Mirelle E.E. Bröker

SOLID BENIGN LIVER TUMOURS

Biological behaviour and management

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SOLID BENIGN LIVER TUMOURS
Biological behaviour and management

Goedaardige solide levertumoren
Het biologisch gedrag en de behandelstrategieën

Proefschrift

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Aan mijn ouders

CHAPTER 1

General Introduction

Due to the widespread use of modern radiological imaging, focal hepatic tumours are detected more often with up to 33% being detected on ultrasound screening, CT or MRI (1, 2). Although most of these incidentally detected focal liver lesions are benign, a malignancy needs to be ruled out. To discriminate between a benign and malignant lesion the imaging findings must be interpreted in the clinical context, including the patient's medical history, previous malignancies, chronic liver disease and family history. Discrimination between benign and malignant liver lesions is essential as their management greatly differs.

Benign liver tumours do not spread outside the liver and rarely lead to a fatal outcome. The most common liver tumours are simple liver cysts, haemangiomas, and solid benign liver tumours. The most important solid benign liver tumours include Focal Nodular Hyperplasia (FNH), Hepatocellular Adenoma (HCA) and Hepatic Angiomyolipoma (HAML). In this thesis, we will focus on the two most common solid benign liver tumours, FNH and HCA.

FOCAL NODULAR HYPERPLASIA

FNH is a benign liver tumour with an incidence of 0.6-3% in the general population (3, 4). The tumour mostly occurs in young women, with a male-female ratio of 1:12 (5). The potential mechanism explaining this gender difference is unclear. There is no data in the literature suggesting an increased risk or growth of FNH on oral contraceptives, and cessation of this hormonal treatment is therefore not recommended (6). FNH is defined as a hyperplastic reaction resulting from vascular malformation or trauma (7, 8).

Macroscopically, FNH is a pale, firm lesion distinct from the surrounding liver without a tumour capsule (figure1) (9). It is most often lobulated and in most cases, it has a central stellate scar which radiates into nodules of normal hepatocytes (10). The central scar contains a malformed vascular structure, the central artery. From this anomalous central artery, the arterial blood flows centrifugally (stellate) which is in contrast to HCA (11).

FNH tends to be detected as an incidental finding at imaging and although abdominal pain and discomfort are being described, the majority of FNH lesions are asymptomatic (3). FNH is a benign lesion with no malignant transformation, a very low incidence of bleeding and if symptomatic, complaints resolve during follow-up (12, 13). Therefore, if the diagnosis is well established, treatment is rarely indicated (10, 13). The biological behaviour of FNH is described in chapter 2.

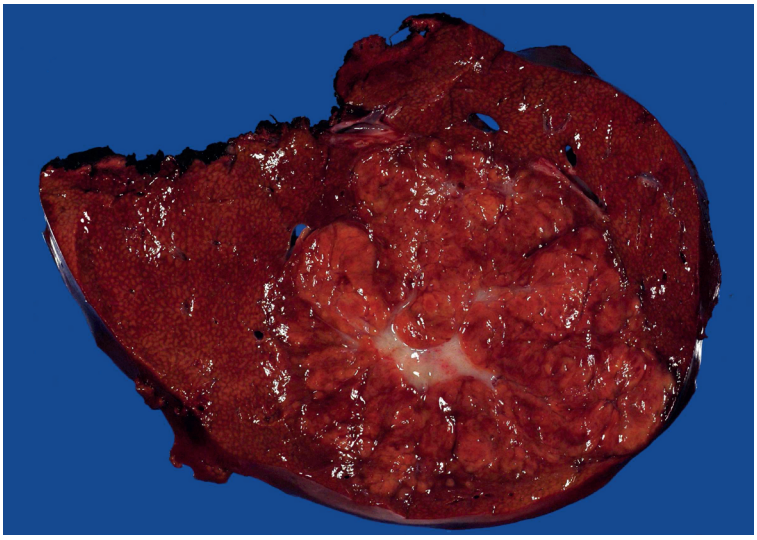

Figure 1. Focal Nodular Hyperplasia (FNH)

HEPATOCELLULAR ADENOMA (HCA)

A hepatocellular adenoma is a benign tumour that occurs most often in women in their second or third decade of life (14). The incidence is reported as 1-1.3 per 100.000 in women who have never used oral contraceptives (OC) and 30-40 per 100.000 in those on long-term OC-use (15, 16).

Macroscopically, HCA is a well circumscribed lesion, ranging in colour from brown to white but mostly yellowish (figure 2). Large plates of hepatic cells, resembling normal hepatocytes, are separated by dilated sinusoids. These sinusoids are fed only by peripheral subscapular vessels which causes diffuse homogenous arterial filling (17). Bile ducts are absent; an important histological feature that helps to distinguish it from FNH (18).

HCA and the association with the use of oral contraceptives was first described in 1973 by Baum et al. (19). In the subsequent years, many authors supported the potential role of sex hormones although the mechanism by which they contribute to the development of HCA was, and still is, not understood (16, 20, 21).

Unlike FNH, HCA may require invasive treatment in selective cases as HCA can be complicated by rupture, bleeding or malignant transformation (22, 23). The most important reason for treatment of HCA is the size of the lesion, as rupture and malignant transformation are rarely described in lesions smaller than 5cm in diameter (24, 25). The first step in the treatment of HCA, independently of the size of the HCA, is cessation of OC use. If the HCA still exceeds a diameter of 5cm 6 months after cessation of the OC, surgical treatment is recommended. (25-27)

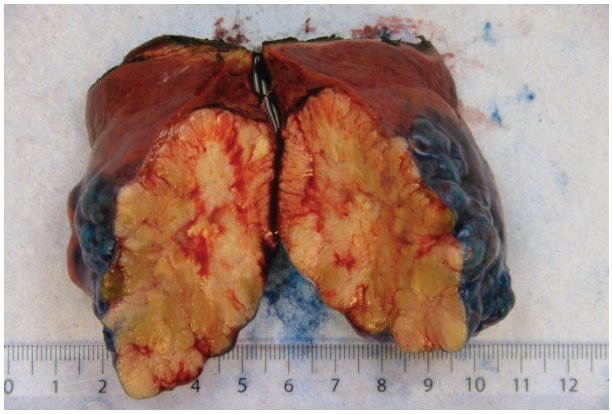

Figure 2. Hepatocellular adenoma (HCA)

The management of multiple HCAs is more complex and even treatment by total hepatectomy and liver transplantation has been described (28). In chapter 3 we will describe characteristics of patients with multiple HCA lesions and compare them to patients with single lesions. Furthermore, we will investigate whether the presence of multiple HCAs should influence management strategies.

Although resection is indicated in HCA lesions larger than 5cm, a conservative management might be chosen in case of multiple HCAs as the risk of surgery may increase while remnant lesions will remain in situ. In addition, centrally located lesions, lesions in patients with comorbidity, or patient's wishes may influence clinical management. A surveillance study on the natural course of HCAs larger than 5 cm is described in chapter 4.

As differentiation between HCA and FNH has a major clinical impact imaging techniques should have a high sensitivity and specificity to support reliable diagnostics. In chapter 5 we will explore the opportunities of using contrast enhanced ultrasound to characterise HCA and FNH. In chapter 6 we will compare the outcome of contrast enhanced ultrasound with contrast-enhanced Magnetic Resonance Imaging (MRI); the latter considered the gold imaging standard to differentiate between benign liver lesions.

In chapters 7 and 8 the impact of MRI in a more detailed diagnostic work-up of HCA will be discussed and compared to a pathological classification system, the Bordeaux-classification (29, 30). Using this system, 4 subclasses of HCA can be identified. We will perform studies to determine whether specific MRI features can be used to identify these different subgroups. (26)

In addition to using imaging or pathological characteristics to diagnose liver tumours, using serum markers to discriminate between benign and malignant liver tumours may have a clinical impact. In chapter 9 a new serum marker is investigated

to differentiate solid benign liver tumours from solid malignant liver tumours as HCC.

The biological behaviour of HCA in women during pregnancy is studied in chapters 10 and 11. HCA has the potential to show hormone-induced growth, leading to haemorrhage in larger lesions. An investigation into whether surgical resection should be recommended before or even during pregnancy will be performed (16, 31). 12 pregnant women with HCA were monitored closely (32) as described in chapter 10. In addition, a multicentre study on the natural course of HCA smaller than 5 cm in selected pregnant women will be introduced to confirm the hypothesis that pregnancy may be allowed in cases of smaller HCA (chapter 11).

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CHAPTER 2

Growth of FNH is not a reason for surgical intervention

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ABSTRACT

Background/Aims

When a liver lesion diagnosed as Focal Nodular Hyperplasia (FNH) increases in size it may cause doubt about the initial diagnosis. In many cases additional investigations will follow to exclude hepatocellular adenoma or malignancy. This retrospective cohort study addresses the implications of growth of FNH for clinical management.

Methods

We included patients diagnosed with FNH based on ≥2 imaging modalities between 2002 and 2015. Characteristics of patients with growing FNH with sequential imaging in a 6-month interval were compared to non-growing FNH.

Results

Growth was reported in 19/162 (12%) patients, ranging from 21-200%. Resection was performed in 4/19 growing FNHs, histological examination confirmed FNH in all patients. In all 15 conservatively treated patients additional imaging confirmed FNH diagnosis. No adverse outcomes were reported. No differences were found in characteristics and presentation of patients with growing or non-growing FNH.

Conclusion

This study confirms that FNH may grow significantly without causing symptoms. A significant increase in size shouldn't have any implications on clinical management if confident diagnosis by imaging has been established by a tertiary benign liver multidisciplinary team. Liver biopsy is only indicated in case of doubt after state of the art imaging. Resection is deemed unnecessary if the diagnosis is confirmed by multiple imaging modalities in a tertiary referral centre.

INTRODUCTION

Focal Nodular Hyperplasia (FNH) is a benign liver tumour with an incidence in the general population of 0.6-3% [1]. FNH is especially common in young women, with a male-female ratio of 1:12 [2]. So far, no explanation has been found for the gender bias; female hormones or the use of oral contraceptives do not seem to play a role in prevalence [3, 4].

An FNH lesion consists of benign hepatocytes surrounding a central fibrous scar with a prominent dystrophic artery. The underlying mechanism of FNH formation is thought to be due to a vascular malformation and injury [5]. Patients do not have an underlying liver disease and are mostly asymptomatic [6].

With the current availability of highly sensitive imaging techniques, FNH is diagnosed more often as an incidental lesion. Magnetic Resonance Imaging (MRI) with liver specific contrast agents has a very high specificity of almost 100% in larger lesions (>3cm) but is less accurate with a sensitivity of 70-80% to diagnose smaller lesions where the central scar may be missing. In these cases the combination of MRI and contrast enhanced ultrasound (CEUS) provides the highest diagnostic accuracy[7].

This year the European Association for the Study of the Liver (EASL) issued the first clinical practice guideline for benign liver tumours [8] in which they state that treatment of FNH is not recommended because of the benign character of FNH, the low incidence of intralesional bleeding [9, 10] and the absence of malignant transformation [11]. In case of doubt about the diagnosis FNH a biopsy may be considered[8]. The guideline describes treatment is only persued in exceptional cases such as expanding FNH.

It has been documented that FNH lesions may show a slow and incidental increase in size during follow-up. However change in size may cause doubt about the diagnosis and the benign character of the liver lesion [12]. Growth of FNH has been suggested to be an indication for resection [13-15], although evidence for this approach is weak. The aim of this study was to evaluate how often a FNH grows, what are the implications for management and compare the patient characteristics of those with and without growing FNH.

Material and Methods

To evaluate the course of disease of FNH lesions increasing in size during follow-up we performed a retrospective cohort study including all patients who had been diagnosed with FNH in the Erasmus University Medical Centre, a tertiary referral centre for focal liver lesions. Inclusion started in 2002, from the moment that we had the availability of two imaging techniques with high sensitivity and specificity to establish the diagnosis FNH and ran until 2015. Diagnosis FNH had to be confirmed

on at least two radiologic modalities, including at least one contrast-enhanced MRI and one contrast-enhanced CT-scan or CEUS and established in a multidisciplinary tumour board committee. Sequential imaging had to be available with at least a six month interval.

Baseline characteristics, including gender, age and body mass index (BMI), were collected from electronic patient records. Patients were scored as symptomatic if abdominal pain or general discomfort was reported in history. Information on the number and size of the FNH lesions were collected from radiological and histological reports. Data on clinical management were obtained from the reports of the multidisciplinary tumour board committee and correlated with data obtained from surgical, radiological and pathological reports.

The radiological reports of all patients were re-examined and growth was established if an increase in size between the diagnostic scan (T1) and follow-up scan at least 6 months after the initial scan (T2) was found. The diagnostic and follow-up scans were reassessed independently by two experienced radiologists (R.D. and I.P) from two tertiary referral centres. Because of the imprecise measurements of size in small lesions and potential bias in outcome, patients with lesions <20mm in both diagnostic and follow-up scan were excluded. We defined growth as an increase in size of at least 20% according to the RECIST criteria for solid liver tumors [16], as no other criteria have been validated. To evaluate whether lesion growth was related to weight gain additional thickness of the subcutaneous fatty layer in the abdominal wall was measured on initial and follow-up imaging. Measurements were performed by both radiologists separately in the midline (linea alba) on the level of the origin of the celiac artery.

Radiology

In patients with a diagnosis of FNH who were found to have an increase in size, the diagnostic and follow-up scan were reviewed. MR imaging was performed with 1.5-T MR systems using a standard MRI protocol of T1-weighted, T2-weighted sequences and a dynamic contrast enhanced series after intravenous administration of a bolus of 30 ml of non-liver-specific gadolinium chelate (gadopentetate dimeglumine, Magnevist; Schering, Berlin, Germany). CT scans were performed with 16- and 64-detector machines with a multiphase CT protocol consisting of plain, arterial- and portal-venous dominant phase scans of the liver after iv administration of 120 cc (Visipaque, General Electric Healthcare Medical Systems, Milwaukee, Wisconsin, United States). The lesions were scored as typical FNH if they were lobulated, a central scar was present, the aspect of the lesion was homogenous on the diagnostic MRI conform generally accepted classical imaging features of FNH. If there was no consensus on diagnosis or MR imaging showed no typical FNH, pathological

examination had to have been performed for patients to be included in this study. If all imaging had been performed in collaborating hospitals according to our protocol the outcome was reviewed in our hospital.

Data analysis

All analyses were performed using the Statistical Package for the Social Sciences (SPSS) version 20.0 (SPSS, Chicago, IL, USA). Continuous variables were summarized as median and interquartile range and categorical data as n (%) in case of a denominator >50 or a proportion/n in case of denominator <50. Differences between groups were assessed using the Mann-Whitney U test for continuous variables and χ^2-test for binary variables. Correlation between variables was analysed using Pearson product-moment correlation coefficient. Statistical significance was considered at a p-value < 0.05.

RESULTS & DISCUSSION

Out of 372 patients with a suspected FNH, 162 (44%) were included for growth analysis as sequential imaging was available with at least a six month interval (figure 1). The remaining 210 patients were excluded because follow-up was less than six months, they were discharged when diagnosis FNH was established. Three patients were excluded from growth analysis because the maximum diameter of the lesion was <20 mm on both diagnostic as well as on the follow-up scan. The diagnosis FNH was confirmed by the two radiologists in all cases. In 160 patients the diameter measurements from the first (T1) and last (T2) radiological reports were examined and in 28 patients (18%) an increase in size was found. Confirmation of increase with at least 20% was obtained in 19/28 patients as defined by both radiologists (figure 2).

Patients with growing and non-growing FNH did not differ regarding gender, age, BMI, number of lesions, symptoms or use of oral contraceptives (table 1). The number of patients who underwent surgery or embolization of FNH, and underwent follow-up for at least six months, was significantly higher in the growing FNH group compared to the non-growing FNH group (11% and 5% respectively, p = .009) although these patients had no complaints. No adverse events occurred in the patients with an FNH, including patients with growing FNH who did not undergo treatment.

Diagnostic biopsy was performed in 18/162 patients (11,1%), 4 histological examinations were inconclusive and 14 confirmed the diagnosis FNH. Indications for biopsy were growth in 4 and uncertainty about the diagnosis on imaging in 14.

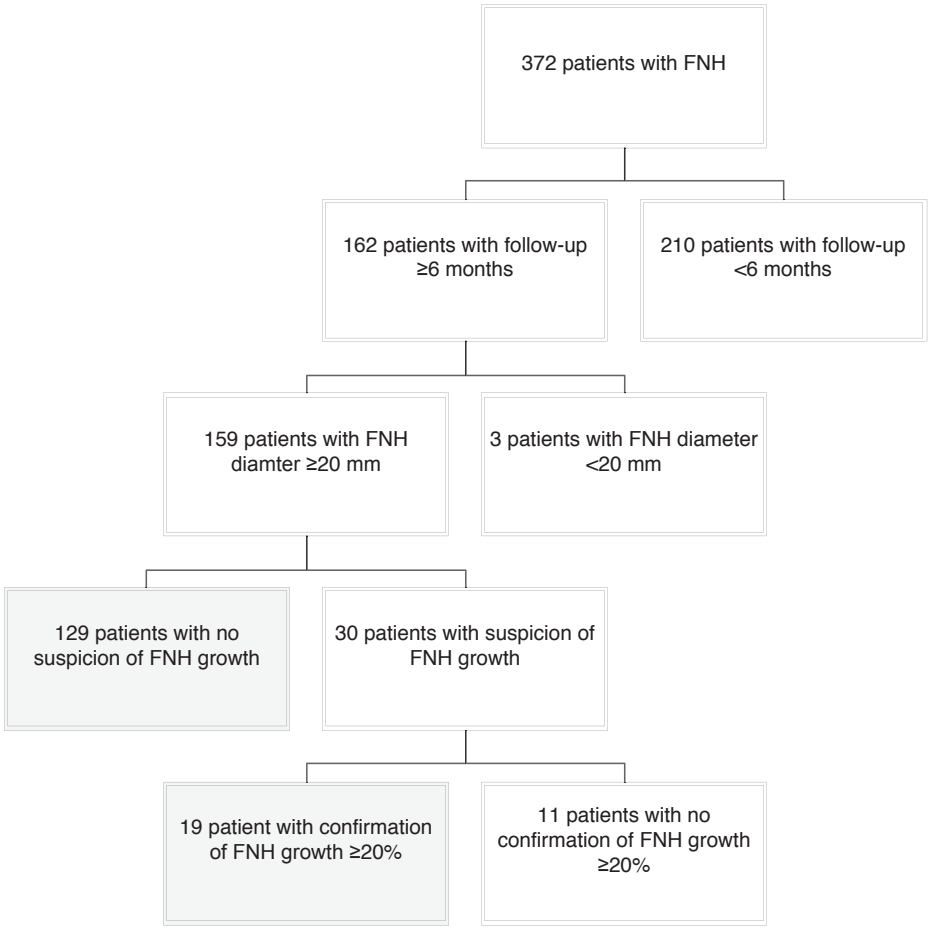

Figure 1. Flowchart inclusion

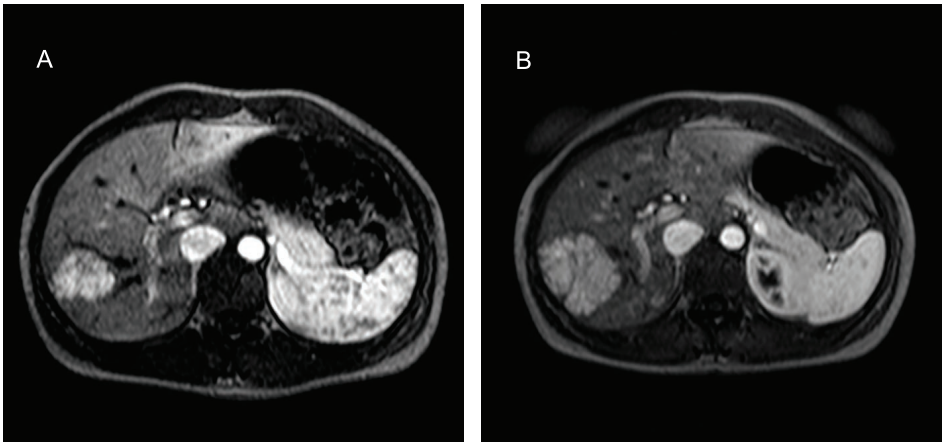

Figure 2. Example of a growing FNH.

Table 1. Patient and lesion characterics

	Growing FNH (n=19)	Non-growing FNH (n=143)	p-value
Female	19/19	137 (96%)	.363
Age	33 (24-42)	34 (27-43)	.248
BMI	25,5 (24-29)	24,7 (22-30)	.351
Lesions			.677
Solitary	12/19	76 (53%)	
Multiple	7/19	67 (47%)	
Symptoms			.962
None	5/19	38 (27%)	
Upper abdominal pain	10/19	73 (51%)	
Atypical complaints	3/19	18 (13%)	
Elevated liver enzymes	1/19	10 (7%)	
Unknown	0/19	3 (2%)	
Treatment			.009
No	15/19	136 (95%)	
Yes	4/19	7 (5%)	

In total 11/162 (6.7%) patients underwent resection (n=9) or embolization (n=2) of FNH. In all resected cases the diagnosis FNH was confirmed by histological examination of the specimen. In 4/9 the radiological diagnosis was uncertain, in the remaining 5/9 patients the reason for resection was abdominal pain or dyspepsia. Abdominal pain only resolved in 1 patient who underwent treatment because of symptoms thought to be caused by FNH, in the remaining 4 patients the surgery did not provide symptom relief. The indication for embolization was abdominal pain in both patients, neither of them experienced symptom relief.

Growing FNHs

Characteristics of growing FNH are summarized in table 2 and 3. In the growing FNH group the median follow-up time was 31 months (IQR 25 – 42). Growth percentage ranged from 21.1% to 200% (figure 3). The majority of lesions (10/19) was located in the right hemi liver and 9/12 was left sided. Four patients underwent resection, three because growth caused doubt about the diagnosis and one because of a symptomatic lesion. Three resected FNH were located in the right lateral liver and one in the left lateral liver. Pathology reports of the resected lesions all confirmed benign FNH. None of the patients who underwent resection had a diagnostic biopsy of the lesion before surgery.

In all 15 patients treated with a wait and see policy additional imaging was performed (MRI with liver specific contrast or CEUS) which confirmed the lesions to

be FNH. Thirteen out of these 15 were discharged from follow-up or were referred back to their initial hospital, two patients were kept in follow-up every 2-3 years according to their own wishes.

There was no statistically significant correlation between the growth percentage of the FNH and the percentage difference in subcutaneous fat (r =-.214, p =.340).

