables were analyzed using the Kaplan-Meier method. As there were six patients who were lost to follow-up shortly after diagnosis of HCC, they were excluded from all survival analyses. Likewise, the three patients who had received conventional chemotherapy were excluded from survival analyses based on therapy for the reasons given above.

Survival was taken here as meaning time from the initial diagnosis of HCC until patient death (for overall survival) or until progressive disease (for disease-free survival), the latter as defined by the RECIST criteria. This was employed in the univariate analysis of survival in terms of baseline patient characteristics (e.g. liver function), baseline tumor characteristics (e.g. lesion size), staging systems, and types of therapy. Survival was expressed in terms of 1, 3 and 5-year

survival, median survival, or as Kaplan-Meier curves. The logrank test was used to validate the results of Kaplan-Meier analysis.
In order to ascertain the factors that predicted prognosis when all significant univariate parameters were considered together, multivariate analysis using the Cox regression model was performed. The most significant variables in a multivariate context were identified using the backward elimination (likelihood ratio) method. These variables were then further tested using the standard Cox regression model.

Unfortunately, several variables that were found to be significant in univariate analysis had too many missing cases to be included in the multivariate analysis. These included AFP level, ECOG score, CLIP score, Okuda stage, and TNM stage for the analysis of overall survival. For disease-free survival, only the CLIP score variable had to be excluded. In order to be able to analyze the staging systems in a multifactorial context, a patient subgroup was created which included only patients where data concerning all four staging systems was available.
The results of the multivariate analyses were displayed as hazard ratios. In all statistical tests performed, a significance level of ≤ 0.05 was considered significant.

3 Results

3.1 Baseline patient characteristics

3.1.1 Gender, age and ethnicity

The group of 136 patients included in the study at baseline was composed of 105 males (77%) and 31 females (23%). The age upon diagnosis ranged from 33 to 85 years and the median age was 67. As would be expected from the German population studied here, there was a predominant Caucasian group of 123 patients (90%). The remaining 13 patients were of Turkish, Soviet, Asian, or Middle Eastern origin (0.7 - 3.7 %).

3.1.2 Liver cirrhosis, causes, and Child-Pugh stage

Underlying liver cirrhosis was identified in 112 patients (82%) at the time of HCC diagnosis. In most cases it could be attributed to an underlying cause of alcohol abuse (42%), HCV infection (28%), or HBV infection (14%). Other causes of cirrhosis found were cryptogenic (7%), fatty liver disease/NASH (4.5%), and hemochromatosis (3.5%). One younger patient (49 years) presented with Abernethy malformation (a congenital malformation of the portal vein) as the cause of cirrhosis, classified as ‘other’ in the table below (0.9%).

For 11 patients a secondary cause of cirrhosis could also be identified and in 10 of these cases (9%) this was alcohol abuse with a primary cause of HCV or HBV infection. The remaining case was a patient with hemochromatosis and a secondary cause of HBV infection (0.9%). Child-Pugh scores were calculated for the 112 patients with liver cirrhosis and showed 66 patients with Child A cirrhosis (59%), 32 with Child B (29%), and 5 with Child C (4.5%).

Risk factors for the development of liver cirrhosis/HCC were also recorded for the 23 non-cirrhotic patients. The main risk factors in this patient group were diabetes mellitus, which was present in 13 patients (56.5%), followed by fatty liver disease/NASH (13%), alcohol abuse (8.7%), and viral hepatitis B and C in isolated cases. In eight of the non-cirrhotic patients (35%), no risk factors for liver cirrhosis or HCC could be identified.

3.1.3 Portal hypertension and activity status (ECOG score)

Portal hypertension was found in 100 patients (73.5%) upon initial diagnosis of HCC, and was absent in 30 patients (22%). In the remaining 6 patients (4.5%) the presence of portal hypertension had neither been confirmed nor negated in the medical records.
Patients’ activity status at time of diagnosis was recorded using the ECOG scoring system (see section 2.1 above). This showed 51 fully active patients (37.5%), 33 slightly restricted patients

(24%), 25 restricted patients (18.5%), and one patient where self-care was limited (0.7%). Unfortunately, as the ECOG score was not always recorded or indirectly obtainable in the medical records, the status of 26 patients (19%) remained unknown.

Table 10: Baseline patient characteristics

Variable	Nr.	(%) (n=136)
Gender		
Male	105	77.2
Female	31	22.8
Age category (y)		
< 50	8	5.9
50 - 59	22	16.2
60 - 69	55	40.4
70 – 79	44	32.4
80 - 89	7	5.1
Ethnicity		
Caucasian	123	90.4
Turkish	5	3.7
Former Soviet state	5	3.7
Asian	2	1.5
Middle Eastern	1	0.7
ECOG score		
0 (Fully active)	51	37.5
1 (Slightly restricted)	33	24.3
2 (Restricted, self care possible)	25	18.4
3 (Limited self care)	1	0.7
Unknown	26	19.1

Table 10 (continued): Baseline patient characteristics

Variable	Nr.	(%) (n=136)
Underlying liver cirrhosis		
Y	112	82.4
N	23	16.9
Unknown	1	0.7
Portal hypertension		
Y	100	73.5
N	30	22.1
Unknown	6	4.4
Cause of liver cirrhosis		**(n=112)**
Alcohol abuse	47	42.0
HCV	31	27.7
HBV	16	14.3
Cryptogenic	8	7.1
Fatty liver disease/NASH	5	4.5
Hemochromatosis	4	3.5
Other	1	0.9
Secondary cause of liver cirrhosis		
Alcohol abuse	10	9.0
HBV	1	0.9
Child-Pugh score for cirrhosis		
Child A	66	58.9
Child B	32	28.6
Child C	5	4.5
Child-Pugh score unknown	9	8.0
Risk factors for HCC in non-cirrhotics		**(n=23)**
Diabetes mellitus	13	56.5
Fatty liver disease/NASH	3	13.1
Alcohol abuse	2	8.7
HBV	1	4.3
HCV	1	4.3
No known risk factors	8	34.8

3.2 Baseline tumor characteristics

3.2.1 Tumor nodularity, size and extent of liver infiltration

Tumor nodularity and extent of tumor infestation in terms of being greater or less than 50% of liver volume were assessed in order to characterize tumors. Approximately half of the patients presented initially with unifocal disease (48.5%), and the other half with multifocal or diffuse disease (50%). Extent of disease exceeding 50% of liver volume was noted in 23 patients (17%). Lesion number was further classified into clinically relevant categories to assist in the survival analysis. One-hundred-and-two patients (75%) presented with 1-3 lesions upon diagnosis, a further 23 (16.9%) had 4-5 lesions, and 9 patients (6.6%) presented with more than 5 lesions. The size of the largest lesion was also categorized in groups of ≤ 2 cm (10% of patients), > 2-6 cm (49%), and > 6 cm (36%). The median size of the largest legion was 5.2 cm and the median number of lesions was two.

3.2.2 Vascular invasion and metastasis

Vascular invasion was found in 35% of patients upon diagnosis of HCC. In 15 patients (11%), invasion was only of the smaller blood vessels, but in 33 cases (24%) the portal or hepatic veins were already involved at the time of diagnosis.
Metastasis was present in 26 patients (19%), with the intra-abdominal lymph nodes being the most common site affected (found in 11% of all patients), followed by the lungs (3.7%), bone (1.5%), adrenal glands (1.5%), peritoneum (< 1%), and the abdominal wall (< 1%).

3.2.3 Tumor differentiation (G score) and AFP levels

Histologic examination of tumors was unfortunately only performed in slightly more than half of all patients. Nineteen patients (14% of all cases) showed well-differentiated tumors (G1), 42 patients (31%) showed tumors of moderate differentiation (G2), 11 cases (8%) were poorly differentiated (G3), and two patients (1.5%) had undifferentiated tumors (G4). In order to facilitate statistical analyses of this data, patients with G3 and G4 tumors were grouped together.

AFP levels were firstly categorized as being < or > 400 ng/ml, as per the CLIP staging system. There were 23 patients with AFP levels elevated above this threshold (16.9%) and 63 with low AFP (46%). For the remaining patients there was no information available concerning AFP as at diagnosis (37%). In terms of having an AFP level considered normal (< 13.4 ng/ml), 29 patients had levels below this threshold (21%) and 57 had levels above it (42%).

Table 11: Baseline tumor characteristics

Variable	Nr.	(%) (n=136)
Nodularity		
Uninodular	66	48.5
Multinodular	68	50
Unknown	2	1.5
Number of lesions		
1-3	102	75
4-5	23	16.9
>5	9	6.6
Unknown	2	1.5
Size of largest lesion (cm)		
≤ 2	14	10.3
> 2-6	67	49.3
> 6	49	36.0
Unknown	6	4.4
AFP level (ng/ml)		
< 400	63	46.3
> 400	23	16.9
Unknown	50	36.8
AFP in normal range (< 13.4 ng/ml)		
Y	29	21.3
N	57	41.9
Unknown	50	36.8
Extent of liver infiltration		
< 50%	111	81.6
> 50%	23	16.9
Unknown	2	1.5
Metastasis		
Y	26	19.2
N	106	77.9
Unknown	4	2.9

Table 11 (continued): Baseline tumor characteristics

Variable	Nr.	(%) (n=136)
Organ of metastasis		
Lymph node	15	11.0
Lung	5	3.7
Bone	2	1.5
Adrenal gland	2	1.5
Abdominal wall	1	0.7
Peritoneum	1	0.7
Vascular invasion		
Y (involving portal/hepatic veins)	33	24.3
Y (not involving portal/hepatic veins)	15	11.0
N	83	61.0
Unknown	5	3.7
Tumor differentiation (G score)		
G1	19	14.0
G2	42	30.9
G3	11	8.1
G4	2	1.5
Unknown	62	45.6

3.2.4 Tumors classified as per Okuda, TNM, CLIP and BCLC

Tumors were classified in accordance with the four staging systems mentioned in section 1.7. Patients were distributed almost evenly between the Okuda stages 1 and 2 (46% and 42% respectively), with a further 5 patients (4%) diagnosed with Okuda stage 3. Given the small number of stage 3 patients, these were grouped together with stage 2 for further analysis.

The TNM staging system also showed an almost even distribution of patients across the stages T1-T3b (between 20% and 29% of patients at each stage), with just 3 patients (2%) diagnosed in the T4 stage. These were likewise grouped together with T3b patients for the further analyses.

CLIP scores were calculated for patients where possible, but unfortunately, due to a lack of relevant data (e.g. no record of AFP measured at time of diagnosis), the total score could not be calculated for almost 40% of patients. Fifty percent of patients were found to have CLIP scores

of 2 or below. As there were only 18 patients (13%) in the CLIP groups 3-5 (with no patients in the category CLIP 6), the subgroups CLIP 0-1, CLIP 2-3, and CLIP 4-5 were employed.

The parameters required for the BCLC classification were fortunately more readily available than those required for the CLIP system, with the majority of patients in BCLC stage C (82 patients, 60%), followed by 22 patients (16%) in stage A, 16 in stage B (12%), and six in stage D (4%). In spite of the small number of stage D patients, these were nevertheless treated as an independent group in further analyses, given their significantly poorer prognosis.

Table 12: Tumor classification in terms of TNM, Okuda, CLIP and BCLC systems

Variable	Nr.	(%) (n=136)
Okuda stage		
1	63	46.3
2	57	41.9
3	5	3.7
Unknown	11	8.1
TNM stage		
T1	40	29.4
T2	30	22.1
T3a	27	19.9
T3b	32	23.5
T4	3	2.2
Unknown	4	2.9
CLIP stage		
0	17	12.5
1	21	15.4
2	29	21.3
3	12	8.8
4	5	3.7
5	1	0.7
Unknown	51	37.5
BCLC stage		
A	22	16.2
B	16	11.8
C	82	60.3
D	6	4.4
Unknown	10	7.4

3.3 Types of therapy

As several patients who were included in the baseline data ended up either being treated at another clinic or not returning for follow-up, we were compelled to remove six patients from all statistical analyses that follow, leaving a total of 130 patients. A further three patients were

excluded from survival analyses based on therapy, as they had received conventional chemotherapy, a strategy that has since been proven ineffective in prolonging patient survival. Only the first three rounds of treatment were considered for analysis as subsequent rounds only contained a small number of patients. Within these three rounds, slightly more than half of all patients received just one therapeutic modality (54%), over a third received more than one type of therapy (37%), and approximately 9% of patients were not eligible for any therapy.

3.3.1 First round of therapy

The most common therapy employed initially was interstitial brachytherapy with afterloading, which accounted for 25% of patients. This was followed by equal rates of liver resection and transarterial chemoembolization (TACE), each accounting for just under 20% of all patients. Treatment with sorafenib was the next most common therapy at 16% of patients. Cases where disease was too advanced to treat or where patients expressly wished to receive no therapy made up a further 9% of total patients, and the remainder was accounted for by radiofrequency ablation (RFA), selective internal radiotherapy (SIRT), transarterial embolization (TAE), and percutaneous ethanol injection (PEI), all at rates under 5%.

Factors affecting the choice of initial therapy were studied using the chi-squared test. Variables that appeared to influence initial therapeutic decisions were number of lesions ($p = 0.001$), lesion size ($p = 0.043$), vascular invasion ($p < 0.001$), extent of liver infiltration </> 50% ($p = 0.001$), Child-Pugh score ($p = 0.035$), and portal hypertension ($p = 0.016$). Variables that appeared not to influence therapeutic decisions were ECOG score ($p = 0.523$) and metastasis ($p = 0.162$).

3.3.2 Second round of therapy

Approximately 60% of patients underwent a second round of therapy, although this was often with the same type of therapy as the first round. Interstitial brachytherapy with afterloading also dominated this round (20% of all patients), followed by TACE (18%), and systemic treatment with sorafenib (8.5%). RFA accounted for 4% of second-round therapy and all other modes of treatment accounted for 1-3% of patients.

3.3.3 Third round of therapy

Approximately one third of patients underwent a third round of therapy. Sixteen percent of patients were treated with TACE, 8% with interstitial brachytherapy, and 3% with sorafenib. Treatment of metastases as a third-round therapy was necessary for 2.5% of patients.

Table 13: First three rounds of therapy

Treatment multimodality	**Nr.**	**(%) (n=127)**
> 1 modality	47	37
One modality	69	54.3
No treatment	11	8.7
First treatment	**Nr.**	**(%) (n=130)**
Liver resection	24	18.5
Interstitial brachytherapy	32	24.6
RFA	6	4.6
PEI	1	0.8
TACE	25	19.2
TAE	3	2.3
SIRT	4	3.1
Chemotherapy with sorafenib	21	16.2
Conventional chemotherapy	2	1.5
No treatment possible, best supportive care	12	9.2
Second treatment		
Liver resection	3	2.3
Liver transplantation	1	0.8
Interstitial brachytherapy	27	20.8
RFA	5	3.8
TACE	23	17.7
TAE	1	0.8
SIRT	1	0.8
Chemotherapy with sorafenib	11	8.5
Conventional chemotherapy	2	1.5
Metastasis treatment	1	0.8
No second therapy	55	42.2

Table 13 (continued): First three rounds of therapy

Third treatment	Nr.	(%) (n=130)
Interstitial brachytherapy	10	7.7
RFA	1	0.8
LITT*	1	0.8
TACE	21	16.2
TAE	1	0.8
SIRT	2	1.5
Chemotherapy with sorafenib	4	3.1
Metastasis treatment	3	2.3
No third therapy	87	66.8

*LITT = Laser-induced interstitial thermotherapy, a local thermoablative technique that involves the insertion of a laser applicator into tumor tissue to apply high levels of heat in an effort to destroy malignant tissue.[137] As this is an uncommon therapy, used in only one patient within this cohort, it was not presented with the more common therapies described in the Introduction section.

3.4 Univariate survival analysis

3.4.1 Overall survival and disease-free survival of entire cohort

Kaplan-Meier analysis of the entire patient cohort showed a median overall survival (OS) of 42 months (95% confidence interval, CI: 28.9-55.2) and a median disease-free survival (DFS) of 9 months (95% CI: 6.2-11.8). The overall 1-,3-, and 5-year survival was 79% (95% CI: 70.9-86.1), 54% (95% CI: 43.6-64.8), and 38% (95% CI: 24.3-50.7) respectively. Disease-free survival rates were 37% at 1 year (95%CI: 28.3-45.9), 11% at 3 years (95% CI: 4.6-16.6), and 4% at 5 years (95% CI: -0.7-8.9).

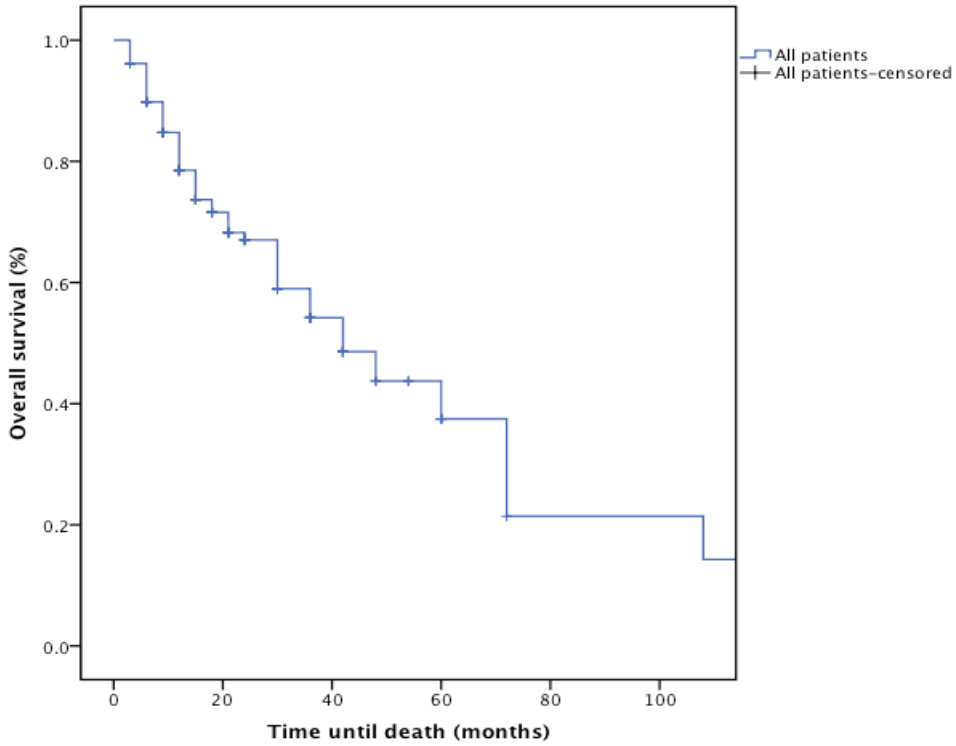

Figure 2: Kaplan-Meier curve of overall survival of entire patient cohort

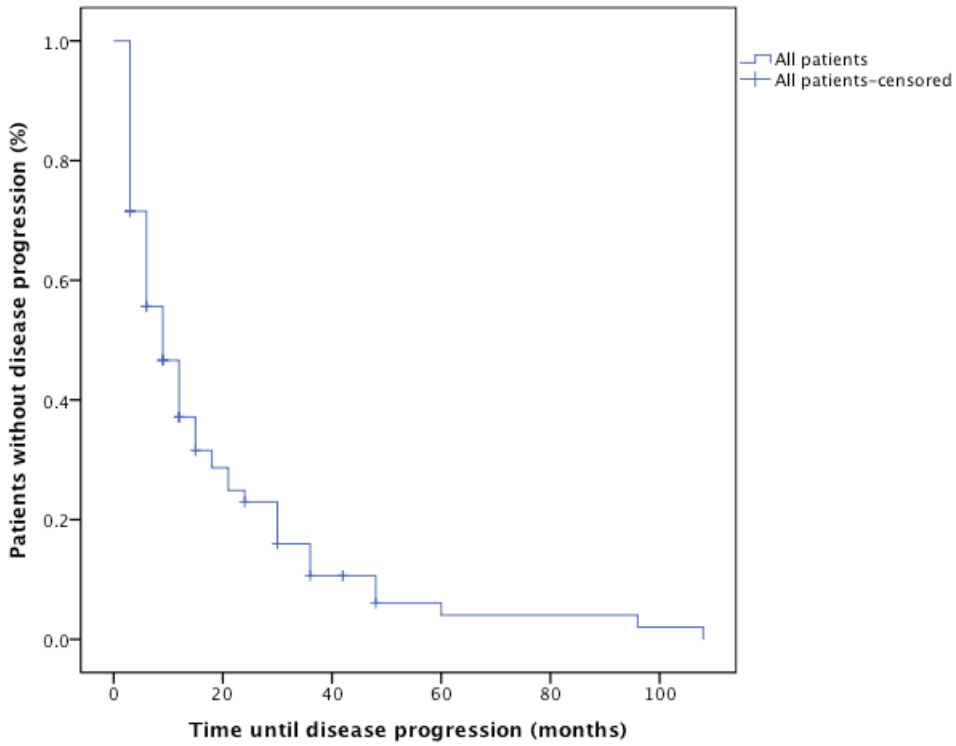

Figure 3: Kaplan-Meier curve of disease-free survival of entire patient cohort

(Two patients at far end not included)

3.4.2 Overall and disease-free survival in terms of gender, age, and ethnicity

There were no significant differences in OS of patients in terms of age (p = 0.707), gender (p = 0.414), or ethnic origin (p = 0.204). Disease-free survival was however significantly longer in the youngest age category of < 50 years (p = 0.032), with a median DFS of 96 months (95% CI not available due to small group size, n=8), compared to 6-12 months in all other age categories.

3.4.3 Overall survival in terms of liver function and patient performance status

Patient OS appeared to be strongly influenced by both liver function and patient performance status. In terms of liver function, there were significant differences in OS for patients grouped according to bilirubin level (p < 0.001), Quick score (p < 0.001), albumin level (p = 0.015), presence of encephalopathy (p = 0.026), and presence of ascites (p < 0.001). Predictably, the Child-Pugh score of liver cirrhosis was therefore also significantly linked to OS (p < 0.001), with Child A patients having a median OS of 48 months (95% CI: 31.2-64.8), as opposed to patients with cirrhosis at the stages Child B (12 months, 95% CI: 7.2-16.8), and Child C (6 months, 95% CI not available due to small group size, n=5). Surprisingly, the presence or absence of cirrhosis did not show a significant association to OS (p = 0.317), although prolonged survival was seen for non-cirrhotics, with a median OS of 60 months (95% CI: 0.7-119.3). The presence of portal hypertension showed a marginally insignificant relationship to OS (p = 0.079), although there was a noticeable difference in median OS for portal hypertensive patients (36 months, 95% CI: 25.4-46.6) versus patients without portal hypertension (60 months, 95% CI: 41.3-78.7). The cause of cirrhosis did not appear to be associated with OS (p = 0.446).

The patient performance status, understood here as ECOG score, appeared to play a significant role in OS (p = 0.003), with fully active patients achieving a median OS of 42 months (95% CI: 26.6-57.4) as opposed to patients who were restricted in their daily activities, where median OS was 15 months (95% CI: 8.0-22.0). Patients in the slightly restricted group appeared to survive at similar rates compared to fully active patients (estimated mean of 42 months, 95% CI: 32.8-51.7).

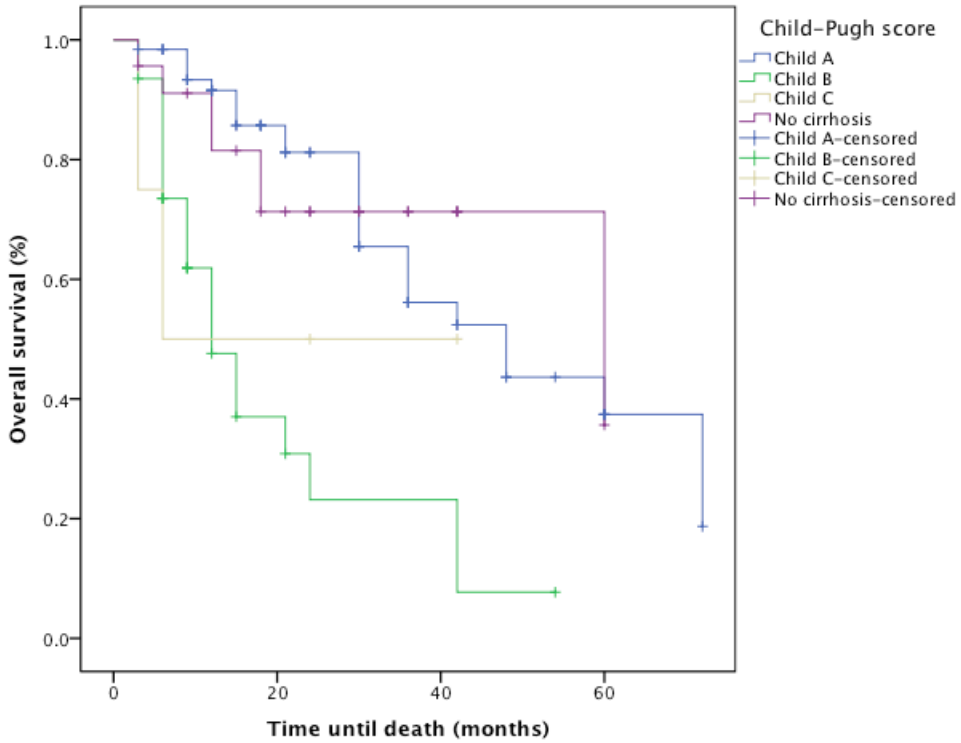

Figure 4: Kaplan-Meier curve of overall survival in terms of Child-Pugh score

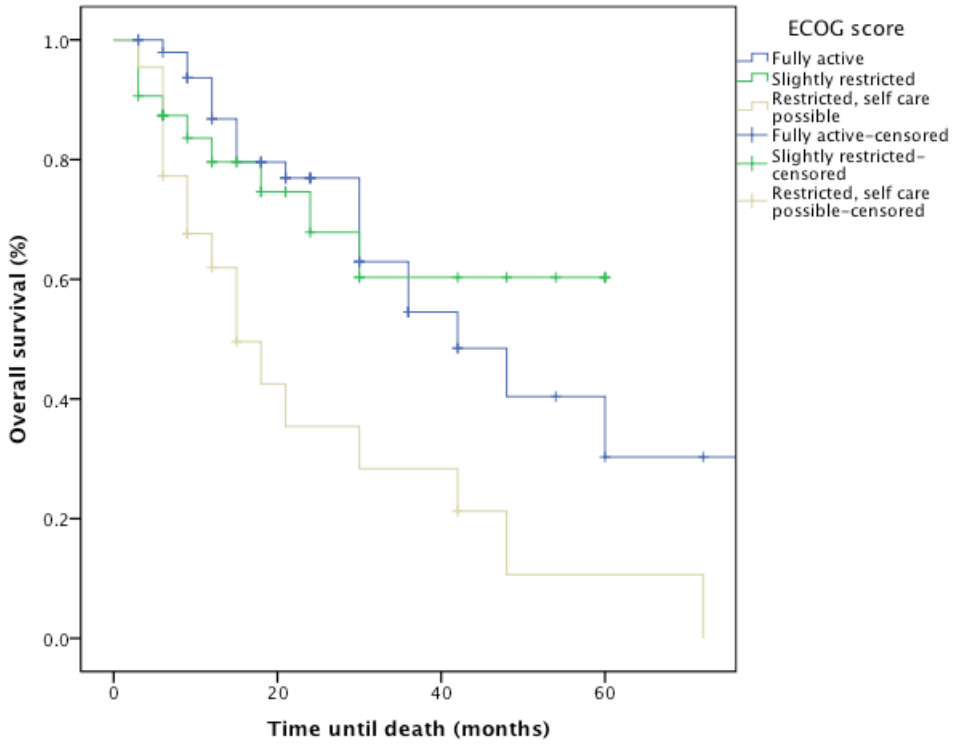

Figure 5: Kaplan-Meier curve of overall survival in terms of ECOG score

(One patient at far end not included)

3.4.4 Disease-free survival in terms of liver function and patient performance status

Poor liver function also appeared to negatively influence DFS. With the exceptions of albumin level (p = 0.598) and the presence of encephalopathy (p = 0.125), there were significant differences between patients grouped according to the liver function parameters, bilirubin (p = 0.026), Quick (p = 0.041), presence of ascites (p = 0.017), and Child-Pugh score (p = 0.012). Child A patients showed a median DFS of 12 months (95% CI: 8.2-15.8), compared to Child B (6 months, 95% CI: 2.9-9.1), Child C (3 months, 95% CI not available due to small group size, n=5). Patients with no cirrhosis had a median time to progression of 9 months (95% CI: 5.6-12.4). Portal hypertension also appeared to impact time to disease progression (p = 0.022), although median DFS was 9 months for both groups (95% CI for portal hypertension = Y: 6.4-11.6, and for portal hypertension = N: 0-21.5). However, upon analysis of the patient percentiles for DFS, the absence of portal hypertension corresponded with more than double the median survival at 25% DFS (36 months vs. 15 months), and double the median DFS of patients with portal hypertension at 75% DFS (6 months vs. 3 months).
Patient performance status was not significantly linked to DFS (p = 0.516).

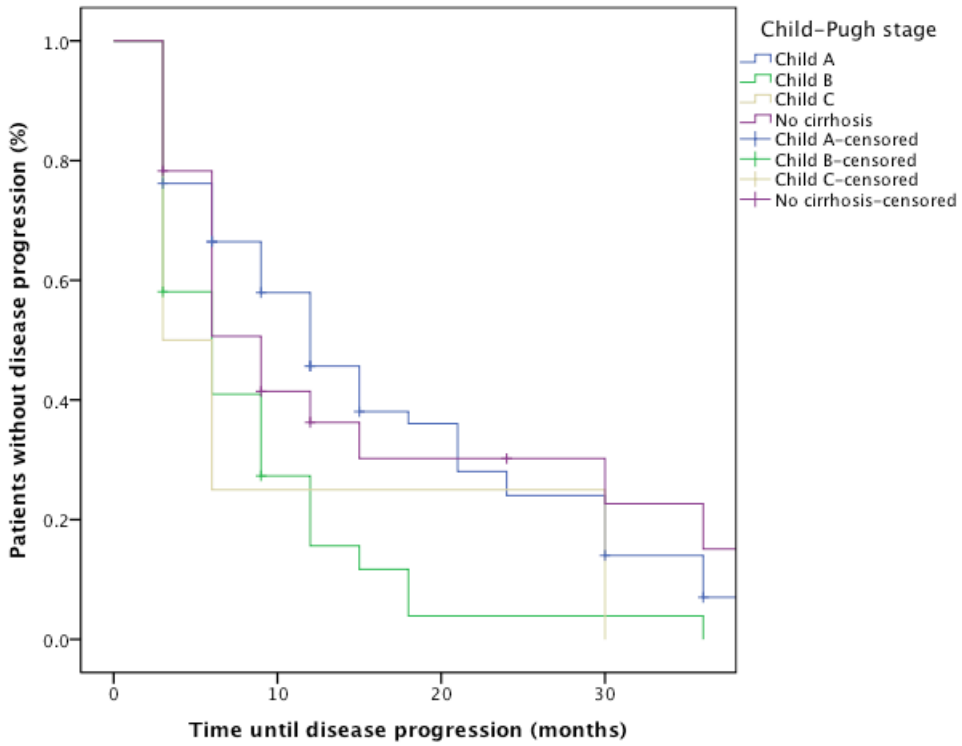

Figure 6: Kaplan-Meier curve of disease-free survival in terms of Child-Pugh score
(Four patients at far end not included)