Table 2. Lesion characteristics of growing FNH

Patient	Time between imaging sessions (weeks)	Number of lesions	Maximum diameter first imaging session (mm)	Maximum diameter last imaging session (mm)	Percentage increase T1 – T2	Increase Subcutis mm (%)
1	136148	7	34*26	44*37	29,4%	20,50 (141%)
2	137	3	76*58	92*64	21,1%	2,50 (14%)
3	149	1	35*25	57*47	62,9 %	-1,00 (-13%)
4	319	1	61*57	86*74	41,0 %	-4,50 (-13%)
5	185	1	8*7	24*23	200,0 %	,50 (2%)
6	118	1	77*71	97*87	26,0 %	-,50 (-2%)
7	258	5	66*48	83*53	25,8%	3,00 (9%)
8	235	1	54*46	76*65	40,7%	-1,00 (-3%)
9	135	1	28*24	35*31	25,0 %	5,50 (46%)
10	151	2	22*21	58*43	163,6 %	-4,50 (-21%)
11	111	1	45*36	61*52	35,6%	4,00 (24%)
12	50	1	53*36	65*49	22,6 %	13,50 (75%)
13	137	1	34*30	48*45	41,2 %	3,00 (15%)
14	115	1	33*24	54*40	63,6 %	7,50 (26%)
15	108	1	46*34	61*50	32,6%	6,50 (25%)
16	53	1	28*33	46*40	64,3 %	-5 (- 9%)
17	164	2	92*60	112*68	21,7 %	-1 (- 5%)
18	435	2	24*21	45*44	87,5 %	-1 (-7%)
19	118	1	52*41	64*46	23,1 %	4 (11%)

Table 3. Summary of characteristics of growing FNH

Median follow-up time (months)	31 (IQR 25-42)
Location	
Right hemiliver	10
Left hemiliver	9
Conservative treatment	18/19
Resection	4/19
Doubt about diagnosis due to growth	3
Symptomatic lesion	1

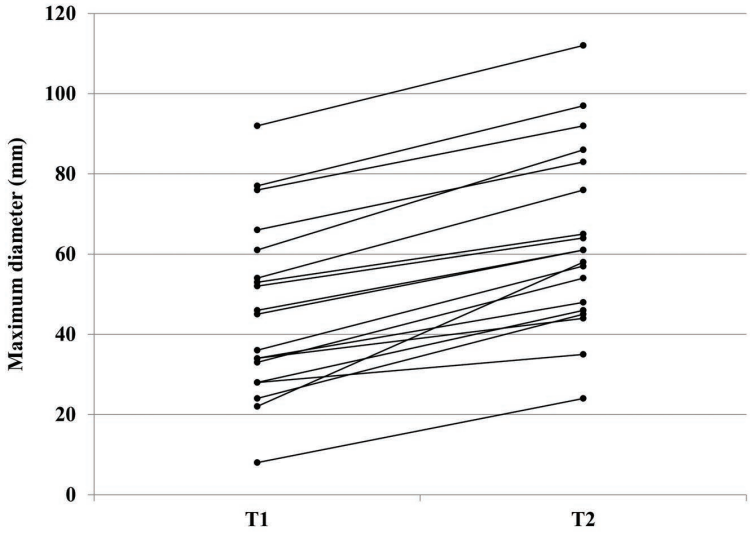

Figure 3. Size growing FNH
This figure shows the size (mm) on T1 (diagnostic scan) and T2 (follow-up scan) of the 19 FNH in which growth is confirmed.

DISCUSSION

This study reports on a large series of patients with FNH and their follow-up. A specific focus of attention in our study was to evaluate if growth of FNH should have implications on clinical management, as growth may cause doubt about the initial diagnosis. In our study population 12% of the lesions showed growth over a period of at least six months. It should be noted that this figure most probably overestimates the incidence of growing FNH and there may be a bias in observation as the patients included in our analysis were referred to a tertiary referral centre because of uncertainty about the diagnosis and management.

The diagnosis FNH was confirmed by resection in 4 patients and additional imaging in the form of MRI with liver specific contrast agents or CEUS in the rest of the patients. No adverse events were reported in the group of growing FNHs. In line with the studies of Weimann et al. [15] who observed 5 patients with growing FNH and Perrakis et al. [14] who described 13 patients with growing FNH, we were unable to identify risk factors for growth.

In the 18 biopsies that were performed in our cohort, 14 (77.8%) confirmed FNH, while in a recent study from Sannier et al. a diagnostic accuracy of 95% in 19 patients was reported[17]. This could be explained by the fact that the accuracy for histologically diagnosing FNH and especially the distinction from other solid liver tumors such as hepatocellular adenoma and hepatocellular carcinoma has improved

significantly in the study period. In 2009 Bioulac-Sage et al. [18] published a paper in which they were the first to describe abundant expression of Glutamine Synthetase as a marker to distinguish FNH from other hepatic lesions.

Our results suggest that growth of FNH is quite common and that growth in itself should not have any implications for clinical management. Growth may cause doubt about the initial diagnosis, but if imaging characteristics are typical for FNH this is not necessary. MRI with liver specific contrast agents in combination with CEUS has the highest accuracy for FNH diagnosis [19-21]. Growth on itself may not be an indication for biopsy: in our center the final recommendation on whether or not . Growth on itself may not be an indication for biopsy: in our center biopsy is deemed necessary is made in a multidisciplinary liver tumour board meeting. In general, our recommendation is to only perform a biopsy when a discrepancy in diagnosis exists between the two imaging modalities. accurate . This could imply that some of the tumors were inadequately diagnosed as FNH before 2008. However by including only tumors [22] diagnosed based on two imaging modalities (MRI and CEUS) this proportion was kept to a minimum. . This could imply that some of the tumors were inadequately diagnosed as FNH before 2008. However by including only tumors that were diagnosed based on two imaging modalities (MRI and CEUS) this proportion was kept to a minimum.

Differences in management between FNH and hepatocellular adenomas demand an accurate differentiation. Resection is indicated for hepatocellular adenoma if the tumour exceeds a diameter of 5cm 6 months after the use of Oral Contraceptive is stopped, because of the risk of bleeding [23]. In contrast, for FNH no strict indications for resection are defined. As liver resections may have a peri-operative complication-rate up to 20-25%, a diagnostic liver resection is not advisable [24]. In the case of FNH, the liver-resections are generally performed in young, healthy women. As our study showed no complications of the conservative approach we advise to avoid resection as described in the EASL Clinical Practice Guideline [8], even if the lesion is growing.

FNH is often an incidental finding discovered by various imaging techniques. In our cohort we found that 26.5% of the patients were asymptomatic, while most studies have shown a large percentage of asymptomatic patients ranging from 65% [14] to 90% [25]. One possible explanation could be that the Erasmus Medical Hospital is a tertiary referral centre, and more patients with symptoms are referred. We assume that most of the symptoms are not caused by the presence of FNH, and that FNH indeed could be asymptomatic. If treated, patients need to be comprehensively informed and it should be stressed that it may not be guaranteed that the abdominal pain will resolve . One possible explanation could be that the Erasmus Medical Hospital is a tertiary referral centre, and more patients with symptoms are referred. We assume that most of the symptoms are not caused by the presence of FNH, and that

FNH indeed could be asymptomatic. If treated, patients need to be comprehensively informed and it should be stressed that it may not be guaranteed that the abdominal pain will resolve [26].

The biggest limitation of our retrospective study is the design that is inherent to bias. In addition, it may be questioned whether the sample size of the growing FNH group is large enough to justify the conclusion; however, with 19 patients we are the first to describe such a series of growing FNH and others may be challenged by this report to add new data.

In conclusion, our series confirm that FNH is not a static lesion and that growth may occur rather frequently. It must be noted that patients with a growing FNH do not report more pain or discomfort compared to the patients with non-growing FNH. Moreover, growth in itself should not have any implications on clinical management. In case of doubt, MRI with liver specific contrast agents in combination with CEUS provides the highest diagnostic accuracy. As these imaging techniques are not available in every hospital, patients could be referred to a centre specialized in focal liver lesions. Growth is not an indication for liver biopsy and biopsy should only be considered when the two imaging modalities do not provide the same diagnosis. No adverse outcomes were observed in patients with growing FNHs, therefore we recommend that even growing FNHs should not be resected and follow-up (growing) of FNH after a certain diagnosis made in a tertiary referral centre is not indicated.

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CHAPTER 3

Inflammatory and multiple hepatocellular adenoma are associated with a higher BMI

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ABSTRACT

Aim

To identify patient and lesion characteristics associated with the occurrence of single or multiple HCA.

Methods

Using a tertiary centre database, we retrospectively collected information about patient and lesion characteristics, management and follow-up of all patients with HCA included between 2001 and 2016 Patients were classified in three groups; patients with a single HCA, 2-9 HCA and ≥10HCA.

Results

458 patients were diagnosed with HCA, including 121(26.4%) with single HCA, 235(51.3%) with 2-9 HCA and 102(22.3%) with ≥10 HCA. Significant differences regarding mean Body Mass Index(BMI) were found with the highest BMI in patients with more than 10 HCA; ($p<0.05$). Mean BMI was significantly higher in patients with inflammatory HCA compared to steatotic HCA(31 vs 26 resp., $p<0.05$). Steatotic HCA were more often single lesions(22/55, 40%), while patients with inflammatory HCA were often diagnosed with multiple lesions(122/166, 73%).

Conclusions

Our series demonstrate a significantly higher BMI and frequency of inflammatory HCA in patients with multiple HCA compared to single HCA.

INTRODUCTION

A validated molecular and pathological classification of hepatocellular adenoma (HCA) was introduced by Bioulac-Sage et al in 2009[1]. This classification identifies HCA with a different clinical outcome[1, 2]. One subgroup of steatotic HCA lacks the expression of the liver-fatty–acid binding protein and has a very low risk of bleeding or malignant proliferation (H-HCA, 35-50%)[3]. A second subgroup includes inflammatory HCA (I-HCA, 45-50%), a subtype which is at risk of having a β-catenin mutation associated with an increased risk of malignant transformation and bleeding[4]. A third subgroup, is characterized by a β-catenin mutation (B-HCA, 15-18%). Finally, a group is being defined as unclassified as it does not show any specific features or mutations (U-HCA, 10%)[1, 5, 6].

All these different subtypes may present as a solitary lesion on imaging. A small minority of patients with HCA presents with liver adenomatosis (LA), defined by Flejou and colleagues as the presence of more than 10 adenoma lesions in an otherwise normal liver parenchyma [7]. Only several case-reports and small case-series with patients with more than 10 HCA's have been described[8]. However, as estimation of the exact number of HCA's appears to be difficult, the term liver adenomatosis has been replaced by multiple HCA [9]. Multiple HCA's have been described to be present in approximately 50% of all HCA.

Studies describing risk factors HCA are mainly based on analysis of a solitary HCA and include the long-term use of estrogen containing oral contraceptives, female gender and obesity[4, 10, 11]. It has yet to be studied whether risk factors for multiple HCA differ from single HCA.

It may be questioned whether patients with multiple HCA's must be treated according to the same guidelines, as those with solitary lesions. The EASL guideline on the management of benign liver tumours suggests to treat these patients based on the size of largest nodule as the risk of complications is not related to the number of HCA.[3, 12]. However, this might be challenging if there are multiple HCA > 5cm. With the availability of advanced imaging techniques and their increased use, liver lesions, including multiple adenomas, seem to be diagnosed more often. The management of these lesions may be a challenge for physicians as the guidelines may not always be applicable.

We studied which patients are at risk for multiple HCA and whether patients and lesion characteristics between single or multiple HCA differ. Furthermore, we investigated whether the presentation of single or multiple adenomas may lead to different management strategies.

MATERIALS AND METHODS

The study protocol was in agreement with the ethical guidelines of the 1975 Declaration of Helsinki and approved by the local Institutional Review Board and Ethical Committee from the Erasmus MC University. Informed consent was waived.

All patients who were diagnosed with HCA in our tertiary referral center for focal liver lesions (the Erasmus Medical Center, Rotterdam) between 1999 and 2016 were included. With the availability of data on the diagnosis of all consecutive patients in this period we selected those in whom the diagnosis had been confirmed on at least one MRI or, if indicated, by histopathological evaluation. The final diagnosis and management strategy had to be confirmed in a multidisciplinary hepatic tumor board committee.

From this database, we retrospectively collected baseline characteristics including gender, age and body mass index (BMI) from all patients. We derived the number (1 HCA, 2-9 HCA or >10 HCA), size and presence of bleeding from the radiological and pathological reports. Tumour size was defined as the diameter of the largest HCA on MRI in mm. Bleeding was defined using MRI criteria: On T1-weighting a hematoma is hyperintense in the beginning becoming more and more isointense in the chronic phase; On T2-weighting a hematoma starts hyperintense and resolves in the chronic phase with zones of signal void (black) due to deposition of hemosiderine, mostly in the periphery.

Patients were subdivided in three groups: single HCA, multiple (2-9) HCA (MA) and >10 HCA or liver adenomatosis (LA). Noninvasive MRI diagnosis of HCA was based typical features including results of using liver specific contrast agents. HCA subtypes (H-HCA, I-HCA, β-HCA, β-IHCA and U-HCA) were based on immunohistochemistry as described by the Bordeaux-group[13] or on typical MRI features: H-HCA diffuse and homogenous fat signal, IHCA hyperintensity on t2–weighted images and T1- hyperintensity on the delayed phase or atoll sign on T2-weighted images. B-HCA and U-HCA have no validated specific sign[14, 15].

Data analysis

All analyses were performed using the Statistical Package for the Social Sciences (SPSS) (IBM Corp. Released 2013. IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY: IBM Corp). Differences between groups were assessed using a one-way ANOVA for continuous variables or chi-square test for categorical variables. Statistical significance was considered at a p-value < 0.05.

RESULTS

Overall, 458 patients were included and 121 were found to have a single HCA (26.4%), 235 multiple HCA's (51.3%) and 102 liver adenomatosis (22.3%). Baseline characteristics are presented in table 1. The median age at presentation was 39 (IQR 15-78). Most patients were female (n=451, 98%) with 12 (2.6%) female patients having no history of oral contraceptive use. There were six male patients, all of which were diagnosed with a single HCA. One was found to have an H-HCA, 4 an I-HCA and 1 a U-HCA. The median follow-up period of all patients was 34 months (IQR 17-49). No malignant transformation of any HCA into a Hepatocellular Carcinoma (HCC) was found in this period.

Table 1. Patient characteristics (N=458)

	HCA (N=121)	MA (N=235)	LA (N=102)	P-value
Age (years)[1]	38 (20-78)	38(20-66)	39 (15-59)	0.527
Female (n)[2]	115 (95%)	234 (99.6%)	102 (100%)	0.002
BMI (kg/m²)[1]	27.7 (17.0-41.0)	30.4 (18.3-62.1)	31.2 (20.3-47.4)	0.001
OC use (n)[2]	116 (94%)	233 (99%)	97 (94%)	0.005
Tumor size (mm)[1]	59 (9-177)	58 (9-200)	67 (12-200)	0.097
Tumor bleeding (n) [2]	18 (15%)	53 (23%)	24 (24%)	0.172

This table shows patients characteristics of patients with single hepatocellular adenoma (HCA), multiple hepatocellular adenomas (MA) and liver adenomatosis (LA).
BMI, body mass index; OC, oral contraceptive.
[1] Data are presented as median with the range between brackets.
[2] Data are presented as numbers with the percentage between brackets.
P-values below 0.05 were considered statistically significant.

Comparison between these three groups showed a significant difference in BMI (kg/m²) with a median of 27.7 in patients with a single HCA, 30.4 in patients with MA, and 31.2 in patients with LA (Figure 1). Pairwise post-hoc analysis showed a significant difference on BMI between single HCA and MA, and HCA and LA. No difference between MA and LA was observed. Female gender and the use of oral contraceptives were significantly different between groups. A pairwise post-hoc analysis showed a difference between single HCA and MA and single HCA and LA. OC use was significantly higher in MA compared to single HCA and LA. There was no difference in age or bleeding of adenomas between groups.

A total of 267 HCA were classified according to the Bordeaux-classification based on MRI findings or pathology reports (table 2). The percentage of H-HCA (17%) was significantly lower in the group with MA/LA compared to HCA(Figure 2). 8 patients were found to have a β-catenin mutation based on pathology. I-HCA was the most

common subgroup in single HCA as well as MA(63%). The median BMI in I-HCA was found to be 30.9 compared with a median BMI in H-HCA of 26.0, and 29.7 in U-HCA 29.7 in B-HCA. Additional analyses were performed in patients in whom the largest lesion exceeded 50 mm as this specific group should be considered for resection

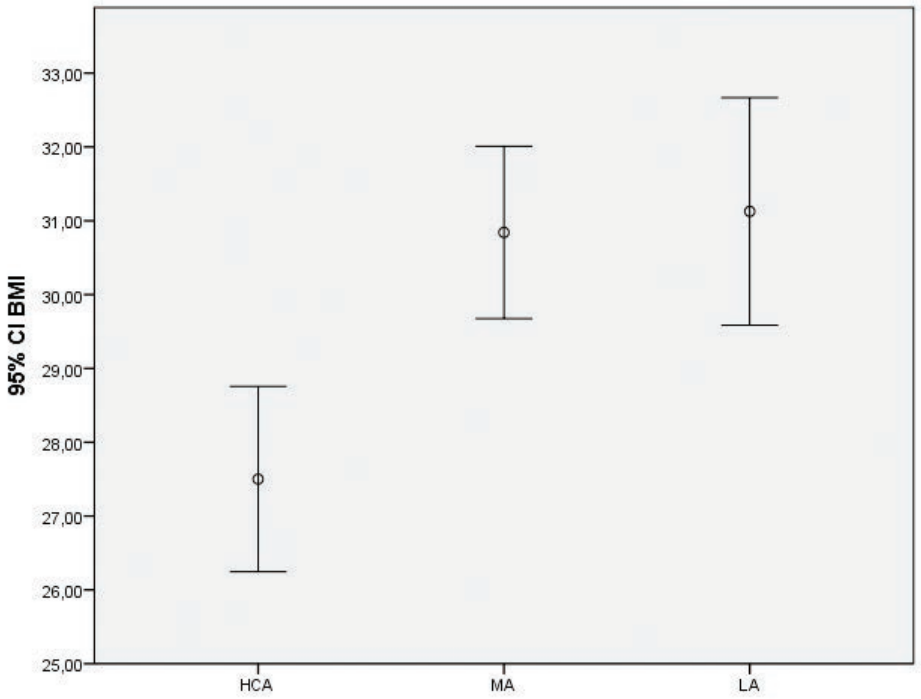

Figure 1. This figure shows BMI of patients with a single hepatocellular adenoma, multiple liver adenomas and liver adenomatosis. HCA, hepatocellular cellular adenoma; MA, multiple adenoma's; LA, liver adenomatosis; CI, confidence interval; BMI, body mass index.

Table 2. Bordeaux Classification

	Single HCA (N=75)	MA/LA (N=192)	P-value
H-HCA	22 (29%)	33 (17%)	0.023
I-HCA	44 (59%)	122 (64%)	0.485
B-HCA	1 (2%)	7 (4%)	0.449
U-HCA	7 (9%)	28(15%)	0.315
I-HCA +B-HCA	1 (1%)	2 (1%)	1.000

This table shows the Bordeaux classification the adenoma of patients with a liver adenomatosis, multiple liver adenomas and a single hepatocellular adenoma. HCA hepatocellular cellular adenoma; MA multiple adenomas; LA liver adenomatosis; H-HCA steatotic HCA;, I-HCA Inflammatory HCA; B-HCA HCA with mutations of the β-Catenin Gene; U-HCA Unclassified HCA without markers.

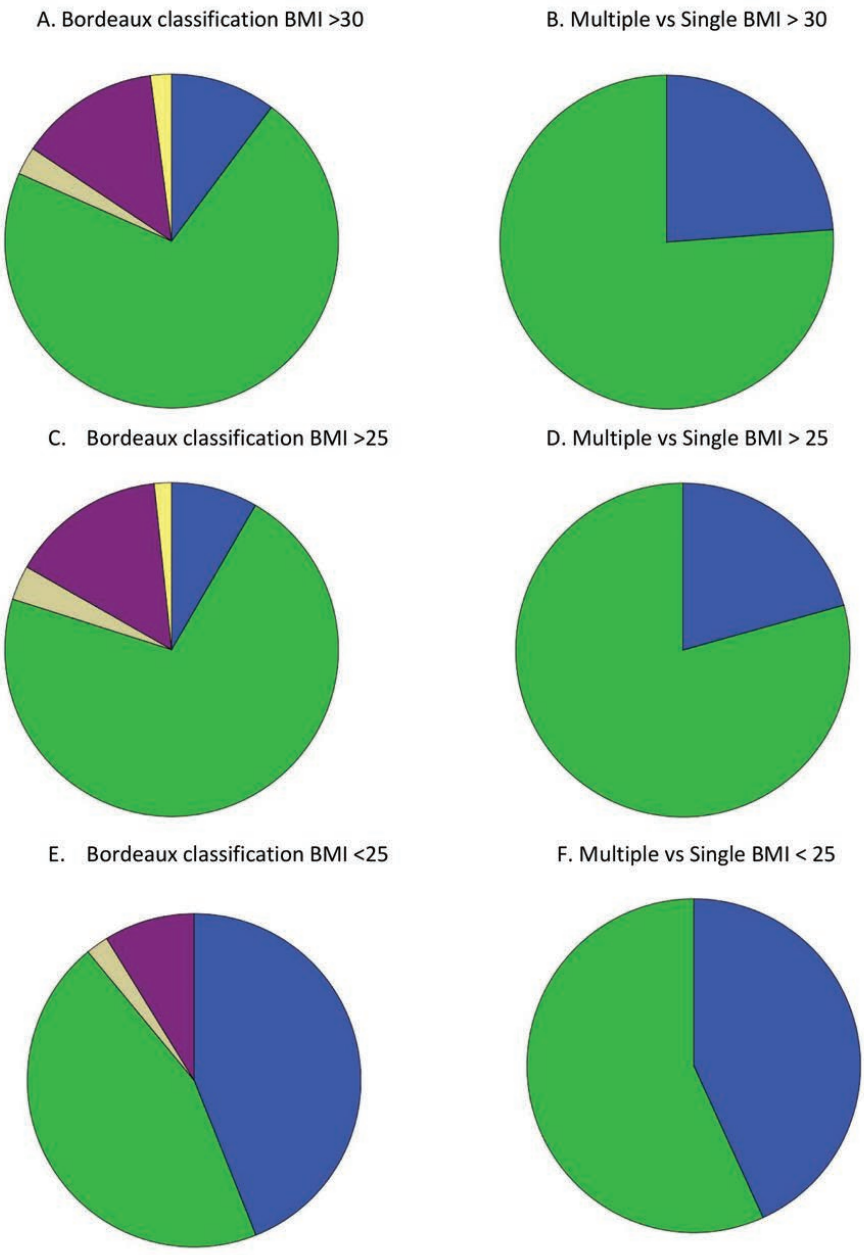

Figure 2. Multiple and subtype distribution of HCA depending on BMI
A,C,E; blue H-HCA; Green I-HCA, Grey B-HCA; Purple U-HCA, Yellow IB-HCA (Inflammatory and B-cat positive HCA)B,D,F; blue; single HCA, green: multiple HCA

or other curative treatment as described in the EASL Clinical Practice Guidelines on the management of benign liver tumours[3]. Larger lesions were found in 56 (46%) single HCA's, 109 (46%) MA's and 54 (52%) LA's. There were significant differences in intervention between the three groups (table 3). More patients with a single HCA underwent resection if the lesion exceeded 50 mm compared to patients with larger lesions in MA or LA.