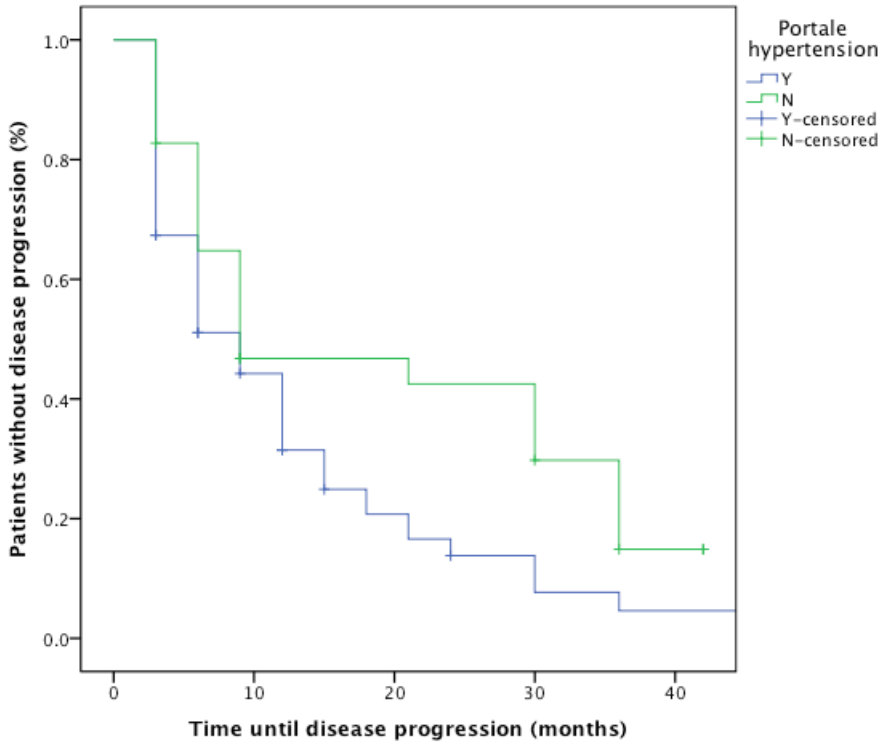

Figure 7: Kaplan-Meier curve of disease-free survival in terms of portal hypertension
(Three patients at far end not included)

3.4.5 Overall survival in terms of tumor characteristics

In contrast to the above-mentioned findings, many attributes concerning HCC itself were not significantly related to OS. There was a significant difference in OS between groups categorized according to number of lesions (p = 0.016) and lesion size (p = 0.02). Patients with 1-3 lesions achieved a median OS of 42 months (95% CI: 30.7-53.3), as opposed to those with 4-5 lesions, who achieved only 15 months (95% CI: 10.4-19.6). Median OS could not be assessed for patients with more than 5 lesions due to the small group number (n=9) containing only one death (at 12 months). The remaining patients were still alive at durations ranging from 3-60 months.

As for survival in terms of lesion size, while the median OS of the group with a largest lesion of ≤ 2 cm could also not be assessed due to the group's small size (n=12), and low mortality (two deaths at 24 and 30 months), the remaining patients showed good cumulative survival ranging from 6-60 months, with an average of 51 months (95% CI: 40.1-61.7). The group with a largest lesion size of > 2-6 cm had a median OS of 48 months (95% CI: 32.1-63.9), and where the largest lesion exceeded a diameter of 6 cm, median OS was 30 months (95% CI: 12.8-47.2).

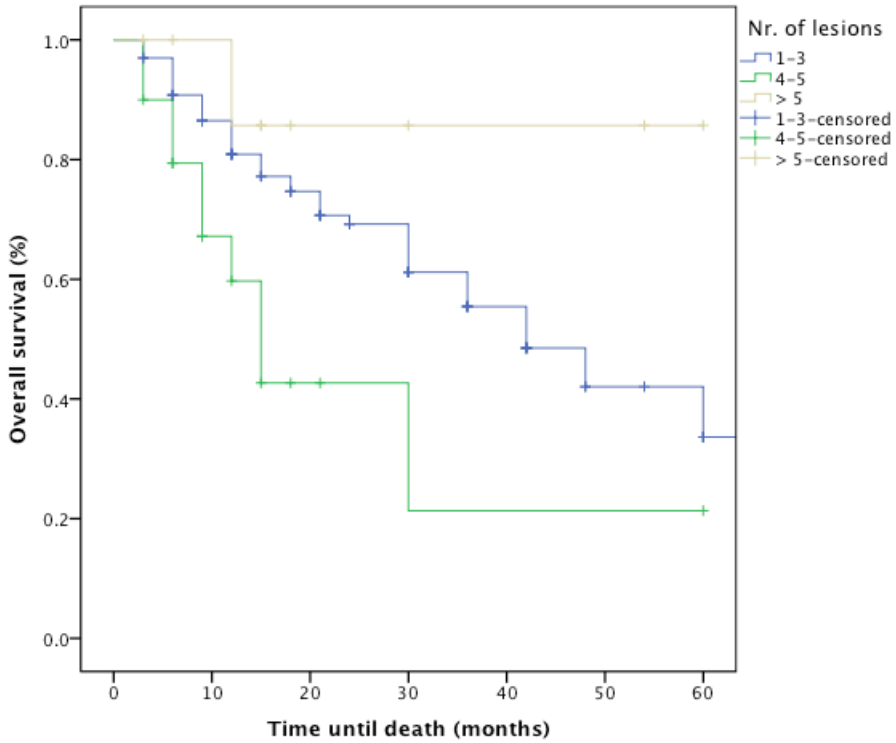

Figure 8: Kaplan-Meier curve of overall survival in terms of number of lesions

(Five patients at far end not included)

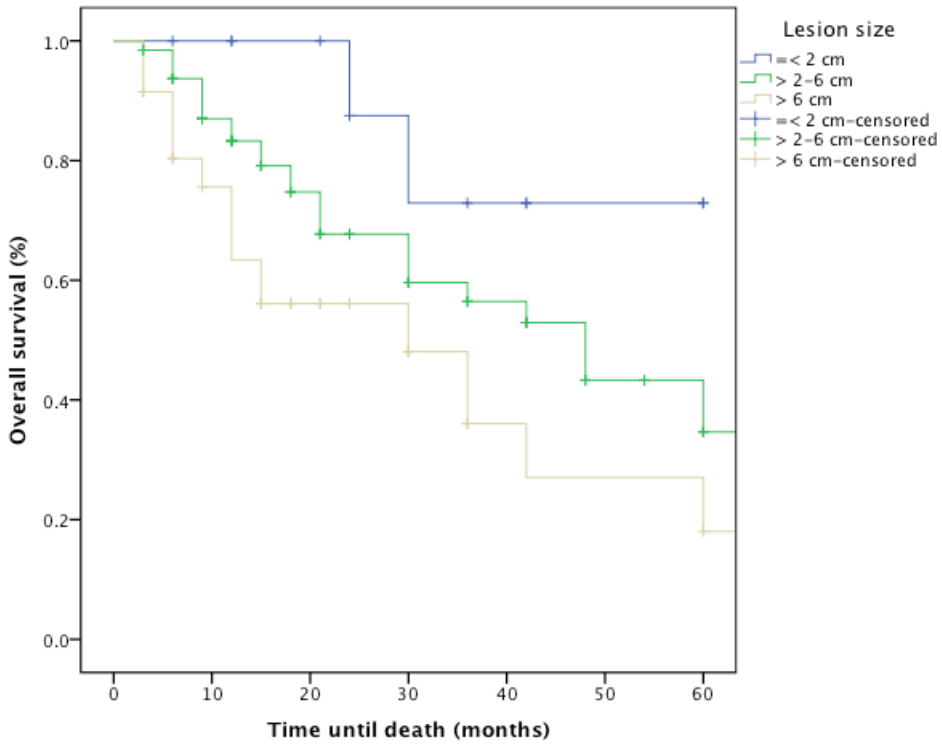

Figure 9: Kaplan-Meier curve of overall survival in terms of lesion size

(Three patients at far end not included)

Having an AFP of < 400 ng/ml upon initial diagnosis of HCC corresponded with better survival (p = 0.001), with patients below this threshold showing a median OS of 42 months (95% CI: 27.9-56.1), compared to 12 months for those above (95% CI: 6.7-17.3). Patients with AFP levels in the normal range (<13.4 ng/ml) did not show a significantly improved survival compared to those above this threshold (p = 0.467). Vascular invasion of the portal or hepatic veins was significantly associated with poorer survival (p < 0.001), with a median survival of 15 months (95% CI: 8.0-22.0), compared to 48 months where no infiltration was found (95% CI: 36.4-59.6). Although median OS could not be calculated for patients with microvascular or small-vascular invasion (due to low mortality and patient number) survival rates appeared to be similar to patients without vascular invasion, with the exception of one patient who was still alive at 192 months. OS was not significantly associated with metastasis (p = 0.662), extent of liver infiltration (p = 0.377), or degree of tumor differentiation (p = 0.903). Interestingly however, AFP levels of > 400 ng/ml were (marginally) significantly correlated with the presence of metastasis (p = 0.052), vascular invasion (p = 0.004), and larger tumor size (taken as the T stage in the TNM staging system, p = 0.01) using the chi-squared or Fisher's test.

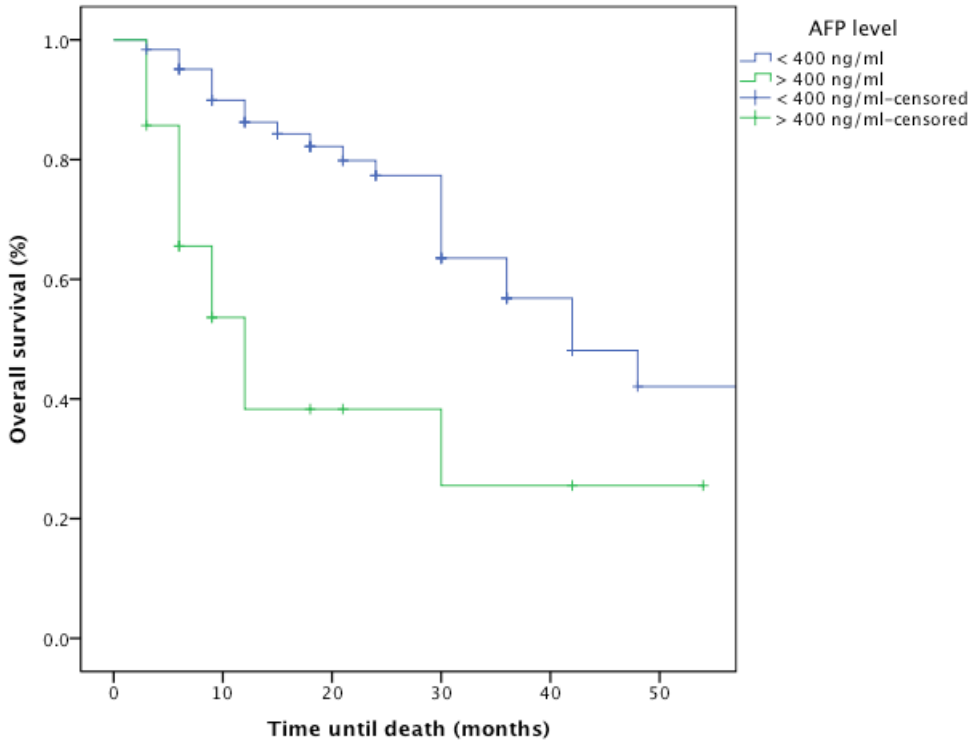

Figure 10: Kaplan-Meier curve of overall survival in terms of AFP level
(Six patients at far end not included)

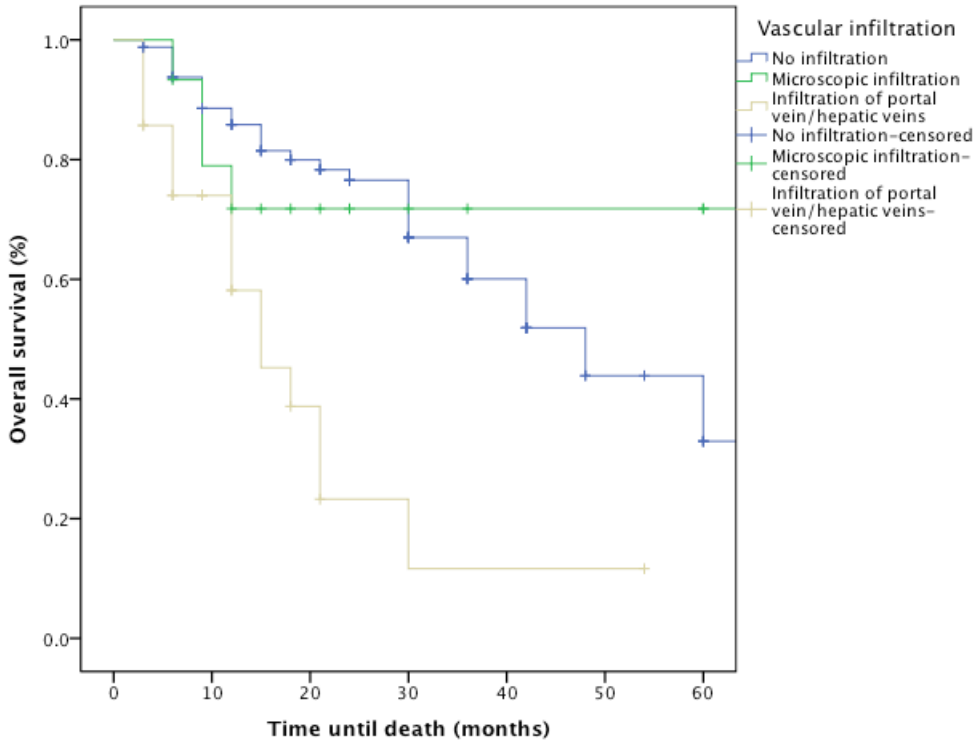

Figure 11: Kaplan-Meier curve of overall survival in terms of vascular infiltration
(Four patients at far end not included)

3.4.6 Disease-free survival in terms of tumor characteristics

The category of lesion size was the only tumor-related characteristic that appeared to influence DFS (p = 0.038). While median time to progression in patients with a largest lesion of > 6 cm was six months (95% CI: 3.9-8.1), the groups of ≤ 2 cm and > 2-6 cm showed DFS of 9 months (95% CI: 0.0-19.2) and 12 months respectively (95% CI: 8.4-15.6). Lesion number (p = 0.651), AFP level < or > 400 ng/ml (p = 0.11), AFP level < or > 13.4 ng/ml (p = 0.181), vascular invasion (p = 0.184), metastasis (p = 0.164), extent of liver infiltration (p = 0.896), and degree of tumor differentiation (p = 0.185) did not correspond significantly with DFS.