DISCUSSION

In this study, we describe the largest series of patients with HCA, MA and LA with a follow-up of more than a decade. A review by Veteläinen et al. described 94 patients from case-reports and case-series with LA[8]. They reported abdominal ultrasound to be the initial imaging in all 94 patients but confirmation of the diagnosis using highly advanced imaging modalities like MRI, with or without contrast, or a contrast enhanced ultrasonography (CEUS) was often missing. Currently, in our hospital all patients with suspected benign hepatic tumour will receive an MRI, in at least four phases (precontrast, arterial, portal and delayed) after administration of an intravenous bolus non-liver-specific gadolinium chelate or a liver specific contrast agent (Gadoxetate disodium, Primovist, Bayer Healthcare, Berlin, Germany or Gadobenate dimeglumine, Multihance, Bracco Imaging, Milan, Italy). Furthermore, patients are assessed with CEUS, using a second generation contrast agent Sonovue (2.4-4.8 mL i.v. Bracco). Both imaging methods provide additional information that improves differentiation of liver lesions [16, 17].

In our series, we did not find MA in male patients. All 6 male patients had a single HCA. No patients had a history of using anabolic steroids.

The aetiology and pathogenesis of HCA is unknown although an association with the use of estrogens was described in 1973[18]. In the following years, many authors confirmed the hypothesis of an association between estrogen containing contraceptives and HCA[19-23]. Withdrawal of oral contraceptive in these patients will usually show regression of HCA[5]. However, we have yet to discover the physiological explanation for the association between estrogen and HCA. The data on sex steroid receptors are rare, inconsistent and some of them used outdated techniques[24]. The largest study which used immunohistochemical analysis, found an estrogen and progesterone receptor in 26% of the HCA[25]. However, they did not draw any conclusions about the correlation between the number of HCA and the presence of the sex steroid receptors. New steroid hormone receptors have been identified in recent years but have not yet been tested on HCA tissue[26-28].

Estrogens are mostly known to be produced by the ovary. However adipose tissue can contribute significantly to the pool of estrogens[26, 29]. Previous studies demonstrated that obese patients have higher estrogen levels compared to healthy individuals[29, 30]. This could explain the relation between BMI and number of HCA in this group of patients. In 2012 Rui et al. conducted a study in which they found that high BMI had a significant positive association with the risk of liver tumours[30]. Bioulac-Sage et al. first suggested a connection between overweight and HCA[31]. Bunchorntavakul et al. found 23 cases of MA in obese patients and suggested a correlation between MA and obesity[32]. We describe a significant difference in BMI between single HCA, MA and LA. Median BMI is highest in the group of patients with LA. We confirmed the suggested association between the number of HCA and BMI in a large group of patients. It has been suggested that HCA could decrease or disappear if patients lose weight[10]. The decrease could be attributed to a lower concentration of hormones due to weight loss[33]. Another explanation could be less inflammation due weight loss as enhanced inflammation in the metabolic syndrome allows cell growth to develop HCA[31, 32]. Currently all patients with HCA are advised to stop the use of OC as well as losing weight. Therefore it is not always clear if the regression is caused by the withdrawal of OC or by the weight loss.

The Bordeaux subtype classification was introduced and included in our data. Subclassification of the largest HCA was conducted in 267 patients. The incidence of the subgroup H-HCA has previously been reported as 30-40% of all HCA [34]. Patients with H-HCA and thereby germline mutations of HNF1A are predisposed to develop LA[34]. However in our cohort, only 8 patients with LA were classified as H-HCA. In patients with I-HCA obesity is a known risk factor. Furthermore the presence of I-HCA is associated with MA as well[8, 31, 32, 35, 36]. I-HCA and a high BMI seemed to cause LA in our cohort as well. The BMI in the patients with I-HCA was significantly higher compared to the patients with H-HCA and LA.

In our cohort the incidence of I-HCA is much higher compared to the distribution between the different subgroups described by the Bordeaux-group[34]. This could be explained by the rapidly rising incidence of obesity in women worldwide[37]. The rising incidence of obesity could lead to a shift towards I-HCA, which will be more frequently observed. Furthermore the prevalence of obesity in women is higher in the Netherlands (46.1%) compared to France (36.9%)[37].

We acknowledge that our study has a limitation. The final diagnoses were not all histologically proven. In those cases, combined imaging was used as the reference method for the final diagnosis, which was made after consensus in our multidisciplinary tumor board committee. In the early years diagnoses of HCA has been differentiated from FNH with at least a conventional MRI. According to signal intensity and dynamic vascular patterns after intravenous aspecific gadolinium injection the

different benign liver tumors are differentiated[14]. However during the inclusion period of this study specific hepatobiliary contrast agents are introduced and the differentiation is now more specific in challenging cases[38].

Biopsy is only approved in our hospital only if there is doubt about the diagnosis or radiological examinations are not in agreement. However MRI approves a highly accurate diagnosis with a sensitivity of 91-100% and a specificity 87-100% for differentiating HCA from FNH[39]. The Bordeaux subtype classification was in most patients performed on MRI, it should be noticed the MRI features of B-HCA are not completly defined[40], therefor there could have been false negatives on MRI resulting in possibly an underestimation of B-HCA.

Further research regarding the role of obesity in HCA and the effect of weight loss needs to be done. Because of the higher risk of surgery and the co-morbidities of fatty liver we suggest to start with weight reduction in all obese patients[3]. If follow up demonstrates no decrease of the HCA treatment should be decided depending on the anatomic location and the steatosis of remaining liver tissue. The management should be discussed by a multidisciplinary committee and strategies may be individualized.

Conclusion

Our series demonstrates a significantly higher BMI and frequency of inflammatory HCA in patients with multiple HCA compared to single HCA. As weight reduction could decrease the size of these HCA, this finding may help to personalize treatment, focusing on tailor-made lifestyle monitoring with OC cessation and body weight reduction in this specific subgroup.

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CHAPTER 4

Retrospective cohort study on timing of resection of hepatocellular adenoma

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ABSTRACT

Background

Hepatocellular adenoma(HCA) is a benign liver tumor that may be complicated by bleeding or malignant transformation. Present guidelines advise cessation of oral contraceptives and surgical resection if the lesion is still >5cm at six months after diagnosis. The aim of this study was to evaluate whether this six month interval is sufficient to expect regression to ≤5cm in large HCA.

Method

This retrospective cohort study included all patients with HCA >5cm diagnosed between 1999-2015 with a follow-up time of at least six months. Medical records were reviewed for patient characteristics, clinical presentation, lesion characteristics, management and complications. Differences in characteristics were addressed between patients kept under surveillance and patients who underwent treatment for HCA>5cm.

Results

Some 194 patients were include, of which 192 were female. Patients in the surveillance group(n=86) had a significantly higher BMI(p=.029), smaller baseline HCA-diameter(p<.001), more centrally located(p<.001) and more frequently multiple lesions(p<.001) compared to the treatment group(n=108). No significant differences were found for sex, baseline-age, symptoms, complication-rates and HCA-subtype distribution. Time-to-event analysis in conservatively treated and patients undergoing treatment >six months after diagnosis showed 69/118 HCA(58.5%) regressing to ≤5cm after a median of 104 weeks(95%-CI 80-128). Larger HCA took longer to regress(p<.001). No complications were documented during follow-up.

Conclusion

This study suggests that a six-month cut-off point for assessment of regression of HCA >5cm to ≤5cm is too early. As no complications were documented during follow-up, the cut-off point in females with typical, non-β-catenin mutated HCA could be prolonged to twelve months irrespective of baseline-diameter.

INTRODUCTION

Hepatocellular adenoma (HCA) is a benign liver tumor occurring mostly in women in their reproductive phase. It has an incidence of approximately one per million per year in the general population compared to 30-40 per million per year in long-term estrogen-containing oral contraceptive (OC) users [1, 2]. Regression of HCA may occur with cessation of OC [3]. Other conditions that have been associated with HCA are obesity, the metabolic syndrome and the intake of androgens [4-7].

Four HCA subgroups have been described based on genetic and phenotype characteristics. These include steatotic (H-HCA), inflammatory (I-HCA), β-catenin activated (β-HCA) and unclassified (U-HCA) adenomas. Another combined group that is both inflammatory and β-catenin activated (β-IHCA) has also been suggested to exist [8, 9]. Distinction between the subtypes can be made immunohistochemically and radiologically. HCA can be complicated by growth and rupture causing potentially life-threatening hemorrhage. The latter is thought to occur mostly in I-HCA [10]. Another possible complication is malignant degeneration to Hepatocellular Carcinoma (HCC) which has been reported particularly in β-HCA [11, 12]. Both hemorrhage and malignant degeneration occur mostly in HCA >5cm [13].

The diagnosis HCA can be made based on contrast-enhanced MRI (CE-MRI), contrast-enhanced CT (CE-CT) or contrast-enhanced ultrasound (CEUS) [14, 15]. In case of inconclusive imaging, a liver biopsy may be considered if the result would have an impact on treatment decisions.

In 2016 the European Association for the Study of the Liver (EASL) issued a guideline regarding the management of benign liver tumors [16]. In females a conservative approach was deemed justified which consists of cessation of OC and weight reduction. Significant growth (>20% according to the RECIST criteria [17]) or a HCA diameter >5cm after six months was stated as an indication for resection. In case of contraindications for resection, trans arterial embolization (TAE) was suggested for consideration as a treatment of larger HCA and radio frequent ablation (RFA) for smaller HCA [16].

As many HCAs regress after cessation of OC, waiting for the lesion to shrink to <5cm might be sensible. Evidence regarding the optimal timing of surgery for HCA is lacking in the world literature and the six month interval as suggested in the EASL guideline is based on expert opinions. In large HCA lesions located centrally in the liver or in multiple bilobal HCA, resection may be challenging. As liver resections may have a perioperative complication rate up to 20% and mortality rate up to 3.1%, which increases with the presence of steatosis, resection should only be considered if necessary[18-21].

The aim of this study was to determine if a 6-month follow-up period is sufficient in large HCA (>5cm) to expect regression to ≤5cm, as is suggested in the EASL guideline. In addition, the differences in clinical and lesion characteristics between patients who were kept under surveillance and patients who underwent treatment for HCA >5cm and the indications for treatment were assessed.

METHODS

This was a retrospective cohort study performed in a tertiary hepatobiliary referral center in the Netherlands. All patients, both male as female, diagnosed with a baseline HCA diameter >5cm between January 1999 and December 2015 were included. The diagnosis of HCA had to be based on imaging (CE-MRI) or histological examination (biopsy or resection specimen). Patients with less than 6 months follow-up time at the authors institute were excluded.

Medical records were reviewed for patient characteristics (sex, age at diagnosis, BMI), clinical presentation (symptoms), OC use, lesion characteristics (size of the lesion, location of the HCA in the liver, number of lesions, HCA-subtype), management (treatment, follow-up) and the occurrence of complications (hemorrhage or malignant degeneration).

Symptoms were scored as no symptoms, upper abdominal pain or atypical complaints at the time of diagnosis. The location of HCA in the liver was described as centrally located in the liver (segment I-IV-V-VIII) or in the left (segment VI-VII) or right (segment II-III) hemiliver. The number of HCAs were documented as solitary or multiple (>1). HCA subtypes were based on immunohistochemistry as described by the Bordeaux-group [22] or on typical MRI features [15, 23, 24] and subdivided in H-HCA (steatotic HCA), I-HCA (inflammatory HCA), β-HCA (β-catenin activated HCA),β-IHCA (combined inflammatory and β-catenin activated HCA) and U-HCA (unclassified HCA). If the HCA subtype had not yet been established by MRI or biopsy in patients, previous available MRI imaging was reassessed by a specialized abdominal radiologist. Hemorrhage of HCA was divided into grade I (intratumoral), grade II (intrahepatic) and grade III (extrahepatic) [10]. Malignant degeneration was based on histological examination of biopsies or resection specimens.

All imaging performed during follow-up was reviewed to assess whether lesions regressed to ≤5cm and how many weeks after diagnosis and cessation of OC this reduction occurred. Size of the HCA was documented at four moments in time: baseline imaging at the moment of diagnosis (T0), at ± 26 weeks (T1), ± 52 weeks (T2) and at last imaging available (T3). Patients were subdivided into two groups: one group of patients who were kept under surveillance with regular imaging and

did not undergo any intervention and a second group of patients who underwent surgery or other interventional techniques as a treatment for HCA. All patients in the surveillance group were advised to stop OC or other systemic hormonal contraceptives (hormonal intrauterine devices were allowed) and in case of obesity to lose weight. In the intervention group the intervention performed was documented, as were the indication for intervention and time from diagnosis to intervention. A sub division was made between patients who underwent an intervention without follow-up imaging beyond T0 (early interventions) and patients who underwent an intervention after imaging at T1, T2 or T3 (late interventions). Time-to-event analysis for the event "regression to <5cm" was performed in patients in the surveillance group and patients in the late intervention group. Patients in the early intervention group were excluded from time-to-event analysis.

Statistical analysis was performed with IBM SPSS software version 21.0 (Chicago, Illinois). Continuous variables were summarized as mean (μ) and standard deviation (SD) in case of normal distribution and as median and interquartile range (IQR) in case of non-normal distribution. Binary variables were summarized as frequency (n) and percentages (%). Differences between groups were analyzed using student T-test or Mann-Whitney U test for continuous variables and $\chi 2$ test for categorical variables. Time-to-event analysis was performed using the Kaplan-Meier method and log-rank test. A p-value of <.05 was considered as the level of significance. This study was approved by the accredited local institutional review board.

RESULTS

A total of 241 patients with an HCA >5cm at baseline were identified. Forty-seven patients were excluded because follow-up time at the institute was <6 months: these patients were either referred back to the initial hospital or lost to follow-up due to patient non-compliance. Of the remaining 194 patients (of which 192 female), 86 were kept under surveillance and 108 were treated with resection or another intervention. In the surveillance group, 70/86 had MRI proven HCA and 16/86 had biopsy proven HCA.

Comparison of clinical and lesion characteristics

The comparison of clinical and lesion characteristics between the surveillance group and intervention group is summarized in table 1. There were no statistically significant differences for sex, median age at diagnosis, OC use, symptoms, hemorrhage or malignant degeneration.

Table 1. Comparison of clinical and lesion characteristics: surveillance vs intervention

	Surveillance (n = 86)	Intervention (n = 108)	p-value
Sex			ns.
Male	0	2 (1.9%)	
Female	86 (100%)	106 (98.1%)	
Median age at diagnosis (yr)	38 (31-46)	36 (30-44)	ns.
Median BMI (kg/m^2)	31,6 (25,8-35,1)	28,5 (24,5-33,0)	.029
Symptoms			ns.
None	30 (34.9%)	34 (31.5%)	
Upper abdominal pain	45 (52.3%)	58 (53.7%)	
Atypical	11 (12.9%)	16 (14.8%)	
Oral contraceptive use			ns.
Never	2 (2.3%)	5 (4.6%)	
At diagnosis	57 (66.3%)	51 (47.2%)	
Before diagnosis	27 (31.4%)	47 (43.5%)	
Unknown	0	3 (2.8%)	
Median diameter of HCA at diagnosis (mm)	71 (60-90)	88 (72-110)	<.001
Location of HCA			
Right hemiliver	25 (29.1%)	52 (48.1%)	.007
Left hemiliver	9 (10.5%)	29 (26.9%)	.004
Central	52 (60.5%)	27 (25.0%)	<.001
No. of lesions			.001
Solitary	13 (15.1%)	39 (36.1%)	
Multiple	73 (84.9%)	69 (63.9%)	
HCA subtype			
H-HCA	11 (12.8%)	16 (14.8%)	ns.
I-HCA	40 (46.5%)	60 (55.6%)	ns.
β-HCA	0	1 (0.9%)	ns.
β-IHCA	0	3 (2.8%)	ns.
U-HCA	5 (5.8%)	11 (10.2%)	ns.
Unknown	30 (34.9%)	17 (15.7%)	0.002
Hemorrhage			ns.
Grade I	18 (20.9%)	25 (23.1%)	
Grade II	5 (5.8%)	4 (3.7%)	
Grade III	2 (2.3%)	0	
No	61 (70.9%)	79 (73.1%)	
Malignant degeneration			ns.
Yes	0	3 (2.8%)	
No	86 (100%)	105 (97.2%)	

This table shows baseline characteristics of patients in the surveillance group and intervention group and whether the characteristics between the groups differ significantly. Values are in median (IQR) or n (%). The HCA-subtypes are explained in the methods section.

Patients who were kept under surveillance had a higher median BMI than patients in the intervention group (p=.029). The median diameter of HCA at diagnosis was higher in the intervention group (p<.001). In the surveillance group, HCAs were more often located centrally in the liver (p<.001) while in the intervention group HCAs were more often located in the right or left hemiliver (p=.008 and p=.003, respectively). In the intervention group more patients had solitary lesions compared to the surveillance group (p<.001).

The distribution of HCA-subtypes in the surveillance and intervention group was not statistically different, although the proportion of unknown subtypes was higher in the surveillance group (p=.002).

Intervention group

Out of 108 patients who underwent an intervention, 94 (87.0%) had a resection, 9 (8.3%) underwent TAE and 5 (4.6%) underwent RFA (table 2). The median time from diagnosis to resection was 5 months (IQR 3,5-17). Seventy-three resections were early interventions, of which the majority I-HCA (56.2%). The most common indications were atypical characteristics on imaging and size >5cm. Twenty-one resections were late interventions of which also the majority were I-HCA and size as the most common indication.

The median time from diagnosis to TAE was 7 months (IQR 2,5-19,5). Out of the nine patients who underwent TAE, three were early interventions, 2 because of hemorrhage and 1 because of size >5cm. The remaining 6 TAE were late interventions and indications were size in 3, previous hemorrhage in 2 and pregnancy wish in 1.

All 5 RFAs were late interventions with a median time from diagnosis to RFA of 34 months (IQR 18,5-46). In all patients the lesion regressed to ≤5cm. In four the indication for RFA was a pregnancy wish and one patient had a residual adenoma after hemorrhage for which RFA was performed.

Time-to-event analysis

The median diameter and IQR of HCA at the four time points is depicted in figure 1A. Out of the 86 patients who were kept under surveillance, four patients did not have follow-up imaging at T1, one due to patient non-compliance and in three patients a one-year interval was decided instead of a six-month interval. Fifteen patients did not have imaging at T2 because a one-year interval was decided after T1. Another fifteen patients did not have imaging at T3: seven were referred back to their initial hospital, six are still in follow-up and two patients were lost to follow-up. 32 patients from the intervention group had imaging beyond T0.

A total of 118 patients were included in the time-to-event analysis. At 26 ± 4 weeks 10-18 out of 118 HCAs (8.5 - 15.3%) showed regression to ≤5cm and at 52

Table 2. Interventions for HCA.

Intervention			n			Mo. from diagnosis tointervention
Resection			94 (87.0%)			5 (3,5-17)
TAE			9 (8.3%)			7 (2,5-19,5)
RFA			5 (4.6%)			34 (18,5-46)
	Early interventions			Late interventions		
	Resection (n=73)	TAE (n=3)	RFA (n=0)	Resection (n=21)	TAE (n=6)	RFA (n=5)
HCA-subtype						
H-HCA	12 (16.4%)	-	-	1	3	-
I-HCA	41 (56.2%)	-	-	18	1	-
β-HCA	1 (1.4%)	-	-	-	-	-
β-IHCA	3 (4.1%)	-	-	-	-	-
U-HCA	9 (12.3%)	1	-	1	-	-
Unknown	7 (9.6%)	2	-	1	2	5
Indication						
Size	24 (32.9%)	1	-	11	3	-
Atypical imaging characteristics	22 (30.1%)	-	-	2	-	-
Pregnancy wish	8 (11.0%)	-	-	1	1	4
Hemorrhage	5 (6.8%)	2	-	1	2	1
Growth	7 (9.6%)	-	-	1	-	-
No regression after cessation of OAC	4 (5.6%)	-	-	4	-	-
Symptoms	1 (1.4%)	-	-	1	-	-
Need for hormonal substitution	1 (1.4%)	-	-	-	-	-

This table shows the median (IQR) time from diagnosis to intervention in months. Interventions were subdivided in early interventions (without follow-up imaging beyond T0) and late interventions (after imaging at T1, T2 or T3). The HCA-subtypes are explained in the methods section.

± 4 weeks this was 28-32 out of 118 (23.7 – 27.1%). At the end of follow-up a total of 69 HCA (58.5%) showed regression to ≤5cm after a median time of 104 weeks (95%-CI 80-128 weeks) (figure 1B).

A sub-analysis based on baseline HCA diameter showed that 38/44 HCA <7cm regressed to ≤5cm after a median time of 63 weeks, 25/51 HCA 7-10cm after a median of 109 weeks and 6/23 HCA >10cm after a median of 208 weeks (p<.001) (figure 1C). No statistically significant differences were found between HCA-subtype and median time for the event "regression ≤5cm" to occur (p=.476, figure 1D). An example of a regressing HCA is shown in figure 2.