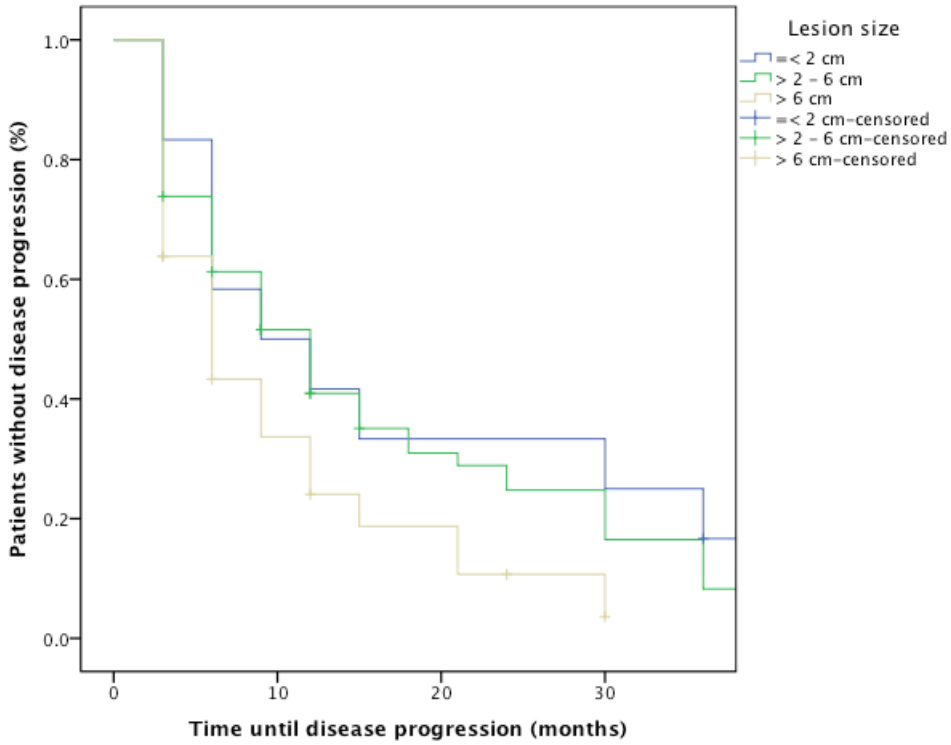

Figure 12: Kaplan-Meier curve of disease-free survival in terms of lesion size
(Five patients at far end not included)

3.4.7 Overall survival in terms of staging system

Of the four staging systems discussed in section 1.7, all were found to be strongly associated with OS except for the BCLC staging system. The Okuda system showed a significance of $p <$ 0.001, with a median survival for stage 1 patients of 48 months (95% CI: 35.4-60.6), compared with 21 months for stages 2/3 (95% CI: 10.3-31.7). The TNM system was analyzed solely according to the T-stage and also showed significance of < 0.001. Patients at stage T1 lived for a median of 48 months (95% CI: 34.6-61.4), and T2 patients survived a median of 30 months (95% CI: 18.2-41.8). Stage T3a patients could not be assessed as less than half of the group had died upon analysis, and mean data were not useful, given the unusually long survival of one patient in this group (192 months). However, using 75% OS, patients survived for 15 months, less than T1 and T2 (42 and 18 months respectively) but more than the T3b/T4 group (6 months). T3b/T4 achieved a median OS of 15 months (95% CI: 8.1-21.9). The CLIP score likewise showed strong level of significance ($p < 0.001$), with a median OS of 48 months (95% CI: 34.0-62.0) for stages 0/1, 30 months for stages 2/3 (95% CI: 20.6-39.4), and 6 months for stages 4/5 (95% CI: 1.3-10.7). Interestingly, the BCLC system did not show the ability to adequately differentiate between groups ($p = 0.491$). Although patients at BCLC stage A did

show the longest OS with 48 months (95% CI: 34.6-61.4), the BCLC stages B and C showed similar rates of median OS of 36 months and 30 months respectively (95% CI: 28.4-43.6 and 10.7-49.3 respectively). BCLC stage D patients survived for a median of 6 months (95% CI unable to be calculated due to small patient group, n = 4). Potential reasons for the unexpected finding of the BCLC staging system's poor predictive ability will be outlined in the Discussion section.

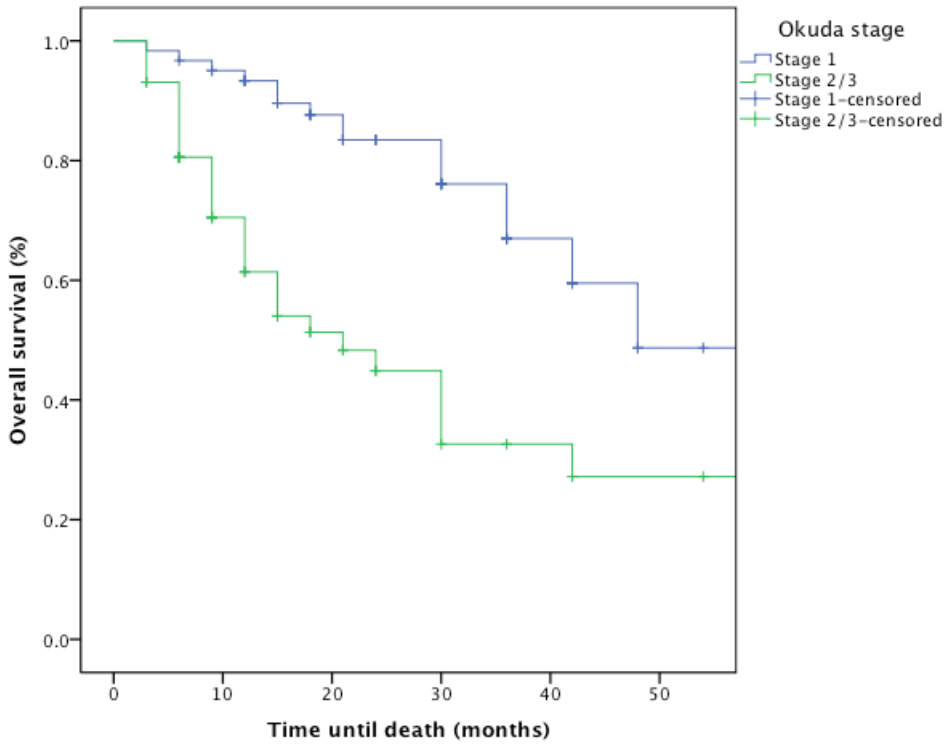

Figure 13: Kaplan-Meier curve of overall survival in terms of Okuda stage

(Nine patients at far end not included)

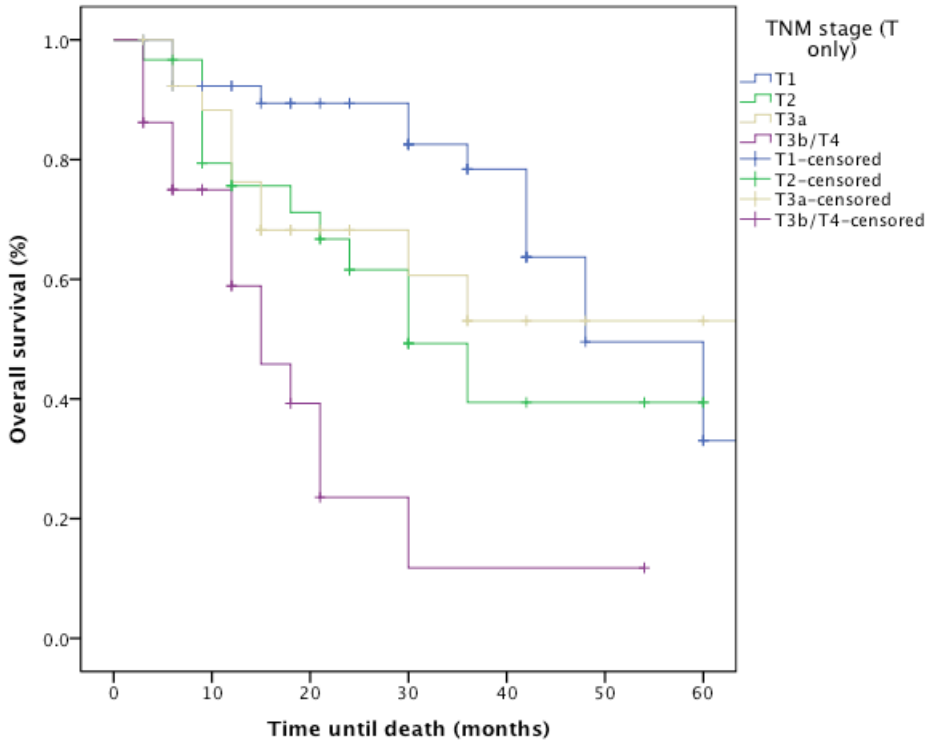

Figure 14: Kaplan-Meier curve of overall survival in terms of TNM stage

(Four patients at far end not included)

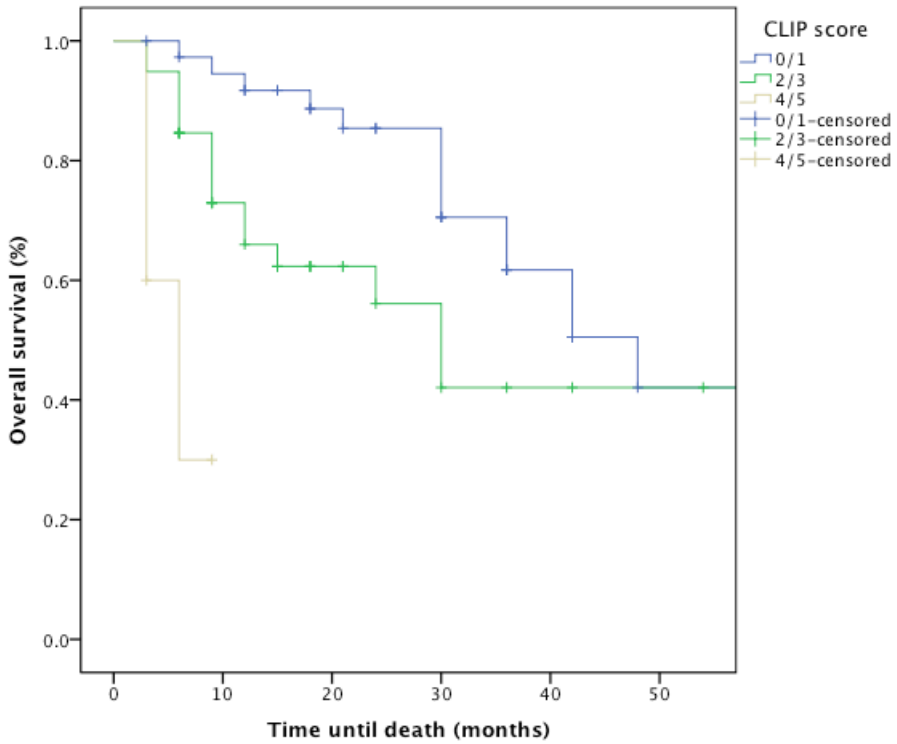

Figure 15: Kaplan-Meier curve of overall survival in terms of CLIP stage

(Six patients at far end not included)

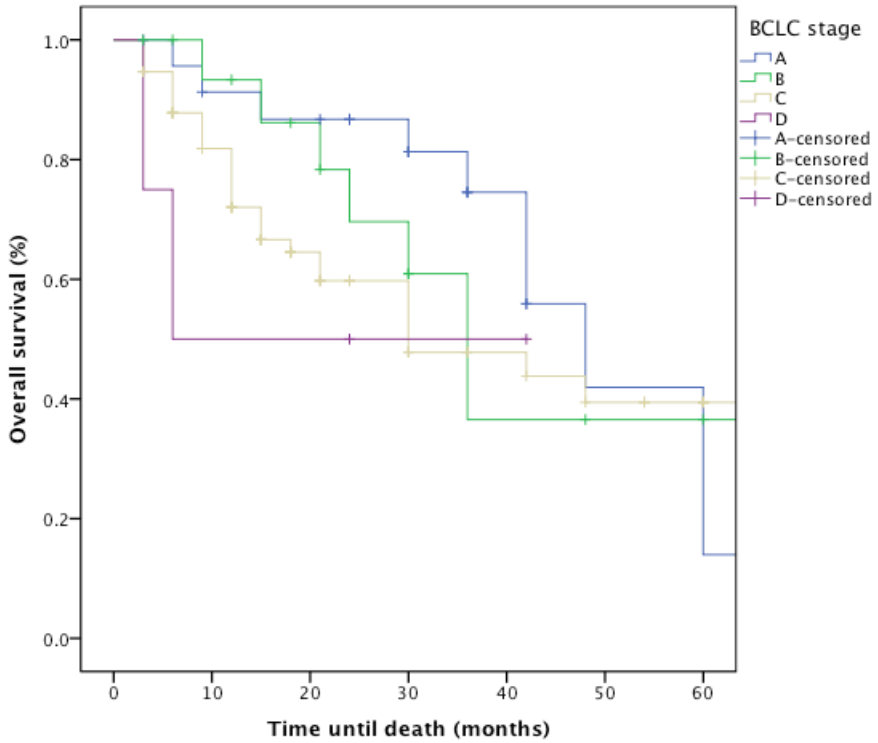

Figure 16: Kaplan-Meier curve of overall survival in terms of BCLC stage

(Four patients at far end not included)

3.4.8 Disease-free survival in terms of staging system

Both the Okuda and TNM staging systems were adequately predictive for DFS (p = 0.007 and p = 0.01 respectively). Stage 1 Okuda patients lived free of disease for a median of 12 months (95% CI: 6.9-17.1), twice as long as stage 2/3 patients (95% CI: 3.9-8.1). As for the TNM system, patients with stage T1 lived free of disease for a median of 15 months (95% CI: 7.6-22.4), T2 showed DFS of 9 months (95% CI: 6.2-11.8), T3a of 12 months (95% CI: 4.5-19.5), and T3b/T4 were disease-free for a median of six months (95% CI: 0.5-11.5).

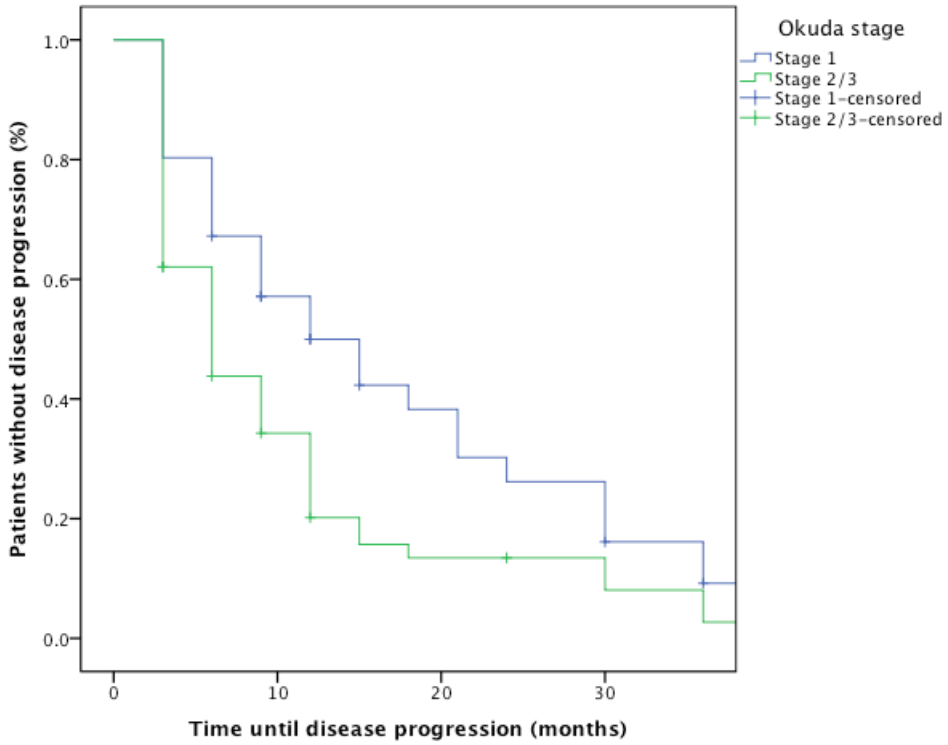

Figure 17: Kaplan-Meier curve of disease-free survival in terms of Okuda stage

(Four patients at far end not included)

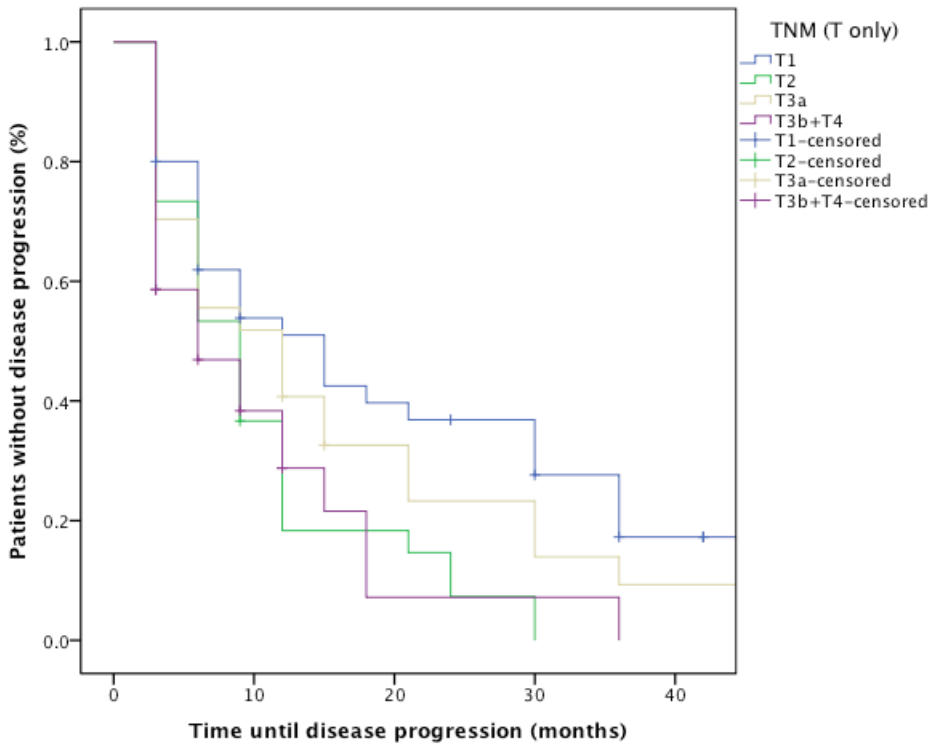

Figure 18: Kaplan-Meier curve of disease-free survival in terms of T stage (TNM)

(Four patients at far end not included)

The CLIP system differentiated median DFS well between groups (CLIP 0/1: 12 months, CLIP 2/3: 6 months, and CLIP 4/5: 3 months, 95% CI: 5.7-18.3, 2.7-9.3, not available for CLIP 4/5 due to small group size, n=6, respectively) but was not significant (p = 0.071). The BCLC system did not achieve significance (p = 0.077) but also differentiated median DFS well between groups (BCLC A: 15 months, BCLC B: 12 months, BCLC C: 6 months, BCLC D: 3 months, 95% CI: 2.0-28.0, 6.0-18.0, 3.6-8.4, not available for BCLC D due to small group size, n=4, respectively).