Out of the 69 patients in whom the HCA regressed to ≤5cm, 45 (65.2%) stopped OAC at the moment of diagnosis, 23 (33.3%) prior to diagnosis and 1 never used

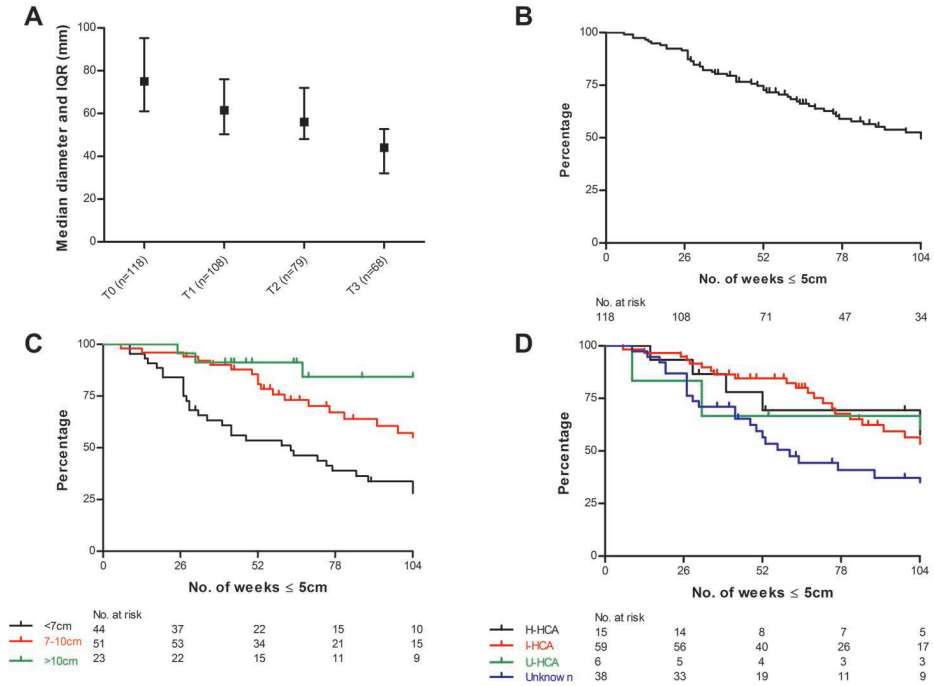

Figure 1. Diameter of HCA and regression to ≤ 5cm.
A: Median diameter and IQR of HCA at four moments in time: baseline imaging at the moment of diagnosis (T0, 75mm), at 6 months (T1, 61.5mm), 12 months (T2, 56mm) and at last imaging available (T3, 44mm).
B: Kaplan-Meier curve for the event regression to ≤ 5cm, all HCAs combined.
C: Kaplan-Meier curve for the event regression to ≤ 5cm, subdivided per baseline HCA diameter.
D: Kaplan-Meier curve for the event regression to ≤ 5cm, subdivided per HCA-subtype.
H-HCA: steatotic HCA. I-HCA: inflammatory HCA. U-HCA: unclassified HCA.

OAC. Out of the 49 patients in whom the HCA did not regress to ≤5cm, 28 stopped OAC at the moment of diagnosis, 19 prior to diagnosis and 2 never used OAC.

There were 22 patients in whom the HCA remained the exact same size at T1 compared to T0 (with a 5mm measurement error). Twelve out of these 22 HCA eventually did regress to ≤5cm . No complications occurred during the surveillance period. In all of the patients who had a bleed from an HCA, that was the initial presentation.

DISCUSSION

This retrospective cohort study including 194 patients with an HCA greater than 5cm at baseline evaluated if a 6-month follow-up period after stopping OC is sufficient

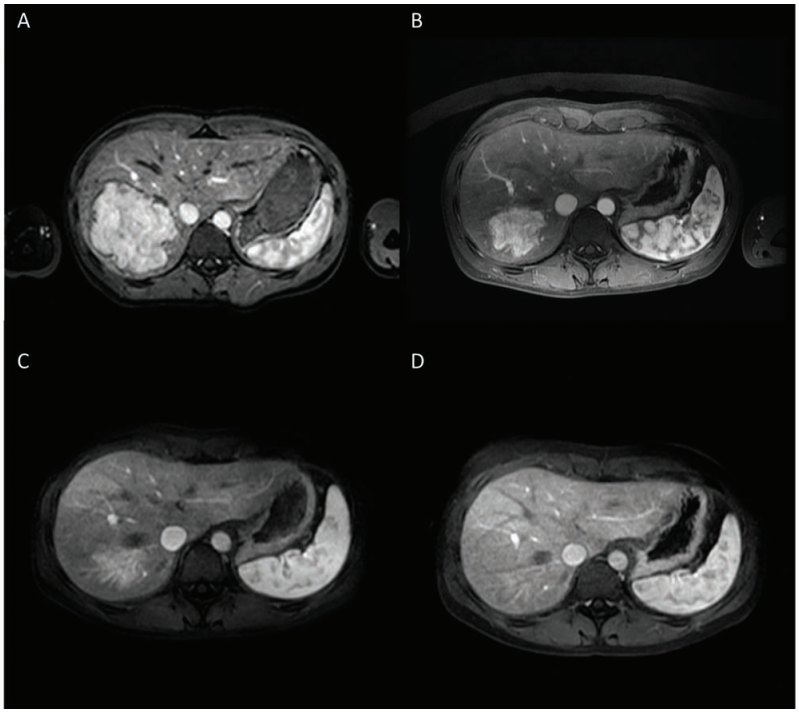

Figure 2. Example of a patient with HCA regression over time.
T1-weighted MRI in the arterial phase after injection of contrast. Twenty-three year old patient who used oral contraceptives, incidental finding on ultrasound.
A: Baseline imaging, 93mm I-HCA in segment 6/7/8.
B: Imaging 7 months after cessation of oral contraceptives, regression of the HCA to 55mm.
C: Imaging 16 months after baseline, regression to 45mm.
D: Imaging 29 months after baseline shows regression to 14mm seen as a small perfusion defect.

to expect regression of the HCA to less than 5cm. This time period is suggested in the EASL guideline on the management of benign liver tumors [16]. As evidence regarding the optimal timing of surgery for HCA is lacking in the world literature, the suggested six-month interval is based on expert opinion [25]. The present results suggest that this interval is too short and that surgery for large HCAs should probably be performed with more restraint.

In this study less than 15% of the HCAs showed regression to less than 5cm after interval half year and only about 25%after a year. At the end of follow-up about 60% decreased in size to ≤5cm after about two years by extending the follow-up time, many unnecessary resections could be avoided. As patients with HCA frequently have obesity and hepatic steatosis 26 and the risk of complications due to surgery is higher in these patients [20, 21], this could provide a considerable health benefit.

Additional analysis showed that HCAs with a larger diameter at baseline take considerably longer to regress to ≤5cm. Hemorrhage was only seen at initial presentation and no rupture or bleeding of HCAs was reported during the follow-up period. There were no differences between HCA-subtypes in median time to regression to ≤5cm. However, this lack of differences in the sub analysis might be a result of the small sample size.

Of the patients in whom regression to ≤5cm was reported, two thirds stopped OAC at the moment of diagnosis and one third prior to diagnosis. Similar numbers were seen in patients in whom the HCA did not regress to ≤5cm.

A comparison between patients kept under surveillance and patients who had intervention for HCA >5cm showed that BMI was higher for patients in the surveillance group. The mean diameter of HCA at diagnosis was higher in the intervention group. Additionally, more patients with centrally located HCA and multiple lesions were kept under surveillance. This could be explained by the fact that in patients who are less suitable for surgery due to a higher BMI, multiple lesions or tumor location, clinicians are more likely to propose a conservative approach due to a higher chance of perioperative complications. Additionally, in patients with larger HCA at the time of diagnosis, clinicians might assume the tumor will not reach the point of regression to ≤5cm and therefore resection is thought to be inevitable. No differences between the surveillance and intervention group were found for sex, mean age at diagnosis, symptoms, HCA-subtype and the occurrence of hemorrhage or malignant degeneration.

Most patients in the intervention group underwent resection of the HCA. A subdivision into early and late interventions showed atypical imaging characteristics and size to be indications for the majority of early resections and size >5cm to be the indication for the majority of late resections. Indications for early TAE were hemorrhage and for late TAE size >5cm, hemorrhage or pregnancy wish. All RFAs were late interventions performed in patients with HCA that had already regressed to ≤5cm. Indications were pregnancy wish or residual adenoma after hemorrhage.

Based on this study, a more conservative approach of HCA seems justified. However, as β-HCA and β-IHCA have a higher risk of malignant degeneration [11, 12], the determination of HCA-subtype becomes increasingly important. In this cohort, all four patients with β-HCA and β-IHCA had early resections. A conservative approach may not be justified if the HCA-subtype is not established. There are still some cases that require early resection and should not be kept under surveillance, for instance in biopsy proven β-HCA and β-IHCA, atypical imaging characteristics and HCA in men. In this cohort, 12 patients with H-HCA also underwent early resection. Given the most recent literature concerning low risk of complications in H-HCA and the results of the present study, a more conservative approach in these patients seems

justified. Unfortunately, the reliability of biopsy for HCA is not well studied and the number of misclassifications is unsure. Therefore, future studies should focus on the diagnostic value of biopsy for subtype classification and the distinction between HCA and well differentiated HCC.

As the risk of complications is higher in large HCAs, it would be advisable to keep patients under strict follow-up. Follow-up every six months when the lesion is >5cm and annually or biennially when the lesion has regressed to ≤5cm, until the occurrence of menopause, seems justified (figure 3) [26].

The strength of the present study is that all results were based on a large, representative cohort with long follow-up. In 2015 Chun et al. performed a retrospective cohort study of 79 patients in which they aimed to validate a surveillance algorithm for women with small (<5cm) HCA [27]. They concluded that patients with HCA <5cm

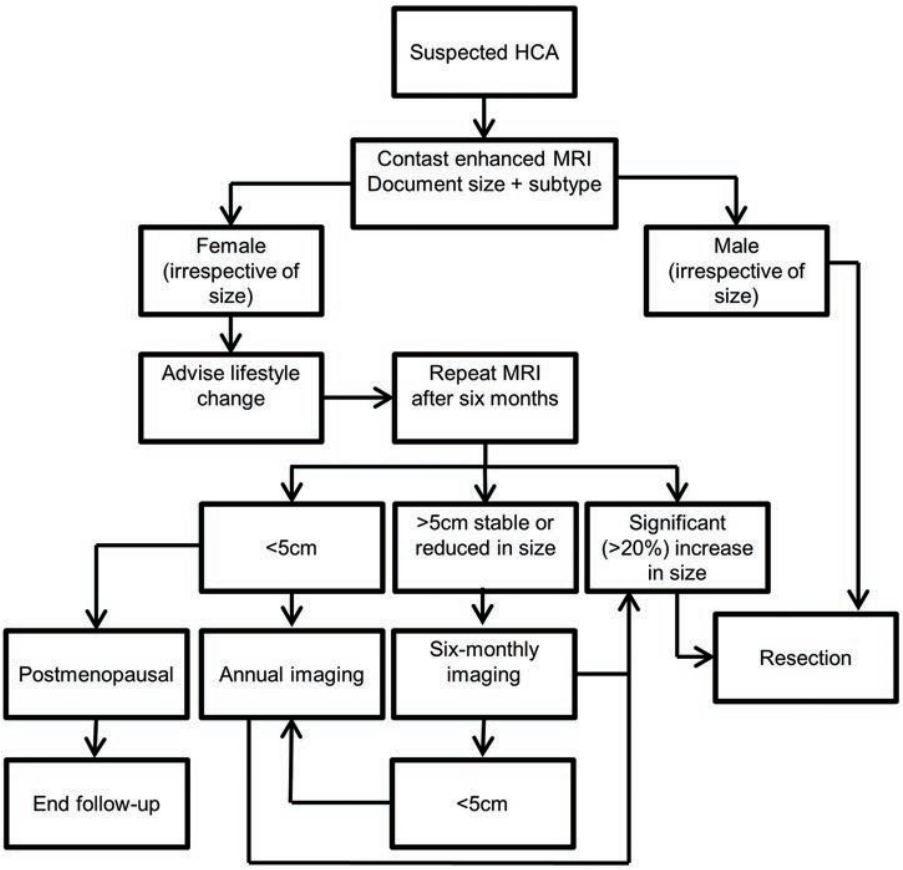

Figure 3. Flowchart for the management of benign liver tumors.
Adapted from EASL Clinical Practice Guidelines on the management of benign liver tumors[16].

can be kept under surveillance at 6, 12 and 24 months after diagnosis and that cessation of follow-up may be considered if lesions remain stable or decrease in size. The present study also assessed the surveillance interval of large HCAs. Future research should focus on the identification of factors that influence the natural course of HCA with the aim of predicting which HCA will regress to ≤5cm and which will require invasive treatment.

The present study might be subject to a few limitations. The retrospective design is inherent to bias. Another limitation might lie in the fact that patients were included between January 1999 and December 2015. In this time frame the quality of the imaging techniques has improved considerably, especially regarding the classification of HCA subtypes. A final limitation of the present study is the interval censoring, as the follow-up scan provided the measurements at a set moment in time. Therefore the exact moment at which the HCA became ≤5cm remains unknown. To overcome this limitation, the number of HCA that became ≤5cm after a half year and a year is reported with a 4-week interval. The present results suggest that a cut-off point of six months for the consideration of resection in HCA >5cm is insufficient to expect regression and that surgery for large HCA should be delayed and exercised with more restraint.

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CHAPTER 5

A model based prediction of the probability of hepatocellular adenoma and focal nodular hyperplasia based on characteristics on contrast enhanced ultrasound

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ABSTRACT

Contrast-enhanced ultrasound (CEUS) is an emerging imaging technique that is increasingly used to diagnose liver lesions. It is of the utmost importance to differentiate between the two most common solid focal liver lesions (i.e. hepatocellular adenoma (HCA) and focal nodular hyperplasia (FNH)), because their management and follow-up differ greatly.

The main objective of our study was to determine how frequently the specific CEUS features of HCA and FNH are visible on CEUS and to define their predictive value for discrimination between HCA and FNH.

In total, 324 CEUS examinations performed on patients with FNH (n=181) or HCA (n=143) were included. Patients with HCA and FNH significantly differed with respect to age and CEUS features of steatosis, echogenicity, homogeneity, the presence of a central scar, central artery, arterial enhancement pattern, necrosis or thrombus, and enhancement in the late venous phase.

INTRODUCTION

Abdominal ultrasound examination is readily available and frequently used in virtually every hospital. Consequently, during examination of complaints that are not directly related to the liver, a lot of patients are misdiagnosed with a focal lesion in the liver on ultrasound. Most of these lesions are of benign origin, such as hemangiomas, simple cysts, focal nodular hyperplasia (FNH) or hepatocellular adenoma (HCA). Some lesions, such as simple cysts, can be diagnosed on ultrasound. However, solid liver lesions, such as FNH and HCA, need further characterization. Accurate diagnosis is of the utmost importance because treatments for the conditions differ greatly. FNH is a benign lesion with no malignant transformation, symptoms that may resolve during follow-up and a very low incidence of bleeding (Behrend and Flemming 2001, Belghiti and Cauchy 2014). Therefore, if the diagnosis is firmly established, treatment is rarely indicated (Belghiti and Cauchy 2014, Terkivatan and Hussain 2006). HCA, on the other hand, has a risk of hemorrhage, rupture and malignant transformation, and treatment might be indicated (European Association for the Study of the Liver . Electronic address 2016).

Macroscopically, FNH tends to be lobulated and in most cases it has a central stellate scar (central element) which radiates into nodules of normal hepatocytes (Terkivatan and Hussain 2006). The central scar contains a fibrous stroma and malformed vascular structure, the central artery. From this anomalous central artery, the arterial blood often flows centrifugally (stellate-type contrast agent distribution), which is in contrast to HCA (Hussain and Terkivatan 2004). HCA tends to have peripheral subscapular vessels, which cause diffuse homogenous arterial filling. These characteristics can be used to discriminate the two conditions.

Until recently, magnetic resonance imaging (MRI) or needle biopsy were needed for characterization (Thomeer and ME 2014, van Aalten and Thomeer 2011). However, recent FDA approval of the contrast agent Lumason (Sonovue, Bracco) will increase interest in, and use of CEUS in clinical medicine.

CEUS is an emerging imaging technique that is increasingly used to diagnose solid focal liver lesions. The use of microbubble ultrasound contrast agents allows detailed assessment of vasculature patterns. The detection and characterization of solid liver tumors has improved considerably using CEUS (Claudon and Dietrich 2013).

The extensively described washout phase, defined as negative enhancement in the tumor 75 seconds after injection of the microbubble contrast agent, is used to differentiate between benign and malignant liver lesions (Bhayana and Kim 2010). Furthermore, a centrifugal hypervascular enhancement pattern (FNH), diffuse arterial enhancement in the arterial phase (HCA), a central scar (FNH), contrast-

enhancement in the late phase (FNH) and the presence of thrombus or necrosis (adenoma) are CEUS characteristics that help to differentiate between FNH and HCA. Moreover, a centrifugal hypervascular enhancement pattern in the arterial phase may be an essential feature for the diagnosis of non-typical FNH (Alberti and Frulio 2014). However, the frequency of the presence of features for HCA and FNH on CEUS and its capacity to differentiate between HCA and FNH have only been described in a few small series (Friedrich-Rust and Klopffleisch 2013, Kong and Wang 2015, Roche and Pigneur 2015). A meta-analysis concluded that a detailed evaluation of HCA by CEUS was not possible because of the low numbers of patients with HCA (Friedrich-Rust and Klopffleisch 2013). Thereafter, 28 patients with FNH and 10 patients with HCA have been described and showed 66% of the lesions using CEUS were correctly diagnosed compared to 40% of the lesions using color Doppler ultrasound (Kong and Wang 2015). Roche (2015) described 40 patients (31 with only FNH, seven with only HCA and two patients with both FNH and HCA) and suggest that it is a useful adjunct tool, especially in assessing smaller lesions, with an almost perfect inter-observer agreement (Roche and Pigneur 2015).

Guidelines outline steps for diagnosing benign solid liver tumors with CEUS and indicate that the specific feature in FNH is a centrifugal hypervascular enhancement pattern in the arterial phase. This specific feature can be used to differentiate FNH from HCA and could even be an essential step for the diagnosis of non-typical FNH (Alberti and Frulio 2014).

HCA, on the other hand, should have a diffuse arterial enhancement in the arterial phase. Other known patterns include central scar (in B-mode as a CEUS late phase), contrast enhancement in the late phase (both patterns described in FNH), or the presence of thrombus or necrosis (adenoma).

According to the literature, describing the characteristics of FNH and HCA in MRI, some findings are more typical than others (Thomeer and ME 2014). For example, the central scar, which is more commonly described in FNH, was also found in 21% of confirmed HCA cases (Hussain and Terkivatan 2004, van Aalten and Thomeer 2011).

How often the specific features described for HCA and FNH are present and visible on CEUS has not been satisfactorily described. Therefore, the main objective of our study was to determine how frequently the specific features of HCA and FNH are displayed on CEUS. We also sought to define the predictive value of features for the discrimination between HCA and FNH on CEUS.

MATERIALS AND METHODS

The study was performed in accordance with the ethical guidelines of the 1975 Declaration of Helsinki and approved by the local Institutional Review Board and Ethical Committee from the Erasmus MC University Medical Center. The need for written informed consent was waived.

Patients

In total, 324 CEUS examinations performed between 2007 and 2014 in patients with confirmed FNH or HCA were available for review in this study. CEUS findings were only included if the diagnosis of the lesion had been confirmed using at least two radiologic modalities, including at least one magnetic resonance imaging (MRI) examination with the use of a liver-specific contrast agent. Consensus on the diagnosis was reached after discussion within our multidisciplinary tumor board committee or if the lesion was histologically confirmed (by biopsy or surgical resection). Patient characteristics were collected from the electronic hospital records.

Contrast-enhanced ultrasound (CEUS)

CEUS was introduced in our hospital as an additional radiologic modality. CEUS was performed by various sonographers, but all exams were reviewed by a specialist with more than 20 years of experience in liver ultrasound and more than nine years of experience in CEUS. The sonographers were blinded to the patients' pre-existing imaging (computed tomography (CT), MRI) information. CEUS was performed using the Hitachi 900 and Hitachi Preirus ultrasound platforms (Hitachi Medical Systems, Japan) with real-time grayscale, contrast-tuned imaging and a 2.5–5.0 MHz probe. The contrast agent used was SonoVue (Bracco, Italy; dose range 1.0–2.4 ml; repeated if needed and flushed by isotonic saline).

Ultrasound examination was performed in a standardized fashion. First, all patients underwent unenhanced abdominal and hepatic sonography using the fundamental color/power Doppler technique. The location, number, size, and sonographic features of the focal liver lesions were recorded. In case of significant hepatic steatosis, identifying a specific liver mass with Ultrasound (US) might be more difficult. However, when switched to the CEUS mode, specific features appear, which can be used to differentiate the different liver masses. Three phases can be observed with CEUS because of the unique network of the hepatic artery and the portal vein, (Claudon and Cosgrove 2008, Jang and Kim 2006, Piscaglia and Lencioni 2010, Piscaglia and Venturi 2010). CEUS was performed during the hepatic arterial (10–40 s post-injection), portal venous (40–120 s post-injection), and late parenchymal phases (> 120 s, bubble disappearance), according to the standardized

EFSUMB protocol (Piscaglia and Nolsoe 2012). The vascularity and enhancement pattern of the lesions were compared with the adjacent liver parenchyma. Accordingly, CEUS was performed five minutes after application of the contrast agent. The flash-replenishment technique was applied when needed. Still images and digital cineloops were saved and later reviewed. Central arteries were defined by the presence of enhancing central arteries with a spoke-wheel appearance. A central scar was defined as a central stellate hypoechogenic area without contrast enhancement in the portal venous phase. Necrosis or a thrombus caused by previous bleeding was defined as an irregular area without contrast filling. Late contrast-enhancement (contrast agent retention) was defined as the presence of hyperechogenic filling (mostly fine) compared to adjacent liver parenchyma in the late portal phase.

Statistical analyses

All statistical analyses were performed using SPSS (version 16.0, SPSS Inc., Chicago, IL, USA). Normality of continuous data was checked by inspecting the distribution. Parametric tests were used for continuous data, as these were all normally distributed. Continuous variables are presented as means with standard deviations and categorical variables as numbers and percentages.

First, a univariate analysis was performed by comparing characteristics between HCA and FNH. All p-values < 0.05 (two-sided) were considered as statistically significant.

Next, a multivariable logistic regression analysis was performed to investigate the association between the different covariates and the definitive diagnosis of HCA. A stepwise regression model with backward elimination was used. All items included in the univariate analysis were initially included as a covariate in the initial model. After each step, the covariate with the worst predictive value was removed until the best-fit model remained. The classification cut-off for elimination was set as $p = 0.05$. The coefficients of the final multivariate model were used to create a formula for the prediction of the probability that the lesion was HCA.

RESULTS

A total of 324 CEUS examinations were performed in patients diagnosed with FNH or HCA. Patients and lesion characteristics of the CEUS examinations of FNH and HCA are summarized in Table 1. Of the 324 patients, 311 patients (96%) were female and 143 (44%) patients had a HCA. The median age at diagnosis for all patients with HCA was 41 years (range 4–63 years). The median age of the 181 (56%) patients with FNH was 37 years (range 17–61). The lesions had a mean diameter of 56 mm, ranging from 10 to 180 mm.