3.4.9 Overall survival in terms of treatment type and multimodality

Treatment was categorized as being either curative (i.e. liver resection, liver transplantation, PEI, RFA, and interstitial brachytherapy), locoregional non-curative (i.e. TACE, TAE, and SIRT), systemic (i.e. sorafenib), or absent (i.e. best supportive care and observation). Survival was analyzed based on the first three rounds of treatment. There were three patients who received conventional chemotherapy within the first three rounds of therapy. Given this small patient number and the now professional consensus that conventional chemotherapy is ineffective in treating HCC, these patients were excluded from the following analyses. The chemotherapy group being studied here thus only contained patients who received sorafenib.

For the initial round of treatment there was a significant difference in OS per therapy group (p = 0.024), with median OS of 60 months for the curative group (95% CI: 41.7-78.3), 42 months for the non-curative locoregional group (95% CI: 26.0-58.0), and 30 months for patients treated systemically with sorafenib (95% CI: 16.7-43.3). Almost half of all patients in the ‘no treatment’ group were deceased at 9 months, with survivors still alive at durations ranging from 9-60 months.

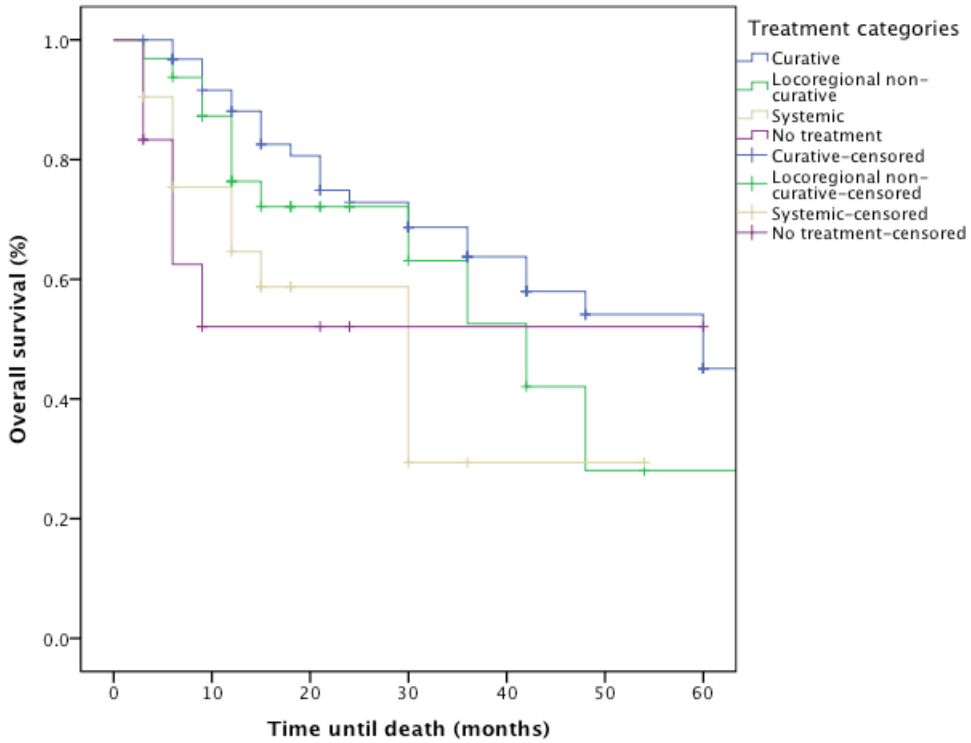

Figure 19: Kaplan-Meier curve of overall survival in terms of first treatment

(Seven patients at far end not included)

In analysis based on multiple treatment types versus just one treatment type or no treatment, a strong correlation was found favoring treatment multimodality (p = 0.001). This group achieved a median OS of 72 months (95% CI: 47.9-96.1), twice that of the group that received only one type of treatment (median OS of 36 months, 95% CI: 19.0-53.0), and an eight-fold increase compared to patients who received no therapy at all (9 months, 95% CI not calculated due to small patient group, n=11).

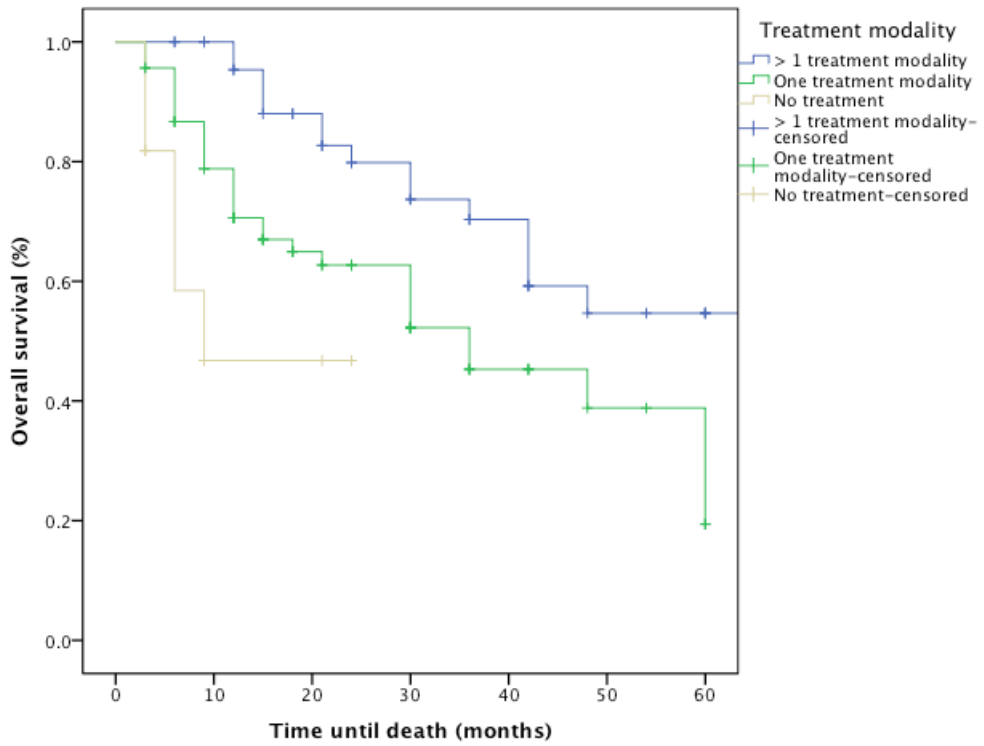

Figure 20: Kaplan-Meier curve of overall survival in terms of treatment multimodality
(Seven patients at far end not included)

3.4.10 Disease-free survival in terms of treatment

A significant association was shown based on analysis of DFS in terms of the first round of treatment (p = 0.002). Patients initially treated curatively survived free of disease for a median of 15 months (95% CI: 7.9-22.1). This was more than double the median DFS for the non-curative locoregional and systemic groups, which both achieved a 6-month DFS (95% CI: 2.8-9.3 and 3.5-8.5 respectively). Progressive disease had occurred in half of the untreated patients by the time of the first follow-up at three months, and the longest time to disease progression was 36 months in this group.

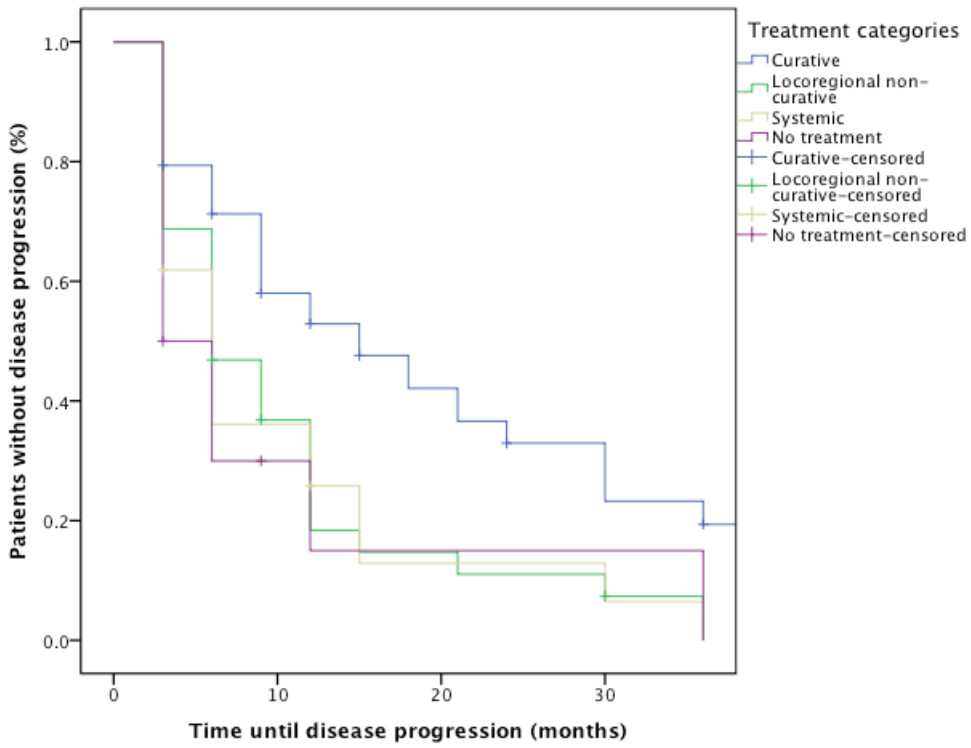

Figure 21: Kaplan-Meier curve of disease-free survival in terms of first treatment
(Eight patients at far end not included)

Similar to the situation with OS, therapy of HCC with multiple treatment modalities also corresponded here with a prolonged DFS (p = 0.028). Patients treated with more than one type of therapy remained disease-free for a median of 12 months (95% CI: 7.2-16.8), compared to 9 months for patients only treated with one type of therapy (95% CI: 6.7-11.3), and 3 months for patients not treated at all (95% CI not calculated due to small patient group, n=11).

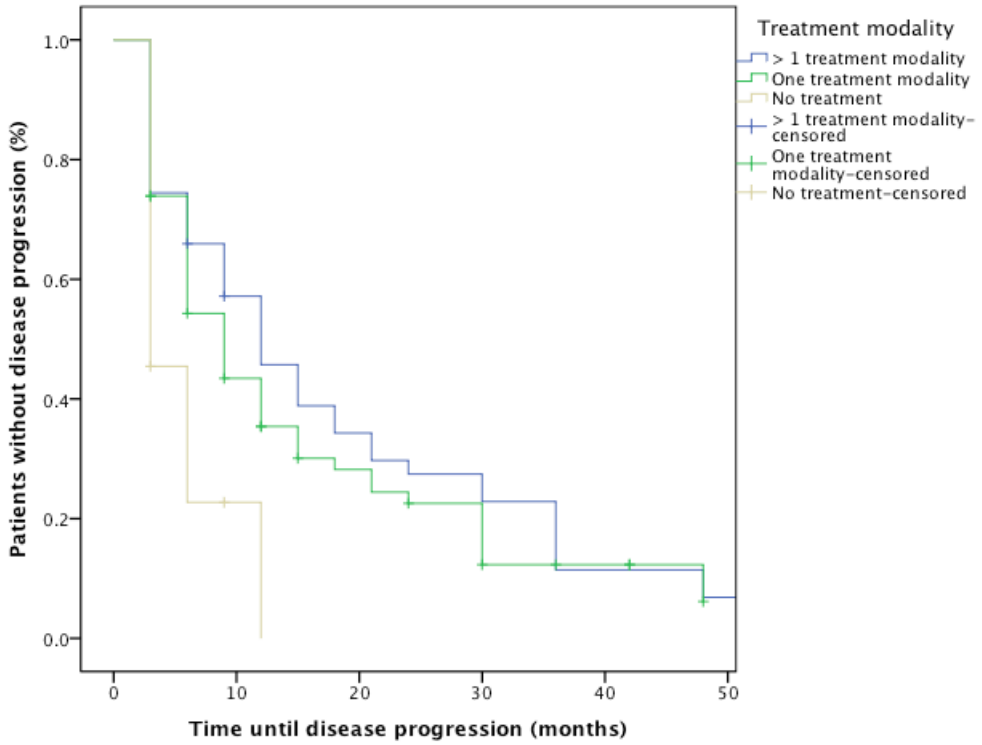

Figure 22: Kaplan-Meier curve of disease-free survival in terms of treatment modality

(Three patients at far end not included)

Table 14: Summary of univariate analysis of baseline variables (p ≤ 0.05 in bold print)

		OS			DFS	
	p	**Median OS (mths)**	**95% CI**	**p**	**Median DFS (mths)**	**95% CI**
Entire cohort (n=130)	-	42	28.9-55.2	-	9	6.2-11.8
Gender	0.414			0.816		
Male		36	20.7-51.3		9	5.3-12.7
Female		72	-		9	6.1-11.9
Cirrhosis	0.317			0.72		
N		60	0.7-119.3		9	5.6-12.4
Y		42	29.7-54.3		9	6.0-12.0
Cause of cirrhosis	0.446			0.867		
Alcohol		36	20.9-51.1		6	2.2-9.8
HCV		72	-		9	5.3-12.7
HBV		48	22.6-73.4		15	9.5-20.5
Other causes		42	12.5-71.5		12	5.6-18.4
Ascites	**<0.001**			**0.017**		
Absent		48	33.2-62.8		12	8.8-15.2
Mild		15	7.8-22.2		6	-
Severe		42	0.0-101.7		6	3.9-8.1
Encephalopathy	**0.026**			0.125		
N		42	29.9-54.1		9	6.3-11.7
Y		21	-		6	3.4-8.6
Child-Pugh Score	**<0.001**			**0.012**		
A		48	31.2-64.8		12	8.2-15.8
B		12	7.2-16.8		6	2.9-9.1
C		6	-		3	-
Portal hypertension	0.079			**0.022**		
N		60	41.3-78.7		9	0.0-21.5
Y		36	25.4-46.6		9	6.4-11.6
ECOG	**0.003**			0.516		
1: Fully active		42	26.6-57.4		12	7.7-16.3
2: Slightly restricted		-			9	4.5-13.5
3: Restricted		15	8.0-22.0		6	3.6-8.4

Table 15: Summary of univariate analysis of tumor-related variables (p ≤ 0.05 in bold print)

	OS			DFS		
Variable	**p**	**Median OS (mths)**	**95% CI**	**p**	**Median DFS (mths)**	**95% CI**
Entire cohort (n=130)	-	42	28.9-55.2	-	9	6.2-11.8
Number of lesions	**0.016**			0.651		
1-3		42	30.7-53.3		9	6.1-11.9
4-5		15	10.4-19.6		6	0.0-13.8
>5		-	-		12	6.0-18.0
Lesion size (cm)	**0.02**			**0.038**		
≤ 2		-	-		9	0.0-19.2
> 2-6		48	32.1-63.9		12	8.4-15.6
> 6		30	12.8-47.2		6	3.9-8.1
AFP (ng/ml)	**0.001**			0.11		
< 400		42	27.9-56.1		12	8.9-15.1
> 400		12	6.7-17.3		6	4.1-7.9
AFP (ng/ml)	0.467			0.181		
< 13.4		42	24.9-59.1		12	9.1-14.9
> 13.4		36	18.3-53.7		6	2.8-9.1
Portal vein invasion	**< 0.001**			0.184		
N		48	36.4-59.6		9	5.8-12.2
Y		15	8.0-22.0		6	0.6-11.4
Liver infestation	0.377			0.896		
< 50%		42	29.7-54.3		9	5.8-12.2
> 50%		-			12	6.0-18.0
Tumor differentiation	0.903			0.185		
G1		48	30.1-65.9		6	1.7-10.3
G2		60	24.0-96.0		9	3.4-14.6
G3 + G4		-			18	0.0-37.1
Metastasis	0.662			0.164		
No metastasis		42	29.9-54.1		12	8.9-15.1
Regional metastasis		18	-		6	3.8-8.2
Distant metastasis		30	7.9-52.1		6	3.1-8.9

Table 16: Summary of univariate analysis of staging systems (p ≤ 0.05 in bold print)

	OS			DFS		
Variable	**p**	**Median OS (mths)**	**95% CI**	**p**	**Median DFS (mths)**	**95% CI**
Entire cohort (n=130)	-	42	28.9-55.2	-	9	6.2-11.8
Okuda score	**< 0.001**			**0.007**		
1		48	35.4-60.6		12	6.9-17.1
2/3		21	10.3-31.7		6	3.9-8.1
T stage (TNM)	**< 0.001**			**0.01**		
1		48	34.6-61.4		15	7.6-22.4
2		30	18.2-41.8		9	6.2-11.8
3a		-			12	4.5-19.5
3b/4		15	8.1-21.9		6	0.5-11.5
CLIP score	**< 0.001**			0.071		
0/1		48	34.0-62.0		12	5.7-18.3
2/3		30	20.6-39.4		6	2.7-9.3
4/5		6	1.3-10.7		3	-
BCLC	0.491			0.077		
A		48	34.6-61.4		15	2.0-28.0
B		36	28.4-43.6		12	6.0-18.0
C		30	10.7-49.3		6	3.6-8.4
D		6	-		3	-

Table 17: Summary of univariate analysis of treatment (p ≤ 0.05 in bold print)

	OS			DFS		
Variable	**p**	**Median OS (mths)**	**95% CI**	**p**	**Median DFS (mths)**	**95% CI**
Entire cohort (n=130)	-	42	28.9-55.2	-	9	6.2-11.8
First treatment	**0.024**			**0.002**		
Curative		60	41.7-78.3		15	7.9-22.1
Non-curative locoregional		42	26.0-58.0		6	2.8-9.3
Systemic		30	16.7-43.3		6	3.5-8.5
No treatment		-			3	-
Treatment multimodality	**0.001**		**(n=127)**	**0.028**		**(n=127)**
> 1 treatment modality		72	47.9-96.1		12	7.2-16.8
One treatment modality		36	19.0-53.0		9	6.7-11.3
No treatment		9	-		3	-

3.5 Multivariate survival analysis

Single variables found to have a significant influence on OS in Kaplan-Meier analysis were further studied in a multifactorial context using the Cox regression model. As mentioned in section 2.2, unfortunately, several variables could not be included in the Cox model of overall survival due to a large number of missing cases. These excluded variables were AFP level, ECOG score, and all staging systems (only the CLIP score had to be excluded for the analysis of disease-free survival). Given the importance of staging systems in the therapeutic decision-making process, multifactorial analysis of a revised subgroup of patient data was thus carried out (see sections 3.5.3 and 3.5.4 below).

In order to simplify the analysis, the individual parameters used in ascertaining patients' Child-Pugh scores were summarized and replaced by the Child-Pugh score alone. After carrying out the backward elimination (likelihood ratio) method, the most significant variables were further analyzed in the standard Cox regression method.

3.5.1 Factors influencing overall survival in a multifactorial context

The variables of Child-Pugh score ($p < 0.001$), lesion size category ($p = 0.025$), and therapy multimodality ($p = 0.011$) proved to have the most influence on overall patient survival in a multifactorial context. The following table displays the correlations found between the subgroups of these variables and hazard ratio.

Table 18: Summary of prognostic parameters for overall survival displaying the most significance in multivariate analysis (p ≤ 0.05 in bold print)

	p	Hazard ratio	95% CI
Level of cirrhosis	**< 0.001**		
No cirrhosis			
Child A	0.227	1.8	0.7-4.4
Child B	**< 0.001**	7.2	2.7-19.1
Child C	0.246	2.6	0.5-13.0
Lesion size (cm)	**0.025**		
≤ 2			
> 2-6	0.260	2.3	0.5-10.0
> 6	**0.047**	4.4	1.0-19.2
Therapy multimodality	**0.011**		
> 1 therapy modality			
One therapy modality	**0.024**	2.1	1.1-4.2
No therapy	**0.005**	4.9	1.6-14.9

The remaining parameters that were found to play a significant role in influencing OS in univariate analysis were not significant using the Cox model. These were category of initial treatment ($p = 0.79$), presence of vascular invasion ($p = 0.388$), and lesion number category ($p = 0.263$).

3.5.2 Factors influencing disease-free survival in a multifactorial context

Using the same method of multivariate analysis for disease-free survival, Child-Pugh score ($p = 0.026$) and lesion size category ($p = 0.051$) proved to be the most significant variables (see following table):

Table 19: Summary of prognostic parameters for disease-free survival displaying the most significance in multivariate analysis (p ≤ 0.05 in bold print)

	p	Hazard ratio	95% CI
Level of cirrhosis	**0.026**		
No cirrhosis			
Child A	0.859	1.1	0.6-1.8
Child B	**0.020**	2.1	1.1-3.8
Child C	0.280	1.8	0.6-5.6
Lesion size	0.051		
< 2 cm			
≥ 2 – 6 cm	0.472	1.3	0.6-2.7
> 6 cm	0.058	2.1	1.0-4.3

The variables of age category (p = 0.247), portal hypertension (p = 0.113), therapy multimodality (p = 0.599), and initial treatment category (p = 0.617) were not significant when studied in a multifactorial context.

3.5.3 Revised analysis for assessment of OS in terms of staging systems

In order to validate staging system accuracy in predicting overall survival in a multifactorial context, a revised analysis was performed for patients whose Okuda, CLIP, TNM, and BCLC data were known. There were 78 patients who fulfilled this condition. Analogous to the main analyses, the four staging systems were first tested against one another in the backward elimination method. This resulted in only the TNM system displaying significance (p=0.01). There was no significance found for the Okuda (p=0.099), CLIP (p=0.288) or BCLC (p=0.566) systems.

The TNM stage was then further analyzed in a backward elimination model along with the variables found to be significant in Kaplan-Meier analysis of the entire patient population that also fulfilled the minimum amount of cases for inclusion (Child-Pugh score, vascular infiltration, lesion number category, lesion size category, and therapeutic multimodality, see above). In this context, the TNM system maintained its significance (p=0.048) alongside Child-Pugh score (p=0.007), lesion size category (p=0.043). The lesion number category was marginally significant (p=0.063). The results of standard Cox regression of these variables are summarized in the table below.

Table 20: Summary of prognostic parameters for overall survival displaying the most significance (p ≤ 0.05 in bold print) in revised multivariate analysis (to accommodate for missing cases in staging data)

	p	Hazard ratio	95% CI
Level of cirrhosis	**0.007**		
No cirrhosis			
Child A	0.071	4.6	0.9-24.0
Child B	**0.002**	21.3	3.0-150.6
Child C	**0.05**	9.0	1.0-80.9
Lesion size (cm)	**0.043**		
≤ 2			
> 2-6	0.355	2.1	0.4-9.6
> 6	**0.037**	6.4	1.1-36.1
Lesion number	0.063		
1-3			
4-5	**0.019**	4.0	1.3-12.4
>5	0.977	-	-
TNM stage	**0.048**		
T1			
T2	0.364	1.6	0.6-4.6
T3a	**0.032**	0.2	0.03-0.9
T3b + T4	0.829	1.2	0.3-4.4

3.5.4 Revised analysis for assessment of DFS in terms of staging systems

Analysis of the revised patient data was likewise performed for the four staging systems in terms of disease-free survival in a multifactorial context. No system displayed significance warranting inclusion into the broader multifactorial analysis in the backward elimination method here. Significance levels were $p = 0.154$ for TNM, $p = 0.234$ for Okuda, $p = 0.795$ for CLIP, and $p = 0.865$ for BCLC.

4 Discussion

4.1 Suitability of baseline characteristics

Statistical analysis of the 136 patients included at baseline showed much consistency with current epidemiological data for HCC, thus rendering this patient pool suitable for comparison with the literature. In terms of gender dispersion, the ratio of males to females in the baseline patient group amounted to just over 3:1. Slightly more than 80% of all patients (112 patients) showed signs of cirrhosis upon primary HCC diagnosis. These findings were consistent with HCC data for central Europe.[4,14] Although the main causes of cirrhosis here were proportioned differently to epidemiological data for Europe,[5] with alcohol abuse being a more prominent cause of cirrhosis than HCV, the growing etiological influence of alcohol in HCC has been documented in recent retrospective HCC studies.[138,139]

The median age upon diagnosis was 67 years old (range 33-85 years), slightly younger than in other low-risk Caucasian populations (e.g. the United Kingdom and North America, where incidence peaks at 70-75 years)[4] but comparable to other recent European studies.[138,140] This age difference could partially be attributed to the effect of several young patients (8 patients under 50 years of age, i.e. 6% of all patients) on the relatively small patient group. Ethnicity of the German population was well represented, with approximately 90% of patients being Caucasian, and the remaining 10% coming from non-Caucasian backgrounds.[141]

4.2 Comparison of overall cohort survival with recent literature

Compared to similar studies from recent years, the survival in this patient cohort was remarkably improved. A German study of HCC patients spanning the years 1988-2007 showed a median OS of 8 months, with 1-, 3- and 5-year survival in the latter decade reaching 42%, 15%, and 9% respectively.[142] Two further studies of German patients from 1994-2008 and 1998-2009 showed median OS of 19 months[139] and 18 months.[143] In the current study, carried out from 2006-2012, a median OS of 42 months was observed, with OS rates of 79%, 54%, and 38% respectively for 1, 3, and 5 years. This striking improvement in survival can be explained in part by key differences in therapies available to patients only in recent years. These, and other aspects that could account for the improved survival here will be outlined in the following.

4.3 Influence of liver function on survival

Liver function can be considered as having a twofold influence on the course of HCC disease. All parameters concerning liver cirrhosis (i.e. bilirubin level, albumin level, Quick score,

presence of encephalopathy, presence of ascites, Child-Pugh score) showed significant correlations with OS and most appeared to also influence DFS. In terms of OS, this strong relationship can be seen to arise from the presence of cirrhosis as a serious comorbidity, which likewise threatens patient survival, often more so than HCC itself.[119,144] In this study, this was illustrated by the large overall survival benefit of non-cirrhotic patients (median OS of 60 months) as opposed to Child A patients (48 months), and Child C patients (6 months).

Compared to recent studies in similar patient populations,[140,145] survival in non-cirrhotics and Child A patients was surprisingly high in this cohort, with approximately a 32-month increase in OS for both groups. However, other recent European literature shows comparable rates of OS for non-cirrhotics.[146,147] Child B and C patients appeared to have similar rates of survival to the current literature.[145] The improved survival of patients with no or mild cirrhosis in comparison to other studies could be explained by their being eligible for more novel therapies here, such as interstitial brachytherapy in a curative intent, and systemic therapy with sorafenib following disease progression. This point will be explored further in section 4.7.

The relationship between DFS and liver function was likewise significantly correlated. Poor liver function affects DFS because it is a contraindication for several effective but potentially liver-damaging therapies (e.g. TACE, SIRT).[78,105] The exclusion from effective therapies limits the possibilities for controlling disease and thus there is a visible reduction in DFS for patients with impaired liver function. In this case, this was demonstrated by a 9-month decrease in median DFS for Child C (3 months) versus Child A patients (12 months). Interestingly, non-cirrhotics showed a lower DFS than Child A patients (median DFS of 9 months vs 12 months respectively), and lower 1-, 3- and 5-year rates of DFS than in the current literature.[146] This could be due to the fact that cirrhotic patients are routinely screened for HCC, leading to lesions being discovered and treated at an earlier stage. Thus, in non-cirrhotic patients, the difference in DFS may be caused by a delayed initial diagnosis of HCC. It can only be speculated as to why this did not also negatively affect OS in the non-cirrhotic group. Perhaps the disadvantage of a later diagnosis in non-cirrhotics is offset in terms of overall survival by a better general patient performance status and a lack of cirrhosis-related comorbidity.

Another cirrhosis-related factor that appeared to influence survival was portal hypertension. This was found in 100 patients (73.5%) upon initial diagnosis of HCC, although it was not necessarily associated with cirrhosis. In cirrhotics without portal hypertension (12% of patients) a good vascular collateralization and an early stage of cirrhosis was made accountable for the absence of portal hypertension. In non-cirrhotic patients with portal hypertension (6% of patients) the hypertension was believed to arise from either tumor invasion (or compression) of the portal

vein or a general, massive tumor infestation of the liver. The presence of portal hypertension was not significantly associated with OS (p = 0.079), but this marginal finding may be due to the relatively small patient population. In Kaplan-Meier survival analysis, patients without portal hypertension had a longer median OS (60 months) compared to patients with hypertension (36 months). There was a significant difference in DFS between patients with and without portal hypertension, although the median survival for both groups was 9 months. These findings reflect the fact that portal hypertension is considered a relative contraindication for operative therapy of HCC and therefore, besides also being an important comorbidity (i.e. with increased risk of bleeding from gastroesophageal varicies), it limits the applicability of curative therapeutic approaches.[69]

4.4 Influence of patient performance status on survival

Patients' general state of health, assessed here using the ECOG performance status scoring system, had a significant impact on the OS of the cohort. There was a 27-month difference in median OS of patients who were fully active (42 months) upon initial diagnosis of HCC, compared to those in the lowest ECOG group in this cohort, namely whose activities were restricted, but who were still capable of self-care (15 months). Univariate analysis of survival for the ECOG groups 0-2 (i.e. fully active, slightly restricted, or restricted patient performance status) showed 1-year survival rates comparable to recent literature with 87%, 80%, and 62% respectively.[128] The positive impact of a good ECOG score on OS can be interpreted in different ways. Fully active patients are less likely to be multimorbid, therefore the likelihood of death from other causes (e.g. cirrhosis, heart disease) is less in this group. In terms of HCC, which generally only becomes symptomatic in later stages, a reduced ECOG score due to disease symptoms is also indicative of poorer prognosis.[148]

Although ECOG score is one of the key factors in the widely-used BCLC treatment algorithm, and could therefore be expected to be indirectly linked to the progression of disease, in Kaplan-Meier analysis it did not appear to affect DFS. It must, however, be noted that the accuracy of patient's ECOG scores was thrown into doubt following Kaplan-Meier analysis of survival in terms of BCLC stage. This point is explored further in section 4.6 below.