Table 1. Univariate analysis of possible predictors for HCA and FNH

		HCA (n = 143) n (%)	FNH (n = 181) n (%)	p value
Patient	Age, years (range)	40.6 (18–77)	36.8 (17–61)	0.001
	Size lesion (mm)	54 (10-180)	59 (15-175)	0.04
Liver	Steatosis	67 (47)	41 (23)	< 0.001
Ultrasound before contrast	Echogenicity (M = 1)			< 0.001
	Hypo	48 (34)	31 (17)	
	Iso	59 (42)	137 (76)	
	Hyper	35 (25)	13 (7)	
	Homogenicity (homo)	107 (75)	156 (86)	0.009
	Central scar	34 (24)	145 (80)	< 0.001
	Central artery (M = 1)	46 (32)	150 (83)	< 0.001
CEUS arterial phase	Enhancement pattern (M = 16)			< 0.001
	Fugal	14 (11)	98 (56)	
	Mixed	19 (14)	40 (23)	
	Petal	99 (75)	38 (22)	
	Necrosis or bleeding (M = 1)	26 (18)	6 (3)	< 0.001
CEUS portal venous phase (M = 3)	Sustained/retention	20 (14)	73 (40)	< 0.001
	Iso	92 (66)	95 (53)	
	Hypo	21 (15)	8 (4)	
	Hetero	7 (5)	5 (3)	

Univariate analysis results for various comparisons for HCA and FNH. $p < 0.05$ was considered statistically significant. Data were analyzed using a t-test or Pearson's chi-squared test where appropriate. Values in parentheses are percentages unless otherwise noted. HCA, hepatocellular adenoma; FNH, focal nodular hyperplasia; CEUS, contrast-enhanced ultrasound.

Steatosis in a non-tumorous parenchyma was observed in 47% of the patients with HCA compared with 23% in patients with FNH. Necrosis or thrombus formation was observed in 18% of the patients with HCA compared to 3% necrosis or thrombus formation in the patients with FNH. There was no acute bleeding or thrombus formation observed.

Results from the univariate analysis of possible patient-related predictors combined with features on CEUS between the group of patients with HCA and FNH are shown in Table 1. HCA and FNH patients differed significantly with respect to age and the CEUS features of steatosis, echogenicity, homogeneity, central scar, central artery, arterial enhancement pattern, necrosis or thrombus, and enhancement in the late portal phase (contrast agent retention).

In a multivariable analysis, the subsequent items were eliminated in the following order: homogeneity ($p = 0.888$), enhancement in the late venous phase (contrast retention) ($p = 0.797$), necrosis or thrombus caused by previous bleeding ($p = 0.527$),

echogenicity (p = 0.108) and steatosis (p = 0.193). The regression coefficients of the final regression model are given in Table 2. Using these coefficients, the predicted probability of HCA was calculated using the following formula:

Predicted Probability (P) =1/(1+e(0.778+(0.36 *Age) + (-1.251*central scar) +(-1.198*central artery) +(0.541*enhancement mixed) +(1.157*enhancement petal)))

A receiver operating characteristic (ROC) curve was plotted and showed an area under the curve (AUC) of 0.854 (Figure 1). Figure 2 shows the predicted probability that the definitive diagnosis is HCA for increasing age and visualization of a central scar and central artery for different enhancement patterns.

Table 2. Multivariable logistic regression analysis for the prediction of HCA based on patient characteristics and features of contrast-enhanced ultrasound examinations

	p value	Regression coefficient	95% confidence interval
Age	0.015	1.037	1.007–1.067
Central scar	0.020	0.286	0.129–0.634
Central artery	0.010	0.302	0.148–0.615
Enhancement pattern			
Fugal	0.056		
Mixed	0.221	1.718	0.722–4.090
Petal	0.017	3.182	1.229–8.239

DISCUSSION

Liver Steatosis

Currently, fatty liver disease is the most common chronic liver disease with an estimated incidence of 30% in Western countries (Browning and Szczepaniak 2004). Liver steatosis is often accompanied by obesity. HCA seems to also be associated with obesity, explaining why significantly more people with HCA have liver steatosis (47%) compared to patients with FNH (23%). Identifying a specific liver mass with US might be more difficult in obese patients. However, specific features can be depicted using CEUS, which can aid in differentiating the various liver masses.

HCA can be hyperechogenic in an otherwise normal liver during ultrasound without the use of contrast ("fat-containing HCA"); however, we also found that 7% of FNHs were hyperechogenic. This rare hyperechogenicity of FNH could be explained by the occasional presence of fat in FNH, which has been previously described and

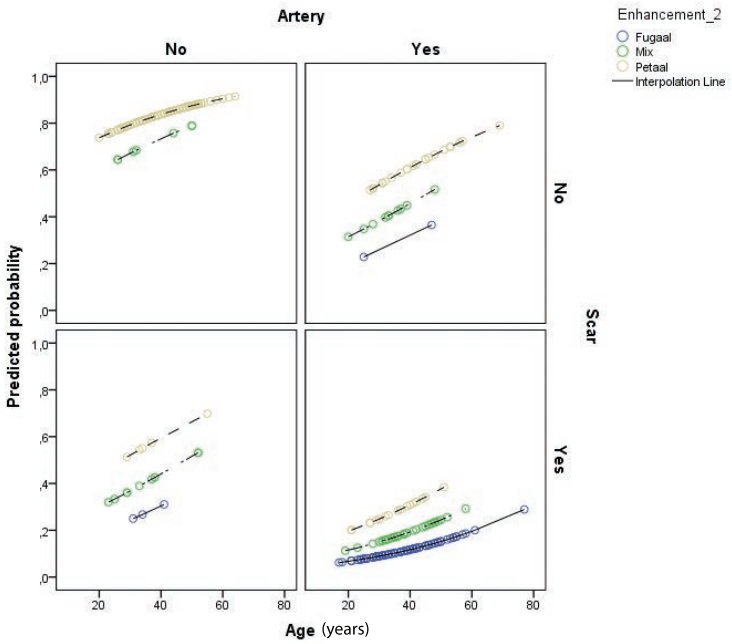

Figure 2. HCA diagnosis
The figure shows the predicted probability that the definitive diagnosis is HCA.
The visualization of a central scar and a central artery on CEUS indicates which quadrant of the figure should be used. The different colored lines in each quadrant stand for the different enhancement patterns (blue, fugal; green, mixed; yellow, petal). By using the age of the patient at the moment of diagnosis on the corresponding colored line in the figure, the predicted probability that the definitive diagnosis is HCA can be determined.

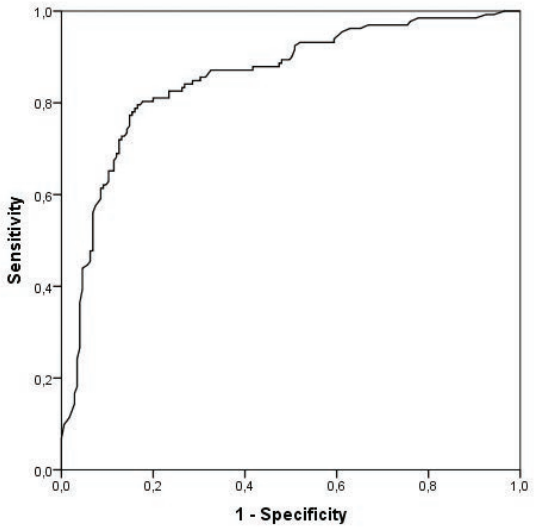

Figure 1. Receiver Operating Characteristic (ROC) Curve
The receiver operating characteristic curve for the prediction of HCA using the formula 1/(1+e(0.778+(0.36 *Age) + (central scar) (central artery) (enhancement mixed) (enhancement petal), showing an area under the curve value of 0.854.

should be considered in ultrasound and imaging diagnostics (Burt AD 2012). When other classical FNH findings are seen, the presence of fat in the lesion can occasionally make the diagnosis less robust.

Central Scar

A classic FNH is composed of nodules surrounded by radiating fibrous septa originating from a central scar (Fukukura and Nakashima 1998, Hussain and Terkivatan 2004). The central scar in FNH is a collection of blood vessels, bile ducts, and fibrosis stroma (Elsayes and Peterson 2007). With CT and MRI, the central scar has been reported in 22 to 85% of FNH cases (Bartolotta and Taibbi 2014, Mortele and Praet 2000). On CEUS, in which the central scar appears as a hypoechogenic area in the delayed phase (Piscaglia and Lencioni 2010), or on B-scan ultrasound, where it appears as a white fibrotic stripe, we identified a central scar in 80% of the FNH cases. However, fibrotic stripes similar to a central scar have been observed in 24% of HCA cases as well. Recently, fibrotic scars have also been described in 21% of HCAs on MRI (Thomeer and ME 2014, van Aalten and Thomeer 2011). The central scar is characterized on MRI by a T2-weighted late enhancement in the delayed phase. As a central scar could also be visible in HCA, differentiation between HCA and FNH should not be based on the appearance of the central scar alone, fibrotic stripes could have the same appearance.

Necrosis or thrombus

A thrombus caused by previous bleeding was present in 18% of the HCA and 3% of the FNH in this study. Thrombus caused by bleeding in HCA is fairly common, with an average overall frequency of 27.2% and a maximum reported frequency of 64% (Bieze and Phoa 2014, van Aalten and de Man 2012). It should be noted that if an irregular area without contrast enhancement is observed on CEUS, no differentiation among necrosis, thrombus from an old bleeding, or a large central scar can be made (Behrend and Flemming 2001, Nguyen and Flejou 1999). The presence of an avascular area (necrosis/thrombus) in FNH is very rare, but can be present and is not exclusively diagnostic for HCA on ultrasound.

Central artery and enhancement pattern

As expected, a central artery with centrifugal (stellate) filling was more common in FNH, and a petal filling was most common in HCA (Kim and Jang 2008).

Arteries in FNH can be abnormally large for the region of the liver they perfuse, and in some nodules, color Doppler examination can be diagnostic. It may sometimes be difficult to localize the central part of the arterial tree with single-plane ultrasound because it can be located eccentrically and not centrally. A subset of patients have

not one, but two or more centers with stellate arterial projections. In these cases, the centers probably tend to be faint and not robust. Further technological development, such as the regular use of 3D CEUS, could be of benefit here.

Contrast agent retention in the late portal phase

This ultrasound sign was confirmed to be predominant in FNH but was found only in 40% of FNH patients. This level was statistically significant but not exclusive; up to 14% of adenomas were hyperechogenic in the late phase. Contrast retention can be confusing because 25% of adenomas are already hyperechogenic on B-scan ultrasound. The sonographer should carefully differentiate between an already hyperechogenic tumor background and an influence of the presence of contrast agent.

Some atypical FNHs may show a washout-like image in late phase – in our series 4%, which is usually seen in Hepatocelullar Carcinoma (HCC) and also in some HCAs (15% in our study).

Limitations

Our study has limitations. First, the final diagnoses were not all histologically validated. In those cases, combined imaging was used as the reference method for the final diagnosis, which was made after consensus was reached within our multidisciplinary tumor board committee. If we had only selected patients with a histologically-proven diagnosis, a bias would have been introduced because a biopsy is only approved in our hospital if there is doubt about the diagnosis or radiological examinations are not in agreement. Second, because the lesions were not biopsied, it was not possible to link the specific features to the Bordeaux classification of HCA subtypes and their ultrasonographic appearance.

The aim of this study was to differentiate FNH and HCA, two solid benign liver tumors. In clinical practice it is also of the utmost importance to exclude hepatocellular carcinoma. The most important feature to differentiate benign and malignant liver lesions on CEUS is the presence of washout (Bernatik and Seitz 2010, Bhayana and Kim 2010). However, this feature showed no additional value in differentiating between FNH and HCA. It is essential to first rule out a malignancy, before using this model, which gives insight into the predicted probability of HCA.

CONCLUSIONS

In conclusion, increased age and CEUS features of liver steatosis, tumor echogenicity, homogeneity, thrombus presence, filling pattern and central scar, central artery, arterial enhancement pattern, and absence of enhancement in the portal venous phase

were found to be predictive in distinguishing between HCA and FNH. A reliable model using age and the presence of a central scar, central artery, and enhancement pattern can predict the probability that the definitive diagnosis is HCA. If the diagnosis of HCA or FNH is equivocal on MRI, CEUS can be used to differentiate the two lesions, as a combination of the two methods provides the highest diagnostic accuracy (Soussan and Aube 2010). This study gives insight about the reliability of the features on CEUS and helps clinicians to decide whether further liver mass biopsy is needed.

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CHAPTER 6

Is contrast enhanced ultrasound comparable to MRI with liver specific contrast agent for the diagnosis of hepatocellular adenoma and focal nodular hyperplasia

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Submitted

ABSTRACT

Purpose

To compare the diagnostic performance of contrast-enhanced ultrasonography (CEUS) with MRI using gadobenate dimeglumine (CEMRI) for diagnosis of hepatocellular adenoma (HCA) and focal nodular hyperplasia (FNH) of the liver.

Materials and methods

Patients referred to a tertiary center for hepatobiliairy diseases with suspicion of HCA or FNH were included. All patients had undergone a prospective work-up of CEUS and CEMRI. Final diagnosis was considered firm when outcome of CEUS and CEMRI were concordant. Histopathologic assessment (PA) followed in case of discrepancy between CEUS and CEMRI. CEMRI was considered as the reference method for final diagnosis when patient refused biopsy. Sensitivity, specificity, area under the ROC curve and predictive values were calculated for CEUS.

Results

One hundred eighty-two patients (155 female, 27 male, mean age 38 years, range 17-76 years) were included. CEUS and CEMRI were concordant in the majority of patients (n=131, 72%). Discrepancy between CEUS and CEMRI in 51 patients (28%). PA followed in 28 cases (55%), in two cases biopsy could not distinguish between HCA and FNH. In the remaining PA- proven cases (n=26) , CEMRI was correct in 20 cases (77 %) and CEUS in 6 cases (23 %) . In the remaining cases (n=23, 45%), CEMRI was considered as reference for final diagnosis. Sensitivity and specificity were respectively 82,5% and 71,7% for CEUS for diagnosis of HCA and FNH with an area under the ROC of 0.766.

Table 1. CEUS outcome and Final diagnosis

CEUS outcome	Final HCA	Final FNH	Total
HCA	49	29	78
FNH	9	81	90
CEUS Other Diagnosis	2	1	3
CEUS Inconclusive	4	7	11
Total	64	118	182

FNH; focal nodular hyperplasia, HCA; hepatocellular adenoma.
CEUS outcome versus final diagnosis.
Sensitivity 82,5% (49/(49+9)), specificity 71,7% (81/(81+29+3)) , Area under the ROC: 0.766

Conclusion

Concordance between CEUS and CEMRI is fair for diagnosis of HCA and FNH. In discordant cases CEMRI is highly accurate and superior to CEUS in histopathology confirmed diagnoses.

INTRODUCTION

Hepatocellular adenoma (HCA) and focal nodular hyperplasia (FNH) are benign solid liver lesions that are mostly found in young women. Although both entities are benign and often asymptomatic, the pathogenesis and clinical management is different. Accurate diagnosis is therefore important (1-3). Contrast enhanced ultrasonography (CEUS) and MRI with liver- specific contrast agent (CEMRI) are imaging modalities with reported specific imaging features for both HCA and FNH that may lead to confident diagnosis (3-7). At present, both CEUS and CEMRI are regarded as best complementary techniques for diagnosing HCA or FNH and final diagnosis is considered correct in case of concordance between outcomes of CEUS and CEMRI (3). In uncertain or atypical cases, final diagnosis with histopathology (PA) is imperative (3, 8). Recent studies have reported CEMRI to be highly accurate for diagnosis of HCA and FNH (1, 6, 9-11). However, CEMRI is costly and time consuming. CEUS on the other hand has the potential to provide confident diagnosis at lower costs than CEMRI (12-14).

The purpose of this study was to compare the diagnostic performance of CEUS with CEMRI for the diagnosis of HCA and FNH.

MATERIALS AND METHODS

Study population

Our institutional ethics committee approved this retrospective study and informed consent was waived.

Patients were selected from prospective collected databases from the departments of radiology and gastroenterology. Inclusion criteria were patients with final diagnosis of HCA or FNH who had undergone both CEUS and CEMRI of the liver between May 2008 and December 2016. Exclusion criteria: final diagnoses other than HCA or FNH, patients with history of cancer or known chronic liver disease, multiple lesions with both FNH and HCA.

Standard work-up procedure

Patients referred to our center with suspicion of HCA or FNH received a standard work-up with CEUS and CEMRI of the liver. CEUS was performed or supervised by a gastroenterologist with 21 years of experience in ultrasound of the liver, including 5 years of experience in CEUS. Subsequently, MRI examinations were evaluated and reported by one of three experienced abdominal radiologists with expertise in liver imaging (respectively 8, 10 and 11 years), without regard to the CEUS outcome. . In

case of uncertainty, cases were discussed between two or three radiologists and consensus was reached on final diagnosis.

CEUS

CEUS was performed using the Hitachi 900 and Hitachi Preirus ultrasound platforms (Hitachi Medical Systems, Japan) with real-time grayscale, contrast-tuned imaging and a 2.5–5.0 MHz probe. Contrast agent used was SonoVue (Bracco, Italy; dose range 1.0–2.4 ml; repeated if needed and flushed by isotonic saline). Examination were executed in a standardized fashion: first, all patients underwent unenhanced abdominal and hepatic sonography using conventional color/power Doppler techniques, with location, number, size, and sonographic features of the focal liver lesions recorded. Because of the unique network by the hepatic artery and the portal vein, three phases can be observed with CEUS (4-7). Acquisition included hepatic arterial (10–40 s post-injection), portal venous (40–120 s post-injection), and late parenchymal phases (>120 s, bubble disappearance) conform the European federation of societies for ultrasound in medicine and biology (EFSUMB) protocol (8). Vascularity and enhancement pattern of the lesion was evaluated for up to 5 minutes post-injection of contrast. Still images and digital cine loops were saved and later reviewed for final assessment and report. Central arteries were defined by the presence of enhancing central arteries with a spoke-wheel appearance. A central scar was defined as a central stellate hypoechoic area without contrast enhancement in the portal venous phase. Necrosis or previous intralesional hemorrhage was defined as an irregular heterogeneous area without contrast filling. Late contrast enhancement (contrast agent retention) was defined as the presence of hyperechoic contrast filling compared to adjacent liver parenchyma in the portal phase.

MRI

MRI was performed on a 1.5 Tesla (T) unit (General Electrics, Signa, Milwaukee, WI) with a four-channel, phased-array body coil. The MRI protocol was identical for all patients: single- shot fast spin echo (SSFSE, slice thickness = 7 mm; repetition time/ echo time (TR/TE) (ms) = 832/80–120; flip angle = 90°), fat-suppressed T2W fast spin echo (FSE) (5–8 mm, 6315/90– 93, flip angle = 90°), and T1-weighted in- and opposed-phase gradient-echo (GRE) sequences (7 mm, shortest/4.6 and 2.3, respectively; flip angle = 80°). Fat-suppressed, dynamic contrast- enhanced T1-weighted GRE imaging (4–5 mm, 2.7–3.5/1.2; flip angle = 12°) was performed in at least four phases (precontrast, arterial, portal, and delayed), following administration of an intravenous bolus (2–2.5 mL/s) of gadobenate dimeglumine (Multihance, Bracco Imaging, Milan, Italy) at a dose of 0.05 mmol per kilogram body weight. The optimal

arterial phase was based on bolus tracking. Finally, the same scan was repeated during a late hepatobiliary excretory phase at 1 to 1.5 hour after injection.

Reference standard

Final diagnosis was established within a Multidisciplinary Hepatobiliary Tumor Working Group. This group consists of one or more specialized radiologists, surgeons, gastroenterologists and oncologists. Diagnosis was considered firm in case of concordance between CEUS and CEMRI. In case of discordance between CEUS and CEMRI, histopathology analysis (PA) of the lesion followed after percutaneous image-guided biopsy. When biopsy was undesirable or contra-indicated, CEMRI was considered as the reference method for final diagnosis in case of confident findings on MRI.

Case evaluation procedure

Patients' age and sex, previous imaging reports, CEUS reports, CEMRI reports, Multidisciplinary Hepatobiliary Tumor Working Group decisions, pathological reports and final clinical diagnoses were registered using a standardized and anonymized clinical reporting form in the online clinical software program 'openclinica'. From the reports the confidence level of diagnosis with CEUS and CEMRI were graded using a five-point scale (5=definite/confident diagnosis, 4=preferable/probable diagnosis, 3=relative uncertain diagnosis, 2=very uncertain diagnosis, 1= no diagnosis). For final analysis, grade 5 or 4 were regarded as conclusive outcomes, and grade 3, 2 or 1 as inconclusive outcomes.

Data analysis and statistical methods

Descriptive statistics were used to describe the study population and outcomes of both imaging modalities. The association of categorical variables were presented by numbers and percentages, and tested by Fishers' exact test. The primary analysis was patient based. Inconclusive outcomes of CEUS and CEMRI (with confidence level scores less than 4) were considered as false positives or false negatives for the statistical analysis. Sensitivity, specificity, area under the receiver operating characteristic/ROC-curve (AUC) were calculated with SPSS (version 21, IBM, Chicago). All tests were regarded statistically significant if the p-value was less than 0.05.

RESULTS

Study population and final diagnosis

A total of 182 patients (155 female and 27 male) were included. Mean age was 38 years; range 17 to 76 years. Final diagnosis was FNH in 117 (65 %) patients and HCA in 63 patients (35%).

Women were significantly overrepresented compared to men (p <0.001) with no significant difference found in sex (p=0.528) or age (p=0.721) between FNH and HCA.

CEUS - CEMRI agreement, PA-proven cases

CEUS and CEMRI were concordant in 131 out of 182 patients (72%) (figure1) , and discordant in the remaining patients (n=51, 28%). PA was obtained in 28 out of 51 (55%) discordant cases, and in the remaining 23 cases (45%), CEMRI was considered as reference for final diagnosis.

From the 28 cases in which the diagnosis differed and biopsies were performed, 2 biopsies were not conclusive. In the remaining 26 PA-confirmed cases CEMRI was correct in 20 cases (77%) and CEUS in six cases (23%). This difference is statistically significant (p=0.03).

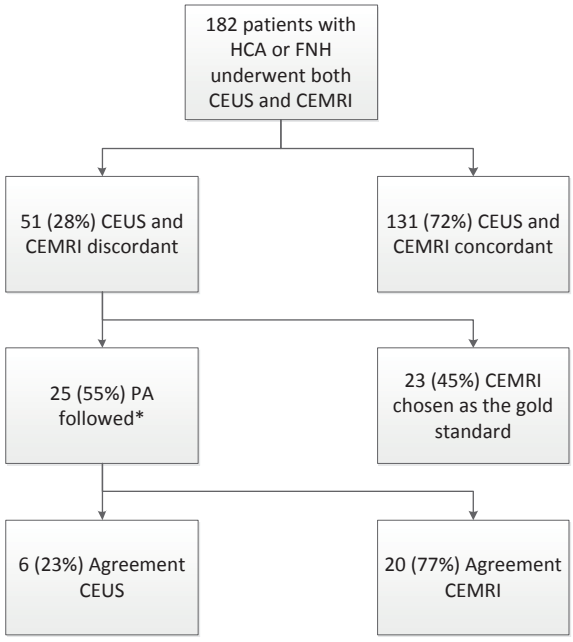

Figure 1. Flow diagram summarizes patient sampling.
*2 biopsies not conclusive

Confidence level of CEUS and CEMRI

For CEUS, the result was conclusive in 171 out of 182 cases (93%), with 148 out of 182 cases (81%) a confident diagnosis (grade 5) and 23 out of 182 cases (13%) a preferable or probable diagnosis (grade 4). Inconclusive diagnoses with CEUS were found in 11 out of 182 cases (6%).

The result of CEMRI was conclusive in 181 out of 182 cases (99.5%). Inconclusive diagnoses with CEMRI were found in 1 out of 182 cases (0.5%).

Diagnostic performance CEUS

When only cases with a conclusive outcome by CEUS are considered sensitivity and specificity are respectively 83% (49/(49+9)) and 72% (81/(81+29+3)) for diagnosis of HCA and FNH, with an area under the ROC of 0.77.

DISCUSSION

We found that agreement between CEUS and CEMRI was fair for diagnosis of FNH and HCA. Furthermore, in twenty-six PA proven cases, CEMRI was correct in all twenty cases, whereas CEUS was correct in six cases. Another important finding is that CEUS has a confident diagnosis in 81% and conclusive result in 93 % and inconclusive result in 7%, whereas CEMRI was conclusive in almost all cases. Based on these results we believe that CEUS is less suitable as a stand-alone imaging modality for final diagnosis of FNH and HCA. CEUS seems more suitable as an adjunct tool for diagnosis in typical cases that were suggested on multiphase CT scan and follow up of lesions that were otherwise confirmed as HCA by CEMRI or PA. The advantage of CEMRI over CEUS can be explained by the lack of hepatobiliary excretory properties of ultrasound contrast medium. While the morphologic characteristics can be assessed by both imaging modalities, including contrast enhancement patterns, the decisive feature for diagnosis on CEMRI is the hepatobilairy excretory phase for differentiating HCA from FNH. Previous studies have shown that 20% of FNH lack typical morphologic features on imaging, including a central scar (15). In addition, a subgroup of HCA (beta- cathenine positive subgroup) may demonstrate scar-like features on MRI with Gadolinium- chelates (16). Furthermore, CEMRI has the advantage of presenting a comprehensive evaluation of lesions, not only in differentiating between HCA and FNH, but also in demonstrating features which may be indicative of transformation to HCC in case of HCA. Our study has limitations. First, because of the retrospective analysis and the relative limited number of PA proven diagnoses. Currently, dedicated CEMRI with confident diagnosis is considered confirmative for diagnosis of FNH and HCA in expertise centers (6, 7, 9-11). Therefore it would be

unpractical and maybe unethical to biopsy all lesions, even though the diagnosis is confident on CEMRI.

Another potential limitation might be selection bias. As the analysis were done retrospectively, patients were selected based on final diagnoses of FNH or HCA. False-positive outcomes in case of other diagnoses, like liver hemangioma, are left out which may culminate in an overestimated specificity for FNH or HCA. However, the purpose of the study was to assess the value of CEUS for diagnosis when compared with CEMRI. We believe that our study design serves this purpose well.

In conclusion, CEUS has fair agreement with CEMRI for the diagnosis of HCA and FNH with a diagnostic performance being inferior to CEMRI in discordant cases.

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CHAPTER 7

Hepatocellular adenoma: when and how to treat? Update of current evidence.

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ABSTRACT

Hepatocellular adenoma (HCA) is a rare, benign liver tumor. Discovery of this tumor is usually as an incidental finding, correlated with the use of oral contraceptives, or pregnancy. Treatment options have focused on conservative management for the straightforward, smaller lesions (<5cm), with resection preferred for larger lesions (>5cm) that pose a greater risk of hemorrhage or malignant progression. In recent years, a new molecular subclassification of HCA has been proposed, associated with characteristic morphological features and loss or increased expression of immunohistochemical markers. This subclassification could possibly provide considerable benefits in terms of patient stratification, and the selection of treatment options. In this review we now discuss the decision-making processes, and associated risk analyses, that should be made based on lesion size, and subtype. The usefulness of this subclassification system in terms of the procedures instigated as part of the diagnostic work-up of a suspected HCA will be outlined, and suitable treatment schemes proposed.

INTRODUCTION

Hepatocellular adenoma (HCA) is an uncommon, solid, benign tumor of the liver, with an estimated incidence of 3-4 per 100,000 women [Bioulac-Sage et al., 2010]; this frequency is based on population research including women using oral contraceptives (OC) [Baum et al., 1973]. A causal role for hormone activity in HCA growth is suggested by data linking adenoma regression to the cessation of OC use, and growth associated with pregnancy [Cobey et al., 2004].

Typically, HCA is treated conservatively, with patients advised to avoid oral contraception. The risks of growth and rupture of HCA during pregnancy has to be underlined, especially in larger HCAs. Tumor progression, suggested by internal bleeding and malignant transformation, necessitates a more aggressive therapeutic approach, with lesions larger than 5 cm considered as the primary risk factor [Marrero et al., 2014]. The introduction of a new subclassification system for HCA has been suggested to help clinicians to stratify patients according to imaging criteria, expression of associated immunohistochemical markers and/or molecular findings. These data may influence the treatment selected [Marrero et al., 2014] since certain subtypes of HCA pose a greater risk of progression to hepatocellular carcinoma (HCC) than others. For example, a subtype of HCA defined by the reduced expression of liver-fatty acid binding protein (LFABP) ordinarily indicates a subtype with a less aggressive course and a tendency towards a benign phenotype.

Based on the recent literature, we will describe the impact of this newly instigated HCA subclassification, and discuss whether this knowledge, combined with imaging data, improves our risk analyses for patients with HCA. Furthermore, we will outline the different therapeutic options indicated by each HCA subtype.

The Bordeaux classification of HCA

In recent years, four distinct subtypes of HCA have been recognized: inflammatory HCA (40-50%, IHCA), HNF1A-mutated HCA (30-40%, H-HCA), b-catenin activated HCA (10-15% b-HCA), and unclassified HCA (10-25%, U-HCA) [Nault et al., 2013]. In these different subtypes, several genetic mutations are identified, causing (benign) proliferation of hepatocytes and in some HCA, malignant transformation [Pilati et al., 2014].

Patients presenting with IHCA demonstrate both serum, and lesional indicators of an active inflammatory response. In these lesions, increased expression is seen of the markers serum amyloid A and C-reactive protein, both classic indicators of the acute phase response [Bioulac-Sage et al., 2007]. Patients within this HCA category frequently demonstrate increased body weight, and a high alcohol intake [Bioulac-Sage et al., 2007, Paradis et al., 2007, Bioulac-Sage et al., 2009]. In approximately 10-20% of these lesions, a b-catenin mutation is found. [Van Aalten et al., 2011].

A second subtype of HCA, H-HCA, is characterized by a downregulation of the liver fatty acid binding protein (LFABP); this phenotype, which is not apparent in the other HCA subtypes, rarely leads to malignant progression [Zucman-Rossi et al., 2006].

Subtype b-HCA is typified by activating mutations of b-catenin that resist phosphorylation-mediated down-regulation by the GSKB/APC/AXIN complex [Nault et al., 2013]. Particularly the exon 3 mutation of b-catenin plays a significant role in malignant progression in contrast to exon 7/8 mutations [Pilati et al., 2014]. The result is an accumulation of nuclear b-catenin which, combined with deletion of APC, favors progression to HCC [Nault et al., 2013]. The comparatively small number of b-catenin positive nuclei can lead to this phenotype being overlooked in small biopsies [Van Aalten et al., 2011]. The b-HCA subtype also demonstrates an overexpression of GLUL (encoding glutamine synthase, GS), which can be used as a sensitive diagnostic biomarker for this subtype [Van Aalten et al., 2011].

The final subtype, UHCA, is not yet defined by any specific genetic mutation, but is instead characterized by various histologic criteria that are unusual in the other subtypes; the under-lying pathogenesis of this subtype remains unclear [Blanc et al., 2015].

Magnetic resonance imaging (MRI) of the different subtypes of HCA

The primary differential diagnosis for HCA is focal nodular hyperplasia (FNH). If in doubt, a biopsy should be taken, especially for larger lesions, as the clinical management will differ for either pathology. In most cases these diagnoses can be differentiated according to signal intensity and dynamic vascular patterns after intravenous aspecific gadolinium injection (conventional MRI)[Van Aalten et al., 2011]. Different patterns can be used for confident diagnosis as proposed by Thomeer et al[Thomeer et al., 2014].

In more challenging cases specific hepatobiliary contrast agents can be used. Two agents are currently available, gadobenate dimeglumine, and gadoxetate disodium.[Grazioli et al., 2013, Thomeer et al., 2014, Mcinnes et al., 2015] If the lesion turns hypointense to the surrounding liver in the hepatobiliary phase FNH can be excluded in most cases. If the lesion becomes iso- to hyperintense the differential diagnosis is FNH or in exceptional cases HCC. However, it should be noted that IHCA can also be isointense in the hepatobiliary phase[Agarwal et al., 2014, Thomeer et al., 2014]. This might be explained by the presence of internal bile duct proliferation, previously thought to be only visible in FNH. This diagnostic pitfall can be visualized when using gadobenate dimeglumine[Thomeer et al., 2014], or gadoxetate disodium[Agarwal et al., 2014]. A recent systematic review about the value of gadoxetate disodium has shown that apart from this pitfall, adequate differentiation is possible in most cases[Mcinnes et al., 2015]. It was reported that the

hepatobiliary phase has a sensitivity of 91-100 % and a specificity of 87-100 % for differentiating HCA from FNH. In the largest study this was only seen in 13 % of the cases[Bieze et al., 2012].

In conclusion, in the vast majority lesions can easily be differentiated based on a combination of typical findings on conventional MRI and features on hepatobiliary phase MRI.

Some typical MRI features allow us to discriminate different subtypes of HCA (table 1): IHCA can be hyperintense on T2-weighted images, with persistent enhancement on delayed imaging in the venous phase [Laumonier et al., 2008]. Ronot et al. validated this feature as being highly specific for IHCA, with a sensitivity of 82% (28/34, CI 65-93%), and an optimal specificity of 100% (12/12, CI 75-100%) [Ronot et al., 2011]. Another diagnostic indicator for IHCA is the atoll-sign [Van Aalten et al., 2011], a hyperintense rim on T2-weighted images at the periphery of the lesion (resembling an atoll) that is enhanced late in the venous phase. This feature is present in 27% of IHCAs in this study [Van Aalten et al., 2011].

Whilst H-HCAs are typically characterized by a large amount of aberrant fat which can be readily appreciated by out-of phase imaging, or on a fat-saturated T1-weighted image [Laumonier et al., 2008, Van Aalten et al., 2011], Van Aalten et al failed to detect fat by MRI for as many as 22% of cases (2/9)[Van Aalten et al., 2011]. Ronot et al. validated the diagnostic feature of diffuse and homogeneous signal dropout on out-of-phase T1 weighted imaging, with a reported sensitivity of 90% (10/11, CI 58-99), and specificity of 88% (32/36, CI 73-96)[Ronot et al., 2011]. The main drawback of this marker is that diffuse intralesional steatosis may also be present in up to 11% (4/34) of IHCAs [Ronot et al., 2011], although, according to the authors, this does not represent a major pitfall as fat is usually distributed heterogeneously within IHCAs (figure 1).

The MRI features of b-HCA are not well defined, principally because these lesions are comparatively rare. Van Aalten et al. reported poorly delimited, high-signal intensity areas, to be typical of this subtype (5/7, 71%), but additional investiga-

Table 1. Typical MRI findings according to suybtypes of HCA. HCA: hepatocellular adenoma, H-HCA: HNF1A-mutated HCA, IHCA: inflammatory HCA, b-HCA: b-catenin activated HCA, UHCA: unclassified HCA.

Subtype	Most typical MRI signs
IHCA	- Hyperintense on T2-weighted images, with persistent enhancement in the venous phase - Atoll-sign
H-HCA	Diffuse and homogenous fat deposition (figure 1)
b-HCA	(Vaguely demarcated scar)
UHCA	No typical sign

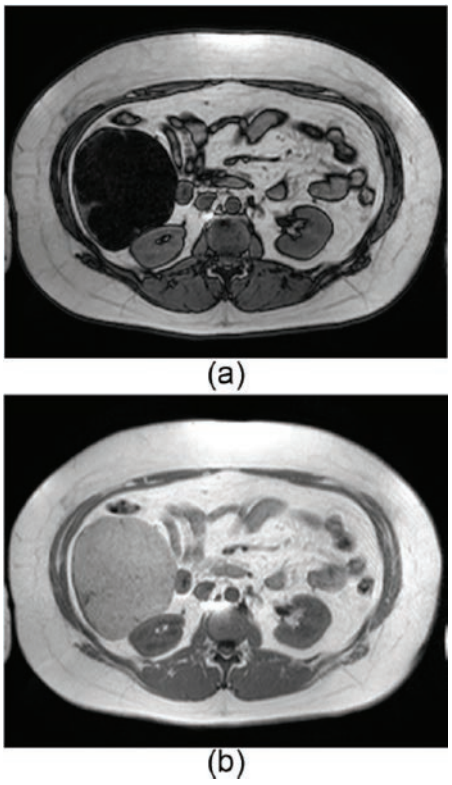

Figure 1a/b. In- and out-of-phase MRI of a typical case of histochemistry proved H-HCA which was resected. Note the diffuse and homogenous signal drop-off on the out-of-phase image (a) versus the in-phase image (b). This correlates with diffuse intralesional fat identified by histology. MRI differentiation between H-HCA and IHCA would not be possible when the signal drop is more heterogeneous.
HCA, hepatocellular adenoma; H-HCA, HNF1A-mutated HCA; IHCA, inflammatory HCA; MRI, magnetic resonance imaging.

tions are warranted [Van Aalten et al., 2011]. Table 2 shows the various MRI features reported in the literature for b-HCA, although, where reported, these features are inconsistent. Despite significant numbers of false negatives, the specificity of these MRI features is very high, leading us to conclude that if any one of these signs are present, a diagnosis of the corresponding MRI subtype can be made with some certainty. Larger datasets will be needed to determine the true value of MRI in HCA imaging for all subtypes; currently, this technique is of most use in evaluating prognosis.

Reviewing the known complications

Intralesional bleeding

On reviewing the recent literature, van Aalten et al. detected evidence of hemorrhage in 27.2% of all patients (315/1160) with one or more HCAs, giving a 15.8% chance of hemorrhage for every HCA (118/748) [Van Aalten et al., 2012]. Acute rupture and intraperitoneal bleeding were reported in 17.5% of patients. A size for the smallest HCAs showing hemorrhage was reported for 13 of the 28 articles reviewed; hemorrhage generally arose in the larger lesions (greater than 5 cm), although

smaller lesions could also bleed (table 3), albeit at much lower rates. These data should be interpreted with caution, as only the resected cases were included. The actual chance of bleeding in the different subtypes is likely to be significantly lower. The risk of bleeding was inconsistent across the subtypes of HCA: IHCA showed a higher propensity for macroscopic hemorrhage (30%), than H-HCA (8%) [Dokmak et al., 2009] which can presumably be attributed to the larger number of venous structures, or telangiectatic changes in this subtype.

Table 2. Recently published b-HCAs with their typical characteristics defined by MRI. Note the low prevalence in the literature of MRI data, with inconsistent findings. HCA: Hepatocellular adenoma, b-HCA: b-catenin positive HCA, T2W:T2-weighted

Authors	Year of publication	Number of b-catenin HCA	MRI findings
Van Aalten et al. [[Van Aalten et al., 2011]]	2012	7	- Vaguely defined scar on T2W sequences (3 cases)
Laumonier et al. [[Laumonier et al., 2008]]	2008	5	- Marked hyperintensity on T2W sequences and persistent delayed enhancement (3 cases) - Isointensity on T2W sequences, with strong arterial enhancement and delayed wash-out (2 cases)
Yoneda et al. [[Yoneda et al., 2012]]	2012	1	- Vaguely defined scar on T2W sequences

Table 3. Summary of the findings of an earlier review of 12 articles in which the percentage of hemorrhaged HCAs, and minimal lesion sizes, were reported. Hemorrhage occurred mostly in larger HCAs (>5 cm; minimally 42.2%), but smaller lesions also showed some bleeding (range 8.3–11.5%).

Series	**Patients with hemorrhaged HCA**	**Size of smallest HCA (cm)**	**Percentage <5 cm of total (%)**
[Reddy *et al.* 2001]	3 of 25	4	–
[Hung *et al.* 2001]	4 of 25	4.2	–
[Toso *et al.* 2005]	10 of 25	1.7	–
[Cho *et al.* 2008]	12 of 41	1	8.3 (1/12)
[Bioulac-Sage *et al.* 2009]	23 of 128	<5	–
[Edmondson *et al.* 1976; Dokmak *et al.* 2009]	26 of 122	<5	11.5 (3/26)
[Edmondson *et al.* 1976]	10 of 42	>5	0
[Leese *et al.* 1988]	2 of 24	5	0
[Ault *et al.* 1996]	4 of 12	6	0
[Closset *et al.* 2000]	7 of 16	7	0
[Deneve *et al.* 2009]	31 of 124	>5	0
[Chung *et al.* 1995]	–	5	0

HCA, hepatocellular adenoma.

Although there may be a difference in prevalence of internal bleeding, all subtypes bear this intrinsic risk [Laumonier et al., 2008, Dokmak et al., 2009, Ronot et al., 2011, Van Aalten et al., 2011], which diminishes the utility of subtype classification in terms of the clinical management of this risk. Furthermore, more data are needed to prove any correlate between reduced bleeding and the H-HCA subtype.

Bieze et al. described a series of 45 patients with 195 lesions. In this cohort, there was a tendency to an enhanced risk of bleeding when the lesion was located in the left lateral liver (11/32 versus 31/163 in other regions), and showed exophytic growth (16/24 versus 9/82) [Bieze et al., 2014] (Fig. 1). The latter phenomenon is probably due to the subcapsular location, with no intrinsic capsule, and minimal surrounding liver with which to prevent rupture of the hematoma into the abdominal cavity. However, no other data are available to support this theory, and preventive treatment in these cases does not appear to be warranted.

As for the clinical application of these findings, there is no evidence that supports the use of subtype classification in the stratification and management of individual patients. Moreover, size still remains the most important marker to predict those at risk for larger bleeding in follow up.

Malignant transformation

Malignant transformation of HCA to HCC is rarely reported, but is an accepted risk, particularly when the diameter of the adenoma exceeds 5 cm (figure 2) [Stoot et al., 2010, Grazioli et al., 2013]. In a systematic review, Stoot et al. reported an overall frequency of malignant transformation of 4.2% for HCAs [Stoot et al., 2010] (67 of 1635 HCAs, interval: 0 -100 %). Only three cases showed malignant transformation for tumors smaller than 5 cm in diameter, which represented 4.4% of the total number of HCCs arising from HCAs (3 out of 67). As suggested for the internal bleeding data, these reports should be interpreted with caution.

Of the four HCA subtypes, (exon 3) b-HCAs are known to trigger a potent mitogenic signaling pathway that is prominent in HCC [Zucman-Rossi et al., 2007, Chu et al., 2013, Pilati et al., 2014], which suggests a positive correlate between the two. Zucman-Rossi et al. reported an incidence of HCCs, or borderline malignant tumors in b-HCAs, of up to 46%; this malignant progression was seldom seen in other subtypes [Zucman-Rossi et al., 2007], and was over-represented for male patents (5 cases, 38%; P =.02)[Hussain et al., 2006]. Since the β-catenin pathway can also be activated in IHCA [Van Aalten et al., 2011], both the b-HCA and IHCA subtypes may necessitate more aggressive treatment than either the H-HCA or U-HCA, although the clinical relevance of this determination has yet to be broadly accepted. In follow-up, malignant progression of HCA to HCC has only rarely been demonstrated, with questionable quality of the imaging data for those rare, reported cases. There-

fore it is presently difficult to prove that HCC is a transition from HCA, although the presence of β-catenin has been suggested as a criterion for the selection of HCA, or well-defined HCC, for resection [Bioulac-Sage et al., 2013]. Interestingly, figure 2 shows a lesion with a typical nodule-in-nodule appearance which suggests a form of

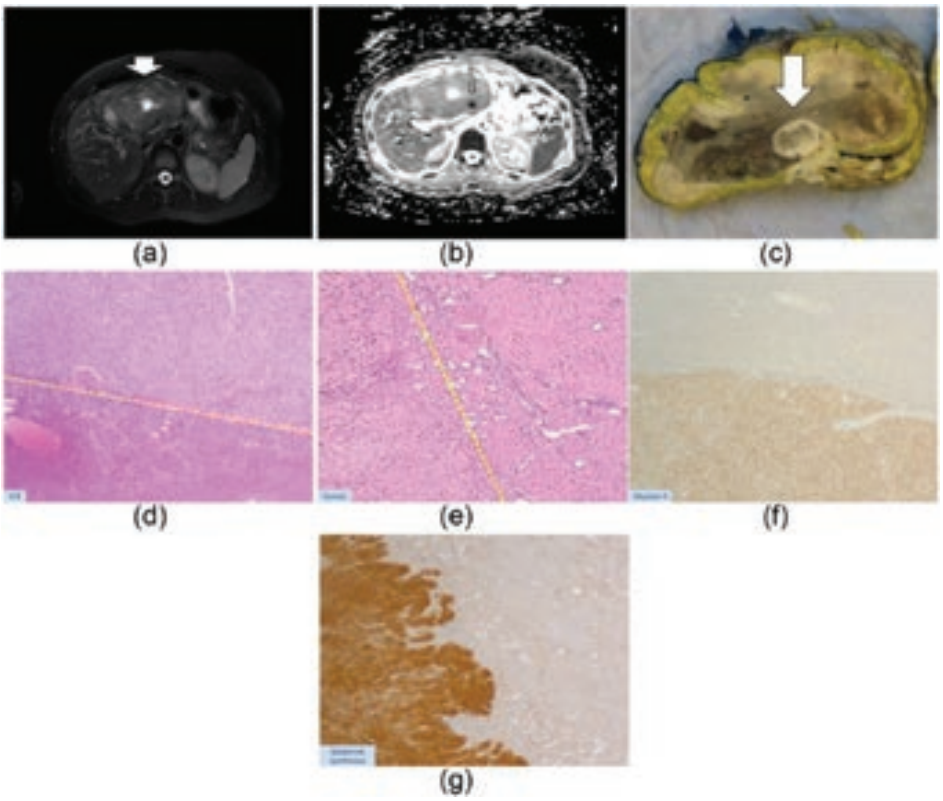

Figure 2. A 32-year-old female using oral contraceptives with a 11 cm lesion in the liver. Based on MRI this lesion was compatible with a HCA. However, both on T2-weighting (Figure 1(a)) as on the images after contrast injection the lesion appeared heterogeneous with a focus of diffusion restriction (typically a low ADC value, (b)). Diffusion restriction is thought to be a typical sign of malignancy in liver lesions. Based on the findings above and because the lesion was larger than 5 cm, the lesion was resected 3 months later. On gross pathology there was a focal nidus (Figure 1(c), arrow, concordant with the nidus on MRI) which appeared to be an HCC in a HCA ('nodule-into-nodule'). On histology (H–E × 25, (d)) at the interface HCA/HCC, the upper part of the tumor showed proliferation of hepatocytes without obvious cytological anomalies, intermingled with thin/isolated vessels (down side of dotted line), favoring an HCA. 'Nodule-into-nodule' consists of clearer cells with mild atypia (above dotted-line, (d)), disorganized or decreased reticulin fibers (e) and obvious positivity for Glypican-3 (f), favoring an HCC (well differentiated). (g) GS-staining pattern at the periphery of the HCA. Glypican-3, Serum Amyloid A and CRP were negative in the HCA. β-catenin staining showed only membranous expression. Based on the above we interpreted this HCA as a b-HCA.
CRP, C-reactive protein; HCA, hepatocellular adenoma; HCC, hepatocellular carcinoma; GS staining, glutamine synthetase immunostaining indicative of b-HCA; MRI, magnetic resonance imaging.

transition from HCA to HCC. Another problem is that corroboration of the pathology is seldom available, due to the fact that biopsies of HCA are rarely performed, with diagnoses generally made with MRI [Hussain et al., 2006]. A final diagnosis of b-HCA based solely on MRI findings would be helpful, but the MRI findings published to date for this subtype are sparse (Table 2). Finally, it should be mentioned that HCA shows a higher risk of malignant transformation in men [Farges et al., 2011]. In these cases, the possibility of hepatitis, an underlying glycogen storage disease (figure 3), or sex steroid hormone abuse, should all be considered as all predispose to HCC [Yoneda et al., 2012]. A more aggressive treatment is advised in such cases, even for lesions smaller than 5 cm.'