4.5 Influence of tumor characteristics on survival

Data concerning the natural history of HCC has shown that, alongside liver function and patient performance status, the most important prognostic factors are related to tumor characteristics, such as the number and size of tumor nodules, the presence of vascular invasion, metastasis,

and AFP levels.[124,144,148,149] Univariate Kaplan-Meier analysis in this patient population confirmed the prognostic importance of most of these factors, with significant logrank tests found for the parameters of largest lesion size, lesion number, vascular invasion, and AFP level with a threshold of 400 ng/ml.

There was not a great difference in OS for the largest lesion size groups ≤ 2 cm and > 2-6 cm (an average of 51 months versus a median of 48 months respectively), but this may be the result of a small sample size for the ≤ 2 cm group (12 patients) and a bias later found in the patient selection strategy of this study, whereby many patients with small, operable tumors and therefore a very good prognosis were unknowingly excluded from the study from the outset (see section 4.6). These groups did however both convincingly outlive the patients with a largest lesion of > 6 cm, where median OS was 30 months. In six cases there was no known baseline tumor size, either due to a diffuse infestation of the liver, or missing imaging information from the time of diagnosis. An analysis of survival in this group is unfortunately not possible given its very small size and the fundamentally different reasons for the missing information. Furthermore, in some other cases, patients presented a mix of diffuse and multinodular infestation of the liver, in which case the largest lesion size was used to characterize the tumor, thereby preventing these patients from being included in survival analysis according to diffuse (i.e. non-measurable) tumor growth.

In terms of tumor size and type of therapy used, chi-quadrat testing showed a significant dependency of first treatment on largest tumor size (p = 0.043), with curative treatments steadily decreasing from 58% of patients in the ≤ 2 cm group down to 43% in the > 6 cm group and an opposite trend of 16% in the smallest lesion category up to 30% of patients in the largest group who received systemic treatment with sorafenib as a first therapy.

The difference between patients based on lesion number was more striking with 1-3 lesions achieving a median OS of 42 months, compared with those with 4-5 lesions, who survived for a median of just 15 months (patients with > 5 lesions could not be adequately assessed due to small group size, n=9). This trend was also demonstrated in Cox regression analysis of a revised patient pool (n=78), where having 4-5 lesions significantly correlated (p=0.019) to a hazard ratio of 4 opposed to the group with 1-3 lesions (95% CI: 1.3-12-4). The Milan criteria cut-off at three lesions in determining patient eligibility for resection or transplantation could be reflected by these data. The chi-quadrat test showed a significance of p = 0.001 for lesion number versus initial therapy which appears to confirm that therapeutic decisions are influenced by lesion number. Approximately 90% of patients who received curative treatment as their initial therapy were in the category of 1-3 lesions, while almost 90% of patients who received systemic sorafenib as their first treatment had more than 3 lesions. The literature on the prognostic and

therapeutic importance of tumor size and lesion number is controversial, however several reports suggest that transplantation could be employed for patients who exceed the Milan criteria,[150-153] and conflicting opinions exist concerning resection,[154,155] particularly as to the acceptable level of survival for such patients.

Vascular invasion also clearly reduced median OS, with patients without invasion surviving a median of 48 months, and those with portal or hepatic vein invasion surviving 15 months. Patients with microvascular invasion but no invasion of the portal or hepatic veins appeared to survive at rates similar to the group without any invasion, with the exception of one patient who was remarkably still alive 192 months after diagnosis. This comparative survival for patients with microvascular invasion has been validated for smaller tumors (≤ 2 cm) in a recent study.[156] For larger tumors with microvascular invasion, this is an unexpected result. It must be noted however, that tumor histology was not available in almost 50% of patients. Thus, a large portion of patients with microvascular invasion would not have been detected, and microvascular invasion was often only detected following examination of a resected tumor. This would lead to selection of patients with better prognosis (i.e. resectable tumors) in the microvascular invasion group. Discounting this sub-population and only comparing patients without vascular invasion and those with portal or hepatic vein invasion, the results mentioned above nevertheless represent an improvement in comparison with another recent German study,[139] which showed a median OS of 20 months for patients without, and 5 months for patients with vascular invasion of the portal vein. This improvement could largely be due to the prominent therapeutic roles of interstitial brachytherapy and sorafenib in our patient cohort (see section 4.7 below). The former was used in 10% of patients with portal or hepatic vein invasion, as it has shown a good therapeutic effect in ablation of tumors close to large blood vessels, as opposed to RFA.[103,104] Sorafenib was employed in almost 30% of patients with portal or hepatic vein invasion. On the other hand, curative therapies (resection, interstitial brachytherapy, RFA, and PEI) were employed in 56% of patients where no vascular invasion was present, and 60% of patients with microvascular invasion, thus accounting for these groups' longer survival. This direct relationship between vascular invasion and therapy was validated by chi-squared testing, which showed significance of < 0.001.

The final tumor-related factor that was a prognostic variable in univariate analysis was AFP level. The cut-off of 400 ng/ml, as used in the CLIP staging system, proved to be useful in predicting survival as opposed to AFP levels defined as normal (< 13.4 ng/ml), which were not significantly associated with survival. Not only did an AFP level below 400 ng/ml correspond with increased OS (i.e. median OS of 42 months versus 12 months for patients with AFP above the threshold), but AFP level in terms of the threshold of 400 ng/ml was also significantly

associated with metastasis, vascular invasion, and tumor size in chi-squared testing. These rates of OS are approximately twice as long as a comparable American study, which showed median OS of 20 months and 6 months respectively for groups below and above the 400 ng/ml threshold.[145] It must be pointed out that the average survival of all patients based on the two groups given above (42 and 12 months for patients below and above the AFP threshold of 400 ng/ml respectively) lies much lower than the actual median survival of the entire cohort. This can be attributed to the large number of missing cases here (n=50).

Although the usefulness of AFP as a diagnostic tool has widely been thrown into doubt by the academic community in recent years due to it lacking sensitivity and specificity,[65,73,74] these data suggest that it is a useful tool for tracking disease activity, given its close association to several important tumor-related parameters, and its prognostic value for OS. It should however be noted here that baseline AFP levels were not available for approximately one third of patients in this study.

Metastasis did not appear to influence survival. This could perhaps be due to the paradox trend of metastatic HCC increasing as a result of increased OS due to the advent of effective novel therapies,[157] or a positive effect of sorafenib on certain types of metastases, as found in isolated cases.[158]

The only tumor parameter that appeared to influence DFS was size of largest lesion. Unexpectedly, the category of >2-6 cm had a longer median DFS (12 months) than the ≤ 2cm group (9 months), although this could possibly be attributed to the latter group's small size (12 patients). The group with the poorest DFS was predictably the category > 6 cm (6 months). As described in section 1.4.1, the size of a HCC lesion reflects the degree of tumor differentiation, invasion, and level of malignancy, with a tumor diameter of 2 cm being considered a threshold for invasive tumor growth[57,58] and neoangiogenesis.[54,59] This appears to explain the difference in DFS, with larger lesions at baseline metastasizing earlier due to invasive growth into vascular structures and growing more rapidly following neoangiogenesis.

4.6 Staging system effectiveness in predicting survival

The staging systems studied here have been under much scrutiny in the literature on HCC and conflicting reports exist as to which system is the most accurate. In this study, the Okuda, TNM, and CLIP systems all showed a strong level of significance ($p < 0.001$) in their ability to predict OS. Unexpectedly, the BCLC system, which is currently the staging system endorsed by European and American expert panels,[8,65] showed a poor correlation to OS ($p = 0.491$). All

systems showed equal duration of median OS for patients in the most favorable disease category (48 months). Patients with poorest OS were identified most accurately by the CLIP and BCLC systems (OS of 6 months), as opposed to the Okuda (21 months), and TNM (15 months) systems. The CLIP score's ability to predict survival in patients with advanced disease has already been observed in other studies, leading to it being recommended in the staging of this patient group by an expert American panel in the past.[122]

In multifactorial analysis of a revised population containing the 78 patients where all staging system data was available, only the TNM system was found to significantly predict survival (p=0.048). Upon closer scrutiny, however, this appeared to be primarily due to an unusually low hazard ratio in the T3a group (HR = 0.2, 95% CI: 0.03-0.9), which could be attributed to several individual cases of unusually long survival in this revised group (reaching 192 months in one exceptional case). This analysis must therefore be regarded as being purely of a descriptive nature, given the large number of patients removed (due to missing data) to attain the revised cohort (n=54 were removed). Multifactorial analyses which include staging systems must also be regarded carefully *per se*, given the complex analytical situation of several similar systems, with similar determining factors being analyzed in parallel to some of the factors themselves (eg. the factor 'cirrhosis' was analyzed here alongside some staging systems where cirrhosis was a determinant, ie. BCLC, CLIP, and Okuda, and one that was not: TNM).

Reasons for the BCLC system not displaying significance in predicting survival in univariate analysis can be found by examining this patient cohort more closely. The hepatological outpatient clinic where this data was collected works in close cooperation with other clinics within the Charité University Hospital. If patients undergo resection or transplantation, the according surgical outpatient clinic generally conducts the follow-up for patients. If HCC disease recurs following surgical therapy, these patients are then referred back to the hepatological outpatient clinic for assessment of other therapeutic options. Through this organizational system, surgical patients who subsequently developed progressive disease were unintentionally selected for this study. Resection or transplantation patients who did not develop progressive disease would have remained in the care of surgical outpatient clinics and thus were excluded from the patient cohort (this would also explain the low level of liver transplant patients in this study at < 1% of patients, compared to as many as 10% of patients in other studies).[145] As the BCLC system is at the same time a staging system and treatment algorithm, it is not unusual that, of the four staging systems studied here, it should be most affected by selection bias based on therapeutic outcome.

A further factor that appeared to have affected the accuracy of the BCLC system is the retrospective collection of patients' ECOG performance status. In spite of efforts to maintain a homogeneous policy of data collection, there was no way of ensuring that the ECOG scores reported for patients were accurate. In comparison to radiological findings or laboratory parameters, the ECOG score - above all other parameters recorded here - was subject to a certain amount of bias, depending on how and by whom it was reported or interpreted. Since a poorer ECOG score instantly qualifies patients for a lower BCLC stage, it is possible that erroneous ECOG scores might have affected the poor prognostic ability of the BCLC system. Given that only 22 patients qualified for the most favorable category of the BCLC system (as opposed to 63 using the Okuda system, 40 using TNM, and 38 using CLIP), but 82 qualified for the third most favorable category (as opposed to 5 using Okuda, 59 using TNM, and 6 using CLIP), the category to which a patient is assigned if the ECOG score is greater than zero, it seems likely that unreliable reporting or poor interpretation of patients' ECOG scores affected the accuracy of the BCLC staging system here. This reliance on the largely subjective ECOG score is a weakness in the BCLC system, which should therefore not be employed alone to make therapeutic decisions or to predict prognosis.

In terms of DFS, the Okuda and TNM staging systems predicted prognosis the most accurately ($p = 0.007$ and $p = 0.01$ respectively), with the TNM system stratifying patients more successfully. Borderline significance ($p = 0.071$) was found in analysis of DFS based on the CLIP system, although it also stratified patients well and once again identified patients with the poorest prognosis (median DFS of 3 months). In spite of the factors mentioned above, the BCLC system, which showed marginal significance of $p = 0.077$ likewise stratified patients well in terms of DFS, also identifying those with the poorest prognosis of 3 months. In multifactorial analysis of the above-mentioned revised patient cohort (n=78), no system appeared to be predictive of DFS.

4.6.1 Reassessment of patient survival in terms of BCLC staging system

As already mentioned, the poor performance of the BCLC system in predicting survival was an unexpected development. In order to assess if this could in part be explained by erroneous reporting or misinterpretation of patients' ECOG status, the data related to the BCLC staging system was reviewed. Patients categorized as BCLC C only on the basis of having a 'slightly restricted' performance status (and who therefore did not present with vascular invasion or metastasis) were identified and reclassified without regarding ECOG status. This led to a classification change in BCLC stage for 21 patients to either stage A or B. Upon performance of Kaplan-Meier analysis with the reviewed patient data, significant findings were found for both

BCLC's prediction of OS (p = 0.04) and DFS (p = 0.011). The following table summarizes the detailed findings of Kaplan-Meier analysis using the reviewed BCLC data:

Table 21: Overall survival (OS) and disease-free survival (DFS) in terms of reviewed BCLC stage

	OS			DFS		
	Median survival (months)	**p**	**95% CI**	**Median survival (months)**	**p**	**95% CI**
BCLC stage:		0.04			0.03	
A	60		44.9-75.1	15		7.9-22.1
B	36		24.8-47.2	12		8.1-15.9
C	30		18.5-41.5	6		3.4-8.6
D	6		-	3		-

4.7 Influence of treatment type on survival

While the type of treatment initially employed undoubtedly plays an important role in determining patients' overall and disease-free survival, it must be noted that more curative therapies are generally only available to patients who have the best prognosis to begin with (e.g. absence of metastasis and vascular invasion). This favorable constellation of 'better prognosis gets better therapy' was reflected in a 60-month median OS for the patient group initially treated curatively, compared to 42 months in the non-curative locoregional group and 30 months in the group that received systemic chemotherapy with sorafenib. Patients who received no treatment only achieved a median OS of approximately 9 months. In comparison to recent literature, there are two unusual outcomes here that require further clarification.

Firstly, while the 60-month OS reported for the curative therapy group is indeed long for HCC in general, compared to the outcomes from a recent American study,[145] where 83.5 months was reported as the median survival of a group treated curatively, this appeared to be an inadequate outcome. Upon closer scrutiny of the patient population in question however, one sees that more than 10% of the American cohort received liver transplantation as a curative therapy (compared to < 1% in this work), which, when considered alone attained median OS of 100 months. Liver resection patients in the same study achieved a median OS of 45 months, and RFA patients 32 months. Another German study showed that curative patients (treated

predominantly with resection and RFA) achieved a median OS of approximately 17 months.[142] Thus a wide spectrum of survival has been reported for this patient group in the literature, and OS for patients treated curatively in this cohort appears to be at the high end compared to other studies. However, given that many resection or transplantation patients were not included in this work (see section 4.6 above), these findings must be interpreted cautiously. For instance, perioperative mortality of this patient group is likely to be underestimated, given that most surgical patients studied here survived long enough to develop progressive disease, whereupon they were referred back to the hepatological outpatient clinic.

The second finding that requires clarification is the conspicuously long OS of patients treated initially with sorafenib (median OS of 30 months). We recall that sorafenib was lauded as the only effective systemic therapy of HCC after it improved survival of advanced HCC patients from approximately 8 to 11 months in a large RCT.[117] An almost tripled survival rate in our observations warrants further examination. Firstly, one must bear in mind the small patient population studied here. Of the 136 patients included at baseline, only 21 received sorafenib as their initial therapy (with a further 15 receiving it as their second or third therapy following disease progression). Approximately half of these 21 patients survived for longer than 12 months, a duration that would have appeared plausible in comparison to the literature. Two factors appear to be responsible for prolonging survival in this group beyond 12 months. The first is an inclusion of several patients into the systemic therapy group who did not initially present with advanced disease (e.g. patients with a ‘fully active’ ECOG status, or only one small solitary lesion). In some cases locoregional or curative therapy was declined by patients, was contraindicated for other reasons, or was carried out as a subsequent therapy. In one case, for instance, lung metastases were detected at baseline, thus qualifying an otherwise robust patient for systemic therapy. In later imaging, the metastases had disappeared but the therapy continued, thereby leaving this patient in the systemic therapy category of our study. The systemic therapy group therefore contained patients with a better prognosis at the outset, whose good survival lengthened that of the group as a whole. This could be highlighted by further survival analysis of the 36 patients who received sorafenib within one of the first three rounds of therapy. Although the relationship between OS and amended (see above) BCLC stage for these patients was not significant (p = 0.208), median OS for sorafenib patients with a BCLC stage of A or B was double that of stage C patients (42 months vs. 21 months respectively, 95% CI: could not be calculated for stage A and B patients due to the low number of cases, n=9, 8.7-33.3 for stage C patients). No difference was found concerning DFS according to BCLC stage. Taking this into account, we advise against direct comparison of the survival of sorafenib patients in this study with that found in the current literature on HCC.