Finally, according to the recent literature, H-HCA almost never degenerate into HCC, although some very rare cases have been reported (Stueck et al 2015). The low risk of H-HCA degeneration may help to simplify the management of liver adenomas as will be discussed later.

As for clinical application, mainly b-HCA and IHCA are prone to malignant degeneration, and mostly if larger than 5 cm. In these instances invasive treatment is recommended.

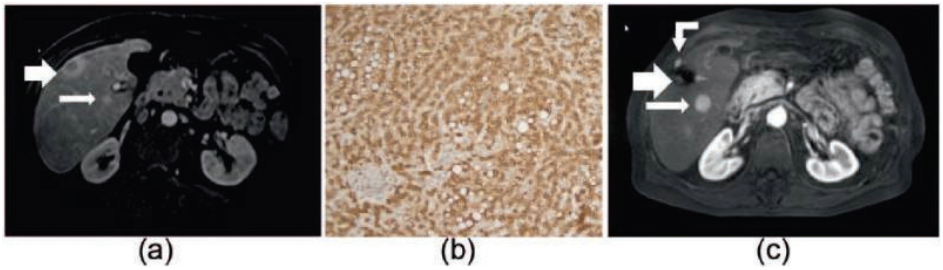

Figure 3. A 50-year-old male with multiple hypervascular lesions. These lesions were diagnosed as HCA or HCC based on imaging and clinical (glycogen storage disease) findings. (a) An axial MR image, with T1 weighting, after contrast injection in the arterial phase. In segment 5, a small lesion with a cystic central portion (large arrow) was biopsied, and subsequently diagnosed as HCC following positive GS staining with negative β-catenin staining. Posteriorly, a second, smaller lesion was visualized (<1 cm, small arrow). Histologic sample of a lesion with diffuse GS-positivity (b). Axial MR image with T1-weighing after contrast injection in the arterial phase (c). In this image, taken 3 years later, the second lesion has grown (now 3 cm, small arrow). The large arrow shows the resection/ablated part of the liver (from lesion 1). A new hypervascular lesion (curved arrow) was also detected outside the liver, which proved to be a trajectory metastasis. These lesions (large arrow, curved arrow) were successfully ablated. This patient is currently being followed at regular, short intervals, and is on the waiting list for a liver transplantation.
GS staining, glutamine synthetase immunostaining indicative of b-HCA, even with a negative β-catenin staining; HCA, hepatocellular adenoma; HCC, hepatocellular carcinoma; MR, magnetic resonance.

Pregnancy

Women with HCA who are pregnant, or wish to become pregnant (figure 4), should be closely monitored for HCA size (with ultrasound or MRI) during their pregnancy, due to the tendency of the lesion to grow, especially during the 3rd trimester when high levels of estrogens are present [Cobey et al., 2004]. Hormone-induced growth, and possible rupture, may result in potentially lethal complications for the mother and unborn child. Treatment of HCA during pregnancy may be indicated when the lesion shows signs of growth or bleeding, however specific figures for the risk of HCA complication during pregnancy are not yet available.

Whether some subtypes are more prone to complications during pregnancy is not known, mainly because the majority is diagnosed non-invasively.

The choice of follow-up, surgery, radiofrequency ablation (RFA) or transcatheter arterial embolization (TAE) for the treatment of HCAs in pregnancy is often a matter of debate. Surgery of lesions located at the periphery of the liver can be performed safely within the first or second trimester, and will usually be indicated by the size of the lesion, and its change in size. Radiation exposure and/or exposure to iodinated contrast media during RFA or TAE may be contraindicated during the early phase of pregnancy, with the treatment of smaller lesions not being indicated. Given the increased risk of hemorrhage in larger HCAs (> 5 cm), or when a previous pregnancy was complicated by either minor or major bleeding, we currently advocate a

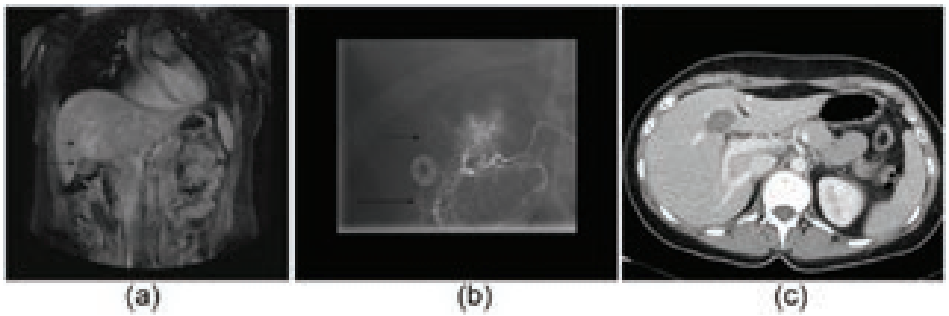

Figure 4. A 25-year-old female with a MRI diagnosis of single HCA, probably inflammatory subtype. As the patient wished to become pregnant, despite growth of her HCA, a decision to treat with TAE was taken. Coronal MR image with T1-weighting of the upper abdomen (a). A hypervascular HCA is indicated (small arrow), adjacent to the gallbladder (long arrow). Ablation was contraindicated due to the close proximity of the gallbladder. Angiogram (b) before TAE showing an arterial tumor 'blush' in the HCA (short arrow), with the gallbladder perfused by the same local hepatic artery division (long arrow). This finding contraindicated TAE due to the possibility of gallbladder necrosis following infarction. Instead, a decision to operate was made, with resection of the gallbladder, and subsequent intraoperative RFA of the HCA. Axial postoperative CT image after contrast injection in the venous phase (c). The gallbladder was resected, in combination with intraoperative RFA (arrow). CT, computerized tomography; HCA, hepatocellular adenoma; RFA, radiofrequency ablation; TAE, transcatheter arterial embolization.

preemptive treatment strategy before pregnancy, as proposed by van Aalten et al. [Broker et al., 2012]. Whenever a HCA is discovered during pregnancy, the second trimester is the optimal moment for invasive treatment, if indicated, as anesthesia is well tolerated at this stage, and the fetus is not yet so large as to interfere with liver surgery [Parangi et al., 2007].

Liver adenomatosis

Hepatic adenomatosis (HCAs more than ten) is regarded by some authors as a different entity[Barthelmes et al., 2005, Frulio et al., 2014]. There seems not to be a strong association with oestrogen or anabolic steroid use[Chiche et al., 2000, Grazioli et al., 2000]. However, there is a strong association with glycogen storage disease[Chiche et al., 2000, Frulio et al., 2014]. Mostly , these adenomas are of the H-HCA and IHCA subtypes[Frulio et al., 2014]. The nodules in hepatocellular adenomatosis are often of the same subtypes. Although one might assume that multiple HCAs increase the propensity for lesional bleeding, previous data have shown no significant difference in macroscopic bleeding between single and multiple HCAs ($p<0.155$) [Dokmak et al., 2009].According to literature there seems no indication to suggest that the risk of malignant transformation is increased in hepatic adenomatosis compared with solitary HCAs[Barthelmes et al., 2005]. However, hepatic adenomatosis are more often found in glycogen storage disease and in man[Chiche et al., 2000],and as such at risk for increased malignant potential. Presently, there is no systematic review available which evaluates the malignant potential of hepatic adenomatosis.As for clinical management, and there is no data suggesting that hepatic adenomatis should be treated differently from solitary HCAs.

Biopsy in the management of HCA

Since the introduction of the HCA subclassification system, several authors have attempted to further refine the diagnostic work-up using additional techniques, including immunohistochemistry [Zucman-Rossi et al., 2006]. The primary motivation for the introduction of additional biopsies was the prospect of identifying HCAs with greater malignant potential (such as exon-3 b-catenin mutated HCAs). There seem to be no unique MRI features with which to assign a b-HCA subtype risk, which offers one argument for the expansion of the use of diagnostic biopsy in order to arrive at a correct diagnosis.

However, at present there is no consensus regarding the diagnostic work-up of HCA [Nault et al., 2013, Marrero et al., 2014]. Nault et al. regard histologic analysis as the backbone of HCA diagnosis, with the detection or exclusion of b-HCA as the main input [Nault et al., 2013]. They argue that biopsy should be offered in all cases of HCA smaller than 5 cm with no typical MRI sign of H-HCA. Lesions larger than 5

cm do not require biopsy since they are all preferably resected. In our opinion, and in accordance with recent American College of Gastroenterology guidelines for liver lesions, the diagnostic work-up of suspected HCA should be based primarily on MRI findings, with biopsy in cases where the lesion cannot be clearly differentiated from FNH [Marrero et al., 2014]. Other indications for biopsy are an atypical presentation of the HCA on imaging, with the main differential diagnosis being HCC in a non-cirrhotic liver [Marrero et al., 2014].

The biopsy of all HCAs (with the exclusion of typical H-HCAs based on MRI) found by imaging would be impractical. Most patients with HCA are young, with minor symptoms on malignant progression; invasive procedures should preferably be avoided. While the risk of bleeding complications is very low when using an 18G core needle biopsy (0.6%) [Haage et al., 1999, Kadri Aribas et al., 2010, Aribas et al., 2012], the risks are not negligible, and deaths due to bleeding complications have been reported [Stattaus et al., 2007]. In our practice a biopsy has not been performed to date, except where the diagnosis of a specific adenoma subtype was expected to alter clinical management.

Unquestionably, a biopsy for further characterization may add important information in well-defined cases. For example, a biopsy with the additional help of immunostaining could facilitate better discrimination between HCA and FNH, as shown in a large retrospective study in France where the investigators compared biopsies against a final diagnosis based on surgical resection [Bioulac-Sage et al., 2012]. A total of 239 needle biopsies were compared with the final diagnosis made on resection. A difference in sensitivity of 74.3% with immunostaining versus 58.6% achieved with routine analyses without GS or other molecular features was reported [Bioulac-Sage et al., 2012]. These data suggest that immunostaining should be made available in centers that routinely treat HCAs.

What is the best approach in cases where differentiation between HCA and HCC is not evident based on MRI? In cases where there is a major suspicion of malignancy (e.g. HCC in non-cirrhotic liver) based on a combination of clinical findings, size of the lesion, increased serum alpha-fetoprotein, and MRI findings (such as heterogeneous presentation with heterogeneous enhancement, wash-out, and true capsula) (figure 2), resection without prior biopsy can be recommended. Although biopsy of each suspect lesion would undoubtedly help in detecting HCC, this approach may be impractical due to the significant level of false-negative findings, the chances of seeding (figure 3), and the enhanced risk of bleeding, which is particularly relevant when multiple biopsies are taken. Furthermore, in cases with a typical presentation, a biopsy will not influence the decision to remove the lesion. Therefore, we suggest to biopsy in selected cases only. Interestingly, the HCC literature documents a similar debate on whether it is acceptable to diagnose HCC in a cirrhotic liver based

solely on MRI findings, or whether the use of routine biopsies should be advocated for all suspected lesions in patients with liver cirrhosis [Sherman et al., 2015, Torbenson et al., 2015]. Even in high grade dysplastic nodes, follow-up by imaging is still preferred above biopsies.

As for daily practice, we recommend biopsy only in very selected cases where HCA cannot be differentiated from FNH with any imaging modality. The clinical repercussion of a wrong diagnosis of either HCA or FNH can have a large influence on a patient's future in terms of treatment and prognosis. When there are signs of malignancy patients should preferentially be forwarded to an experienced referral center for further evaluation. One should be aware that in some cases MRI or biopsy will be unable to differentiate between HCA and well differentiated HCC.

Treatment options for HCA

Historically, HCAs were treated with a wait-and-see policy, with surgical intervention preferred for larger (>5cm) growing tumors. Current management options for HCAs may also include RFA, and TAE, mainly due to the advantages of these minimally invasive techniques. In the following section we will discuss routine, as well as less commonly used HCA treatment options, with a focus on minimally invasive, image-guided, treatment options.

Conservative treatment

When HCAs are smaller than 5 cm, or regress (to < 5 cm) following cessation of OCs, with no further growth detected, a wait-and-see policy is warranted. Although no widely accepted approach has yet been published, we prefer to schedule a patient for follow-up, including MRI, or ultrasound in a yearly follow up until menopause.

Surgery

Surgery has long been considered the treatment of choice because complete surgical removal of the lesion can be achieved in a controlled and relatively safe manner. Elective surgical resection is considered for all lesions greater than 5 cm in diameter. With a mortality of 1.1% (review by Lin et al., n = 170), surgery is a relatively safe procedure. In one review, 93% of patients with ruptured, or non-ruptured HCAs, were primarily treated with surgery, with complications that included two deaths, one biloma, one bile leakage, one infection, and one case of sepsis [Lin et al., 2011]. In another, single-center retrospective analysis of 41 cases, no perioperative mortality was found, and only minor complications arose. These included pleural effusion requiring drainage (n=2), pneumonia (n=1), and wound infection (n=1) [Cho et al., 2008].

In the latter study nine cases were operated on laparoscopically, a technique that is increasingly popular, where appropriate. deÁngelo et al. described 62 HCA patients who underwent either an open procedure or laparoscopy [De'angelis et al., 2014]. They found no difference in postoperative morbidity and zero mortality, with no long-term complications or recurrence of HCA. However, patients with smaller lesions were preferentially treated with laparoscopy (68 versus 9). These authors concluded that laparoscopic liver resections may be limited by lesion size and location, and that the technique requires advanced surgical skills. Given the precision required, robotic surgery may prove to be useful in the future, and could reduce complications; we await further evaluation of its efficacy [Jackson et al., 2015].

In rare circumstances, the treatment of HCA may also involve liver transplantation, a procedure described in a case report by Venarecci et al. [Vennarecci et al., 2013]. Obesity, steatosis, and diabetes, are frequent co-morbidities in patients with HCAs, particularly the inflammatory subtype. These factors, and especially obesity, make surgery less attractive. For those patients who are poor candidates for surgery (centrally located lesions, multiple adenomas, or morbid obesity), RFA and TAE may instead be offered.

Radiofrequency Ablation (RFA)

RFA is a minimally invasive technique used in the treatment of hepatocellular carcinoma (HCC), other liver lesions such as colorectal metastases [Solbiati et al., 2001, Cabibbo et al., 2013], and HCAs [Van Aalten et al., 2010, Van Vledder et al., 2011]. Laparoscopic RFA, or perioperative RFA, may also be considered when the anatomical location (i.e. close proximity to the bowel or gallbladder (Fig. 3)) leads to an increased risk using a percutaneous approach. The use of RFA in the treatment of HCAs has only been described in small case series.

Vledder et al. described one case series including 45 HCAs, in 18 patients, that were ablated in 32 RFA sessions (open, n=4; percutaneous, n=28) [Van Vledder et al., 2011]. Twenty-six of 45 HCAs were successfully treated in one RFA session, with no visible residual disease. A further 9 HCAs were totally ablated following a second RFA session. Three HCAs required 3 or more RFA sessions, with all but 7 of the 45 HCAs totally ablated after three or more sessions. The treated HCAs had a median size of 3.0 cm (ranging from 0.8 cm-7.3 cm). Only minor complications were attributable to the RFA procedure; none of which required additional intervention (class A according to the Society of Interventional Radiology scoring system for complications). A single class D major complication was reported; a cerebrovascular accident during open surgery combined with RFA. Though severe, this complication was linked to anesthesiological and hemodynamic changes during laparotomy, rather

than the RFA procedure. In conclusion, RFA can be effectively and safely used in the treatment of HCA, although multiple sessions may be required for larger lesions.

In a review of HCA cases reported between 1998-2008, Haoming Lin et al. identified 356 HCA patients in reports from China, Europe, North-America, and South-East Asia [Lin et al., 2011]. Only 14 (3.9%) of these cases were treated with RFA, and no severe complications were reported. However, no results in terms of efficacy were provided. In 2008, Rhim et al. assessed the therapeutic efficacy and safety of RFA for HCAs [Rhim et al., 2008], and reported their initial experience in 10 patients with 12 HCAs. Tumor sizes ranged from 1.5-4.5 cm. As no complications were reported after RFA, and no progression or recurrence was noted, RFA was considered a safe and effective treatment option. A minimal ablative margin of 5 mm is recommended during the radical treatment of lesions when using thermal ablation of HCCs. It is presently unclear if a similar margin should be applied to HCAs, as these lesions are assumed to be benign. No data are yet available regarding the ideal ablative margin during thermal ablation of HCAs. In our opinion, volume reduction is more important than an ablation margin, as the former correlates strongly with a risk of bleeding, and malignant transformation. Follow-up imaging after both RFA, and TAE, is routinely performed in our institution by MRI, six months after treatment.

As HCAs requiring treatment are generally large (>5 cm), a promising alternative for RFA may be microwave ablation (MWA). Based on preliminary data, MWA was shown to produce larger ablation zones, in less time, in patients treated for HCC and colorectal metastases. MWA delivers high frequency microwaves (0.9-2.4 GHz) into tumor tissue, which causes fast spinning of molecules and thus destroys tissue. No data are available concerning efficacy and complications after MWA. MWA has specific advantages over RFA, such as larger ablation zones, higher treatment temperatures, and less susceptibility to local cooling by adjacent large blood vessels (heatsink). Although larger zones of ablation can probably be achieved by using single-electrode needles and MWA to treat HCAs, to the best of our knowledge, no data currently exists to substantiate this idea.

Transcatheter Arterial Embolization (TAE)

As HCAs are hypervascular arterial lesions, bleeding may be treated by selective TAE in cases where patients present with hemodynamic instability. Embolization of HCAs is a safe but relatively challenging procedure due to multiple small feeding vessels [Van Aalten et al., 2010]. Nonetheless, in cases of spontaneous rupture and bleeding, TAE should be considered as a first line treatment as it is highly successful and minimally invasive in an acute setting. Although high success rates have been described for TAE, there is only a sparse literature comparing TAE with either surgery, or conservative management. In one study Karkar et al. described 52 patients with

100 HCAs, of which 37% were treated with TAE [Karkar et al., 2013]. In most of these cases TAE was performed in a (semi) elective setting, with rupture and hemorrhage as indications in 20%, and suspected malignancy in 56%. Success rates of up to 92% were claimed for TAE (32), and of the 37 HCAs embolized, only three required secondary interventions (8.1%). All other embolized lesions were treated successfully; some disappeared (5/34), most decreased in size (22/34), or remained stable (7/34). Recurrence rates were also low. It is worth noting that the HCAs embolized were relatively small, with a median diameter of 2.6 cm. However, we feel that resection is indicated if malignancy is suspected and no contra-indications for surgery exist. In a report by Erdogan et al. six HCAs were primarily embolized [Erdogan et al., 2007], four because of bleeding, and two electively, one year after bleeding. No complications were reported, and all HCAs ceased bleeding. Two of the lesions were resected after embolization, two regressed visibly on follow-up imaging, and two HCAs were only seen after resorption of hematoma on follow-up imaging. These last two patients were managed with a wait-and-see policy. In a retrospective study by Dheodar et al., seventeen embolizations where successfully performed in eight patients [Deodhar et al., 2011], with five patients undergoing more than one embolization. The mean size of the treated HCAs was 3.6 cm, and regression was noted in all embolized HCAs after embolization. As noted by Karkar et al., TAE may also be used in an elective setting where no acute intervention is needed [Karkar et al., 2013]. This approach is of clinical interest and deserves further consideration.

Proposed management strategy

One of the major discussions on the management strategy of hepatocellular adenomas involves the clinical application of these recent findings in the dynamic field of adenoma subtyping. How should we take into account these new insights into daily practice? In our view, more data are needed to implement this subclassification in the diagnosis and treatment of adenomas, balancing the risk of an invasive liver biopsy with the additional benefits in terms of individualized therapy and prognostic stratification. A major effort should be made by expert centers involved in the diagnosis and treatment of hepatocellular adenomas to work on this collaboratively, preferably in research setting, to gather more data on the potential benefits for an individual patient.