The second factor that may have prolonged survival in this group is therapeutic multimodality. Of the 11 sorafenib patients who survived for longer that 12 months, six of these received additional locoregional therapy in subsequent rounds (either TACE or interstitial brachytherapy). In Kaplan-Meier analysis of sorafenib patients there was a highly significant ($p < 0.001$) relationship between OS and therapeutic multimodality, with patients who received more than one type of therapy surviving a median of 42 months (95% CI: 19.5-64.5) compared to 12 months (95% CI: 2.1-21.9) for patients who were solely treated with sorafenib. In contrast, disease-free survival of patients did not appear to be linked to therapeutic multimodality for the same patients ($p = 0.478$).

That therapeutic multimodality is an independent prognostic factor of survival for HCC in general has already been observed in another recent German study.[142] In our cohort it was likewise validated in both univariate ($p = 0.001$) and multivariate ($p = 0.011$) analysis of OS and univariate analysis of DFS ($p = 0.028$). As in the case of initial therapy, however, these results must be interpreted cautiously. Patients with superior survival are automatically selected for therapeutic multimodality at the onset, since an increased duration of survival simultaneously increases the chance that the disease manifestation will lend itself to other therapeutic approaches in time. Taken the other way, a patient with poor prognosis and an expected short survival will be an unlikely candidate for multiple therapies and will thus be excluded from this subgroup. Nevertheless, the high level of significance in both Kaplan-Meier and Cox analyses, as well as the doubled median survival for patients with more than one therapeutic modality compared to those with only one type of treatment (median OS of 72 months vs. 36 months respectively) suggest that therapeutic multimodality has a real disease-limiting effect. While research has been done into this area of HCC with encouraging results, there is currently not adequate evidence to support any one particular multimodal therapeutic approach.[159]

In terms of DFS, being treated curatively as the first therapy was significantly associated ($p = 0.002$) with more than double the median time to progression (15 months for curative therapy vs. 6 months for locoregional non-curative and systemic therapy). This may also be interpreted in two ways, namely, both the potential for curative therapy to deliver a complete response (i.e. an absence of all disease), and the fact that patients with early-stage disease (which generally requires a longer time to progression) are more likely to be candidates for curative therapy. As mentioned above, therapeutic multimodality was also associated with superior median DFS of 12 months compared to 9 months for unimodal therapy and 3 months for untreated patients. This benefit can be attributed to the factors mentioned above.

The improved survival in this patient cohort in comparison to recent literature (see section 4.2) can be attributed to one of three factors. Firstly, this cohort was relatively small (n=136) in comparison to other studies used here as references (i.e. n=1010 in an American study,[145] n=441,[142] n=458,[139] and n=405[143] in German studies). Therefore, the statistical analysis of survival in the current work may not be as robust as other examples found in the literature on HCC.

In terms of treatment spectrum however, there are another two key differences between this study and those mentioned above. Firstly, sorafenib became available as the first effective systemic treatment for HCC in 2007, towards the end of two of the above-mentioned studies.[139,142] As it was used to treat 32% of patients in this study (28% within the first three rounds of therapy), one could presume that it played a role in the increased overall survival seen here.

The second key difference between this work and all four studies mentioned above was the introduction of interstitial brachytherapy with afterloading as a potentially curative locoregional ablative procedure for patients not eligible for other curative therapies (i.e. resection, transplantation, RFA, PEI). As mentioned in section 1.6.2.3, interstitial brachytherapy can be used curatively in tumors previously deemed incurable due to the limitations of thermoablative (RFA) or chemoablative (PEI) procedures. In each of the comparable studies, treatment with curative intent involved either liver resection, liver transplantation, RFA, or PEI. When surgical therapy was not possible, one of the latter two methods of local ablation were employed. However, as described in section 1.6.2, these techniques are limited to tumors with diameters measuring less than 5 cm and 2 cm respectively, and RFA is ineffective in treating tumors close in proximity to large blood vessels due to the cooling effects of perfusion. Interstitial brachytherapy overcomes these limitations through the use of high-dose, localized irradiation and has been shown to attain 1-year complete tumor control in 90% of cases.[102,104] In this study, interstitial brachytherapy represented 63% of all performed therapies (53% within the first three rounds of treatment). In the first round of therapy, this novel and potentially curative procedure was used in 25% of patients. The median survival for patients treated with curative intent (composed almost entirely of patients treated with interstitial brachytherapy or liver resection) in this round of therapy was 60 months for OS and 15 months for DFS. One of very few existing studies on the effect of interstitial brachytherapy in HCC has confirmed these positive trends, reporting a median OS up to 59 months following diagnosis in patients with a CLIP score of 0.[102] Findings of DFS in the same study were slightly lower than those reported for the curative group here at 10.4 months. Unfortunately, a direct comparison with the work of Mohnicke *et al* (2010)[102] is difficult here, since they expressed survival in terms of either CLIP score or BCLC

group, two variables which had many missing or erroneous cases in the current study. Nevertheless, in light of the good survival displayed here, which exceeded that of both historical and contemporary studies in similar patient cohorts, and given the comparative survival attained in a recent German study,[102] it appears that interstitial brachytherapy with afterloading offers a very good therapeutic and survival-prolonging effect in suitable HCC patients.

4.8 Limitations of the study

Although the long rates of survival seen in this patient cohort suggest a superior therapeutic approach compared to other recent HCC studies, one must exercise caution in interpreting the findings, and view all things in light of the study's limitations. Firstly, as in any retrospective analysis, the value of the data was limited by its heterogeneity. This resulted in a larger amount of missing information than in a prospective trial, where all required data is collected in accordance with strict study protocols defined prior to the study's commencement. Some of the important variables that were absent in many cases here were tumor histology, AFP level, and ECOG score. This led to an unavailability of related variables such as TNM score, CLIP score, and BCLC stage. Another variable that would have been interesting to analyze, given its growing etiological importance, was the risk factor of BMI > 35 (see section 1.3.3). Unfortunately however, the reporting of this information was too heterogeneous to be of use retrospectively.

The retrospective manner of data collection also impacted the assessment of disease-free survival. In numerous cases, patient contact with our clinic was lost at some point, whereupon primary care physicians were contacted and asked about the patient's status. In such cases, the primary care physicians could usually say whether the patient was still alive or not, but not if the disease had progressed. Thus, when patients were known to be alive but their disease status was unknown, they were classed as having progressive disease, so as to not overestimate DFS (to test that the results were not distorted by this approach, DFS was analyzed with and without such patients and no difference was found in the overall DFS of the patient pool).

Further limitations were the relatively small size of the patient population (n=136) and the collection of data from only one clinic. As mentioned in section 4.6, this unicenter approach resulted in unintended bias in the patient pool, as operative patients without disease progression received their follow-up elsewhere and were thus unavailable for this study. The small patient number also meant that some subgroups were not large enough for an adequate statistical analysis (e.g. tumor size ≤ 2 cm, n=12), and in some cases no analysis was possible at all (e.g. Child C patients, n=5).

4.9 Future areas of research

Given the convincing survival trends in this population of HCC patients treated largely with interstitial brachytherapy, a RCT testing its performance both against other curative therapies (e.g. resection, RFA) and non-curative procedures (e.g. TACE, SIRT) would be of great interest. Although some studies of interstitial brachytherapy have been carried out in a prospective manner for both HCC[102] and liver metastases,[103] these were not RCTs and were therefore more of a descriptive nature.

The strongly significant influence of therapeutic multimodality in prolonging OS and DFS, even in patients with advanced disease being treated systemically with sorafenib, should also be explored further, particularly as this finding has been validated both here and elsewhere.[142] Although an elementary study has already been carried out in this area,[159] conclusive data is still lacking with regards to the most effective therapeutic combinations and the patient groups that benefit most from them.

The usefulness of several other clinical prognostic factors in HCC, such as Child-Pugh score, ECOG score, tumor size, and AFP level has been largely confirmed, both by this study and numerous others.[128,139,142,145] At a molecular level however, knowledge of prognostic markers in HCC is still in its early stages. It would therefore be of interest to validate prognostic molecular markers in a large patient cohort, for instance, as suggested by Villaneuva et al (2010).[160]

As a final suggested area of future research, the complex relationship between metabolic factors and HCC (an aspect of the disease which largely went unconsidered here) should be studied in order to identify metabolic conditions in non-cirrhotic patients that appear to be linked to the development of HCC.

5 Conclusion

We conducted a retrospective analysis of HCC patients treated in the hepatological outpatient clinic of the Charité Virchow Klinikum for the time period of 2006-2012. A total of 136 patients were included at baseline and, of these, 130 were eligible for subsequent survival analysis with overall survival (OS) and disease-free survival (DFS) as the study's endpoints.

At baseline, patients were categorized according to their liver function, general performance status, and level of tumor disease. Patients were then staged in accordance with four of the most common staging systems for HCC, namely, the TNM, Okuda, CLIP, and BCLC staging systems. The rounds of successive treatment that patients received were likewise documented, as well as patients' responses to therapy and the course of disease.

Survival was initially analyzed in the Kaplan-Meier method to identify factors that appeared to affect OS and DFS when considered alone. The variables that displayed significance ($p < 0.05$) were then studied further in a multivariate context using Cox's regression model. Either the chi-squared or Fisher's test were used to assess variable interdependence.

Several important aspects of HCC were highlighted by analysis of patient survival in terms of baseline parameters. Out of all the parameters studied here, liver function appeared to be the variable most closely associated with survival (both OS and DFS), as it displayed the strongest significance in multivariate analysis ($p < 0.001$ for OS and $p = 0.026$ for DFS) and consistently showed highly significant findings in univariate analysis of related parameters (i.e. Child-Pugh score, albumin and bilirubin levels, Quick score, ascites, and encephalopathy). Portal hypertension, a factor often associated with cirrhosis, also appeared to influence both OS and DFS in univariate analysis, although the finding was marginal for OS ($p = 0.079$).

Patient performance status, expressed here in terms of the ECOG scoring system, appeared to influence patient survival in univariate analysis, although the credibility of the ECOG scores obtained retrospectively was cast into doubt by the questionable role that false ECOG scores played in biasing the outcome of analyses related to the BCLC staging system.

Out of the tumor-related characteristics, tumor size was the variable that appeared to influence OS and DFS the most, through significant findings in both a univariate and multivariate context. Other factors that had an impact on OS according to univariate analysis were number of lesions, AFP level, and invasion of the portal or hepatic vein. Interestingly, lesion size and lesion number appeared to influence the type of treatment performed, and AFP level appeared to

predict disease activity, as it was highly associated with several disease-related parameters, such as metastasis, vascular invasion, and tumor size in chi-squared testing.

Out of the four staging systems studied here, the Okuda, TNM, and CLIP systems all showed extremely high levels of significance in predicting OS, and, to a lesser extent, DFS. The TNM and CLIP systems stratified patients most effectively and the CLIP system identified patients with the worst prognosis, both in terms of OS and DFS (although for DFS its significance was marginal, $p = 0.071$). Surprisingly, the BCLC system did not display significance for either OS or DFS. Upon closer scrutiny, it appeared likely that this was due to a biased patient pool (i.e. a comparative lack of surgical patients) and incorrect recording of patients' ECOG scores. After taking the latter into account, survival in terms of BCLC stage was reassessed, whereupon it too showed significance in terms of OS and DFS, albeit to a lesser extent than the other three systems (with the exception of the CLIP score in predicting DFS). Given the BCLC system's reliance on clinicians' subjective assessment of patient status, one must employ its therapeutic algorithm and prognostic forecasts cautiously.

The type of first treatment used (i.e. curative, non-curative locoregional, systemic) seemed to influence OS and DFS in univariate analysis, with patients initially treated in a curative intent predictably surviving the longest. Therapeutic multimodality also appeared to positively influence OS and DFS, even for patients with advanced disease, and showed significance in multivariate analysis of OS. In terms of therapy, a notable difference in this work from other comparable studies in the literature on HCC was the inclusion and predominance of interstitial brachytherapy with afterloading here, which was used in both curative and non-curative intent.

In comparison to the overall survival of similar patient cohorts in recent studies, this patient group appears to have survived remarkably long (between 2-7 times long as other cohorts). To a small extent, this could be due to the inclusion of sorafenib in this patient group, the only known effective systemic drug for HCC (in several studies used for comparison, sorafenib had not yet been approved for the treatment of advanced cases of HCC). In RCTs, sorafenib showed a life-prolonging effect of approximately three months. In this study it appeared to prolong patients' life well beyond this, particularly when used in combination with interstitial brachytherapy or TACE, although this could also be attributed to an inclusion of patients with less advanced disease in the systemic therapy group.

The superior survival in this patient cohort is, however, more likely due to the dominant role that interstitial brachytherapy played in the therapeutic spectrum, as it represented 63% of all treatments, and 25% of first round therapies. With a median OS of 60 months and a median

DFS of 15 months for the patients initially treated curatively here (a group represented largely by interstitial brachytherapy and liver resection), this group of patients convincingly outlived reference patient cohorts studied in recent decades. With cohort survival taken as a whole, patients in the current work showed a median OS of 42 months, compared to 8 months reported in a study spanning from 1988-2007,[142] 19 months in the years 1994-2008,[139] and 18 months for the period of 1998-2009.[143]

This is a very encouraging trend in the recent history of HCC, a cancer increasing in incidence and of worldwide importance. These findings warrant future research into the best role(s) that interstitial brachytherapy could play in disease management, as well as into multimodal therapeutic concepts involving it and sorafenib alongside the more established types of treatment. Furthermore, given that degree of liver cirrhosis and tumor size appeared to be most closely linked to survival here, management of underlying liver cirrhosis and the monitoring of at-risk individuals in order to detect disease at an early stage appear to be of utmost importance in dealing with HCC effectively and prolonging patients' survival.

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7 Eidesstattliche Erklärung

„Ich, Isaac Myers, versichere an Eides statt durch meine eigenhändige Unterschrift, dass ich die vorgelegte Dissertation mit dem Thema: „Verbessertes Überleben von Patienten mit HCC durch neue Therapieoptionen und den multimodalen Einsatz von Therapieverfahren: Erfahrung aus einer großen deutschen Universitätsklinik“ selbstständig und ohne nicht offengelegte Hilfe Dritter verfasst und keine anderen als die angegebenen Quellen und Hilfsmittel genutzt habe. Alle Stellen, die wörtlich oder dem Sinne nach auf Publikationen oder Vorträgen anderer Autoren beruhen, sind als solche in korrekter Zitierung (siehe „Uniform Requirements for Manuscripts (URM)“ des ICMJE -www.icmje.org) kenntlich gemacht. Die Abschnitte zu Methodik (insbesondere praktische Arbeiten, Laborbestimmungen, statistische Aufarbeitung) und Resultaten (insbesondere Abbildungen, Graphiken und Tabellen) entsprechen den URM (s.o) und werden von mir verantwortet.

Die Bedeutung dieser eidesstattlichen Versicherung und die strafrechtlichen Folgen einer unwahren eidesstattlichen Versicherung (§156,161 des Strafgesetzbuches) sind mir bekannt und bewusst.“

Datum Unterschrift

8 Curriculum vitae

Mein Lebenslauf wird aus datenschutzrechtlichen Gründen in der elektronischen Version meiner Arbeit nicht veröffentlicht.

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