Based on our review of the current literature, we propose a management strategy applicable to most cases in which there is a suspicion of HCA (figure 5). This decision tree may not be appropriate for all patients; for some, a more customized approach may be required. In standard situations, mainly when a lesion is larger than 5 cm, oral contraceptives should be stopped and MRI performed after 6 months. If the lesion has contracted to less than 5 cm, clear signs of an H-HCA

should be ruled in or out (figure 1). In scenarios where H-HCAs are subsequently identified, therapy can be less aggressive as inherent malignant progression is very low. Follow-up is then advised, initially every 6 months, and if the lesion shows no further alteration, follow-up can be stopped or simply repeated yearly until menopause [Marrero et al., 2014]. Since typical H-HCAs are easily identified using out-of-phase MRI sequences, intravenous contrast can be obviated at follow-up. A second option is to apply sonography during follow-up which is cheaper and less inconvenient for patients. For small lesions (<5 cm) that are categorized as IHCA, therapy should ordinarily not be altered (in standard cases). However, some may

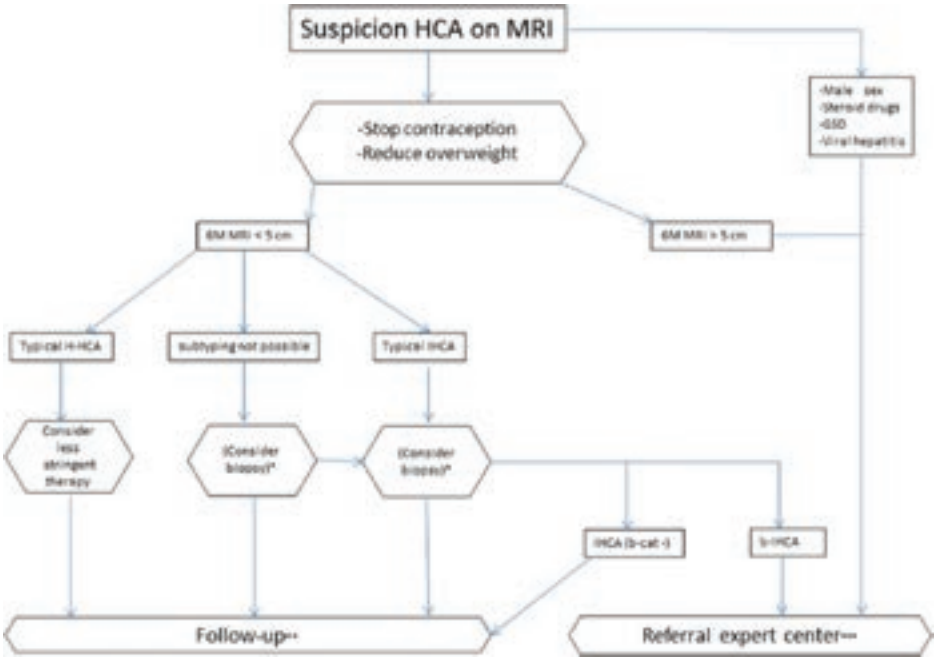

Figure 5. The management decision tree used in our tertiary academic medical center. This decision tree may not be appropriate for all patients; for some, a customized approach should be considered.
*One option is to biopsy those lesions where a subtyping diagnosis by imaging is impossible to achieve, or those lesions with typical signs of IHCA. Currently, this option is considered impractical given the large number of biopsies involved.
**Follow up is advised initially, at 6-monthly intervals. Thereafter, if the lesion shows no further alteration, follow up can be stopped, or repeated yearly until menopause. If the lesion is a typical H-HCA, follow up can be less stringent, possibly involving sonography, or MRI without contrast.
***Referral to an expert center is advised for the evaluation of any indication requiring intervention. This decision should be taken with consideration of contraindications (obesity, diabetes, centrally located tumor, ASA classification). Treatment can be primarily surgical, and in selected cases, RFA or local embolization.
b-HCA,β-catenin activated HCA; GSD, glycogen storage disease; HCA, hepatocellular adenoma; H-HCA, HNF1A-mutated HCA; IHCA, inflammatory HCA; M, months; RFA, radiofrequency ablation.

opt for a biopsy in order to exclude b-catenin mutation. This could also be the case if a subclassification cannot be made with MRI. For larger lesions (>5cm) with a b-catenin mutation or if the patient has an aggravating status such as male sex, steroid use, glycogen storage disease, or underlying viral hepatitis, intervention may be the first alternative. Treatment can be primarily surgical, and, in selected cases, RFA or TAE may be used. Depending on the underlying risks (obesity, diabetes, centrally located tumor), the best option would be to evaluate these patients in an expert referral center.

CONCLUSIONS

MRI is the preferred tool in the management of HCA, its current principle use being size evaluation (cut-off 5 cm), identification of signs of malignancy, and exclusion of H-HCAs, recognized for their benign course and permitting a conservative approach. Until now, there is no reliable MRI characteristic to diagnose non-invasively b-HCA, being the most important lesion to diagnose as it may have the highest malignant potential .

Conservative management remains the strategy of choice for uncomplicated small HCAs, and surgery may be indicated if imaging shows heterogeneous signal and growing smaller lesions suspected of being highly differentiated HCC. Further prospective cohort studies are warranted to support the choices made between these treatment strategies and to determine the role of biopsy in the subclassification of HCAs. In cases where a HCA requires treatment, and surgical resection of smaller lesions (<3cm) carries an unacceptable risk, RFA or TAE may be considered.

CHAPTER 8

Genotype-phenotype correlations in hepatocellular adenoma: an update of MRI findings

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ABSTRACT

Hepatocellular adenoma (HCA) is a generally benign liver tumor with the potential for malignancy and bleeding. HCAs are categorized into four subtypes on the basis of genetic and pathological features: hepatocyte nuclear factor 1α-mutated HCA, β-catenin-mutated HCA, inflammatory HCA, and unclassified HCA. Magnetic resonance imaging (MRI) plays an important role in the diagnosis, subtype characterization, and detection of HCA complications; it is also used to differentiate HCA from focal nodular hyperplasia. In this review, we present an overview of the genetic abnormalities, oncogenesis, and typical and atypical MRI findings of specific subtypes of HCA using contrast-enhanced MRI with or without hepatobiliary contrast agents (gadobenate dimeglumine and gadoxetate disodium). We also discuss their different management implications after diagnosis.

Hepatocellular adenoma (HCA) is a rare, benign tumor of the liver that occurs predominantly in young and middle-aged women (1). In contrast to focal nodular hyperplasia (FNH), HCA may involve complications, such as a life-threatening bleeding and malignant degeneration (1–3). The strong association between the occurrence of HCA and the use of oral contraceptives was first acknowledged in 1970s (4), and the incidence of HCA is now thought to be 30 times greater in oral contraceptive users compared to nonusers (5, 6). A dose-dependent association and spontaneous regression following the withdrawal of estrogens have also been described (4, 7). However, the exact role of estrogen in HCA is still poorly understood.

In this review, we present an overview of the typical and atypical magnetic resonance imaging (MRI) findings of different HCAs compared to FNH, and discuss various pitfalls that may be encountered with MRI.

THE NEW CLASSIFICATION OF HEPATOCELLULAR ADENOMA

A molecular and immunohistochemical classification of HCA has been introduced by the Bordeaux group (Table 1) (8, 9); in this classification, HCAs are divided into four subgroups based on clear genetic differences.

The first group accounts for 30% to 40% of cases and is defined by the presence of hepatocyte nuclear factor 1α (HNF1A) mutations (10). The HNF1A gene controls lipid metabolism and mediates the downregulation of liver fatty acid

Table 1. Immunohistochemical and MRI signs used for differentiating the HCA subtypes

	HNF1A-mutated HCA	β-catenin-mutated HCA	Inflammatory HCA[a]	Unclassified HCA
Immunohistochemical staining				
Glutamine synthetase[b]	–	+/–	+/–	–
β-catenin	–	+	+/–	–
C-reactive protein	–	–	+	–
Serum amyloid A	–	–	+	–
LFABP	–	+	+	+
Typical MRI findings[c]	Diffuse homogenous lesional steatosis	Faint scar strong, diffuse,	Atoll sign and hyperintense signal on T2-weighting	

[a]Inflammatory HCAs may show β-catenin positivity, in these cases most lesions may also show homogenous glutamine synthetase staining.
[b]In exceptional cases, glutamine synthetase can be normal in β-catenin-mutated HCAs.
[c]The MRI signs are preliminary and based on three recent papers (13, 21, 23).
HCA, hepatocellular adenoma; HNF1A, hepatocyte nuclear factor 1α; MRI, magnetic resonance imaging; LFABP, liver fatty acid binding protein.

binding protein (LFABP). LFABP downregulation is typically observed using LFABP staining, which is 100% accurate (8, 11). The most typical presentation of group 1 (i.e., HNF1A-mutated) HCA lesions is the aberrant presence of internal steatosis. It should be noted, however, that internal steatosis is not sufficient for diagnosing this HCA subtype, as other subtypes may also exhibit internal steatosis (12). We prefer to avoid the term steatotic HCA, which is used in some literature on this particular subtype (13). Some patients with HNF1A-mutated HCA have an associated mutation that is thought to be responsible for maturity-onset noninsulin-dependent diabetes (14). Therefore, once this HCA subtype has been diagnosed, the clinician should be warned of the possibility of underlying diabetes.

A second group containing 10% to 15% of cases is identified by the presence of activating mutations of β-catenin (15). While β-catenin is phosphorylated and degraded by proteasomes under physiological conditions, tumors fail to downregulate β-catenin and instead show nuclear accumulation of the protein (16). This accumulation is known to trigger an important signaling pathway in several cancers but is typified by hepatocellular carcinoma (17). Although β-catenin activation is not sensitive enough for immunohistochemical classification due to its visibility in only a few sporadic nuclei (12), another product of the same β-catenin. Therefore, it has been stated that these cases could be at increased risk of malignant degeneration (12).

The final group accounts for 10% to 25% of HCA cases and shows no specific genetic alterations; this group is therefore currently referred to as "unclassified" (16).

IMPLICATIONS FOR DIAGNOSIS

While immunohistochemical staining of LFABP, β-catenin, glutamine synthetase, serum amyloid A, and C-reactive protein has proven to be very effective in differentiating between the four subtypes of HCA, it is also useful in the differentiation between HCA and FNH (9, 20). In a retrospective, multicenter study in France, Bioulac-Sage et al. (20) found that the certainty of biopsy diagnosis of FNH increased from 53% to 87% when additional immunohistochemistry markers and glutamine synthetase immunostaining were used. The certainty of biopsy diagnosis of HCA also increased from 59% to 74% when immunohistochemistry analyses were used. Prior to the introduction of these markers, HCA was often misdiagnosed as FNH during histological examination, especially when inflammatory HCA (formerly known as telangiectatic FNH) was involved (19). The latter lesions include the bile duct proliferation seen in FNH but demonstrate the behavior of a HCA (including the previously described risk of malignant degeneration and bleeding). This confusion should be taken into

account when evaluating older radiologic descriptions of HCA and FNH, as the reference standard has only become significantly more accurate since the introduction of these markers.

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Conventional MRI findings for hepatocellular adenoma

HCA is primarily diagnosed by noninvasive imaging techniques (1), and typical MRI characteristics can be used for differentiating between HCA and FNH. According to recent literature, some MRI findings are more typical than others (Table 2).

Table 2. Classical MRI signs that can be used for differentiating HCAs from FNHs

HCA	FNH
Strong signs	Strong signs
Strong hyperintensity on T2-weighting	Spoke wheel appearance of scar
Hyperintensity on T1-weighting	
Cystic parts	
Hemorrhagic parts	
Diffuse intralesional steatosis	
Atoll sign	
Weak signs	Weak signs
Faint arterial enhancement	Scar
Liver steatosis	Lobular contours
Multiple lesions	Strong arterial enhancement

We distinguish strong signs from weak signs. Strong signs are defined to be characteristic for that lesion. Weak signs are more common in either HCA of FNH, but can occur in both.
FNH, focal nodular hyperplasia; HCA, hepatocellular adenoma; MRI, magnetic resonance imaging.

T1-weighted hyperintensity seems to be prevalent only in HCAs and is most likely caused by blood degeneration products or glycogen storage (21–23).

Other signs of non-neoplastic degeneration can be appreciated in some HCAs through the visualization of internal bleeding cysts, necrosis or fluid. To the best of our knowledge, these finding have not been described for FNH (2). A strong T2-weighted hyperintense band in peripheral areas of the lesion is a typical sign of HCA and was only (possibly) visible in one FNH case in our series to date (see below) (21).

The finding of a central scar is a more commonly described imaging characteristic of FNH (2). However, we also found linear central scars in 21% of confirmed HCAs (21). This sign, which is characterized by a T2-weighted central scar with late enhancement on delayed phase, does not seem to be sufficiently robust to allow differentiation between these two lesions. Additionally, β-catenin-mutated HCAs appear to have a faint central scar in up to 75% of cases (Fig. 1) (21). On the other hand, in our experience a typical 'spoke wheel' appearance of a central scar is only visible in FNH (Fig. 2) (2).

The surrounding liver steatosis, intralesional fat accumulation, faint arterial enhancement pattern and presence of multiple lesions are more or less typical for HCA but can also occur in FNH (21–23). A lobular border is often described as a typical sign for the diagnosis of FNH, but our research group and others have found that the occurrence of a lobular border is not uncommon in HCAs (21, 22).

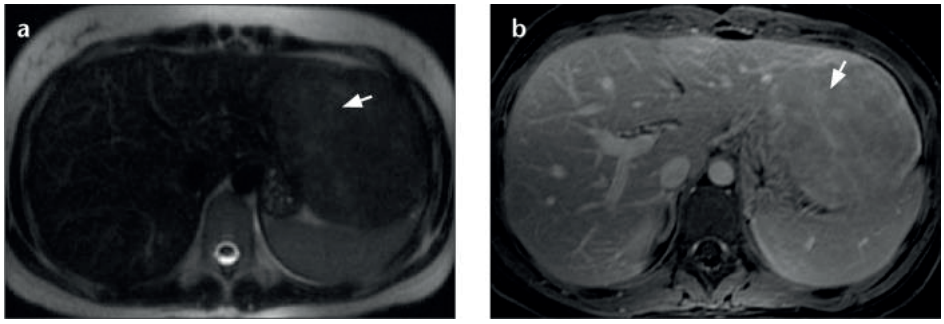

Figure 1a/b. Axial T2-weighted (a) and T1-weighted (b) MR images of the liver after contrast injection. Histologically,β-catenin staining is positive in this patient. The faint scar-like region is T2 hyperintense (a, arrow) with late enhancement after contrast injection of a nonspecific gadolinium-based contrast agent (b, arrow), a finding that is similar to that expected in focal nodular hyperplasia (FNH). In our opinion, a lesion with a scar but lacking a "spoke wheel" aspect is not only typical of FNH, but may also occur in hepatocellular adenoma (HCA) and more typically in β-catenin-mutated HCA.

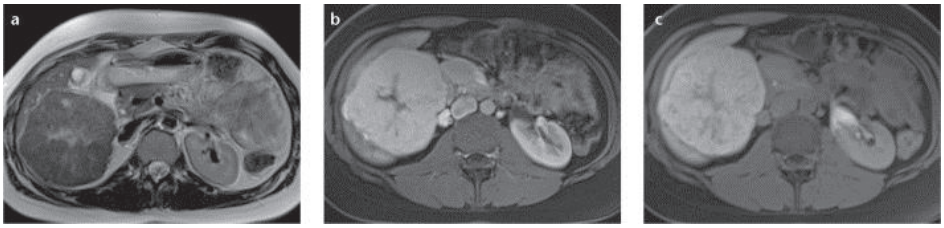

Figure 2a/b/c. Axial T2-weighted (a), venous phase T1-weighted (b), and 20-min T1-weighted (c) MR images of a patient with the "spoke wheel" aspect that is typical for FNH. A central scar with divergences to the periphery is visible on T2-weighting and is reminiscent of a "spoke wheel" (a). These so-called spokes are normally enhanced in the venous phase using a nonspecific contrast agent or gadobenate dimeglumine. However, when using gadoxetate disodium, the central scar and the spokes are hypointense due to pseudo washout (b). After 20 min, the majority of the lesion (except the scar) becomes hyperintense due to internal bile duct proliferation (c).

MRI FINDINGS BASED ON THE NEW SUBCLASSIFICATION

Laumonier et al. (23) were the first to publish the typical MRI features of HCA according to the subgroup classification. A homogeneous dropout of signal on the T1-weighted out-ofphase sequence had a sensitivity of 86.7% and a specificity of 100% for HNF1A-mutated HCA (Fig. 3), whereas this dropout was absent or only focal (heterogeneous) in inflammatory HCA. Moreover, marked hyperinten sity on T2-weighted sequences was found to be typical for inflammatory HCA, with a sensitivity of 85.2% and a specificity of 87.5%.

We found similar presentations for HNF1A-mutated HCA and inflammatory HCA (21). Additionally, we showed that a hyperintense rim on the T2-weighted sequences was diagnostic for inflammatory HCA. This hyperintense rim sign corresponds to si-

nusoidal dilatation (Fig. 4) and is also referred to as an "atoll sign". This characteristic includes a hyperintense rim in the periphery of the lesion on T2-weighted imaging with an isointensity in the center of the lesion reminiscent of the sea within an atoll (24). Small intralesional T2-hyperintense nodules can be found in the center of the lesion (small islands) (21).

Several authors have reported that a faint scar may be a possible sign of β-catenin-mutated HCA (21, 23, 25), but the number of published β-catenin-mutated HCA cases to date is too low to draw any firm conclusions.

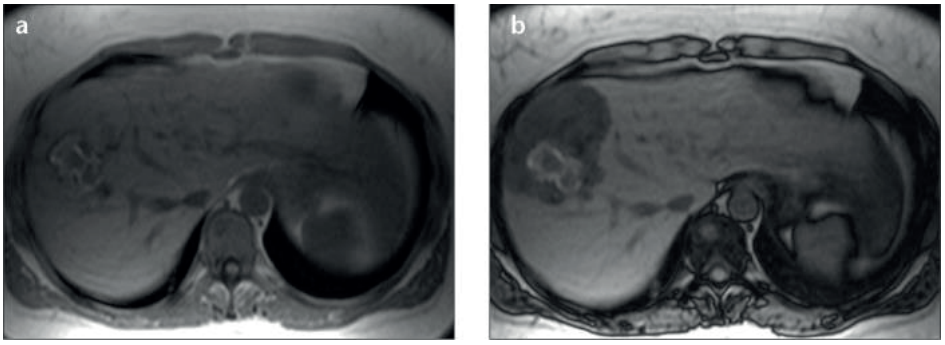

Figure 3a/b. Axial T1-weighted in-phase (a) and opposed-phase (b) MR images of a hepatocyte nuclear factor 1α (HNF1A)-mutated HCA. This lesion shows the typical diffuse and homogeneous suppression of signal in the lesion due to fat accumulation. Also note the blood residue centrally located in the lesion as a T1-weighted hyperintense zone. In addition to steatosis, this lesion showed no liver fatty acid binding protein staining upon histology, which is a very sensitive marker for HNF1A mutation.

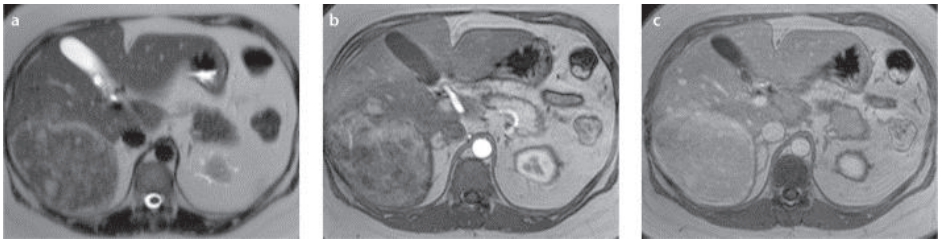

Figure 4a/b/c. Axial T2-weighted (a), arterial (b), and venous (c) T1-weighted MR images of the liver with a typical presentation of an inflammatory HCA. The lesion presents with an "atoll sign" (a), which appears as a T2-weighted hyperintense rim (peripheral island with central sea) with or without central hyperintense islands as can be found inside an atoll. The lesion is hypervascular in the arterial phase (b), with late enhancement of the peripheral rim and central islands (c). Upon histology, these lesions demonstrate positive immunostaining of inflammatory proteins including C-reactive protein and serum amyloid A. The T2-weighted hyperintense rim is thought to be caused by local peliosis.

MRI FINDINGS AFTER USING LIVER-SPECIFIC CONTRAST AGENTS

With the introduction of hepatobiliary contrast agents, an important tool became available for differentiating HCA from FNH (26).

Two gadolinium-based contrast agents are currently available, gadobenate dimeglumine (Multihance, Milan, Italy) and gadoxetate disodium (Primovist, Berlin, Germany; brand name in the USA, Eovist).

Both agents show hepatocyte uptake and biliary excretion, with a hyperintense liver in the hepatocyte phase on T1-weighted imaging as a conse quence. Lesions that involve bile ducts also appear to enhance. This observation is typically the case in FNHs and in some hepatocellular carcinomas (26). The most important finding is that HCAs do not normally show hepatocyte uptake and biliary excretion and are therefore observed as hypointense compared to the liver in the hepatocyte phase.

There are strong arguments for the uptake of gadoxetate disodium in FNHs and some hepatocellular carcinomas by organic anion transporter polypeptide channels (25, 27, 28). However, one study suggested another mechanism of uptake of both gadobenate dimeglumine and gadoxetate disodium in the liver (29).

Yoneda et al. (27) found that organic anion transporter polypeptide-8 is present in the periphery and not in the center of FNH, explaining the peculiar aspect of some FNH cases in the hepatocyte phase (ring-enhancement-type).

Gadobenate dimeglumine is excret ed by the liver at a significantly lower percentage (5%) than gadoxetate disodium (50%) (26). As a result, gadoxetate disodium produces a significantly greater signal intensity change in the hepatocyte phase than gadobenate dimeglumine. This greater change can be helpful in cases where liver activity and thus changes in enhancement are low, as is observed in cirrhosis. One cause of this lower liver enhancement is the competitive uptake between these contrast agents and bilirubin, with uptake of the latter increased in patients with liver cirrhosis. Additionally, lesions that have an intrinsic T1-weighted hyperintensity, as frequently observed in HCAs, may often show insufficient liver enhancement when using gadobenate dimeglumine. This insufficient enhancement results in the hyperintense HCA becoming isointense instead of hypointense compared to the surrounding liver (30).

Whether there is a difference in uptake of hepatobiliary contrast agents between the different HCA subtypes is still largely unknown. Our recently reported initial results noted that inflammatory HCAs range from isointense to hyperintense in relation to the liver in the hepatocyte phase (Fig. 5) (30). However, the underlying cause of this finding was not obvious. Of particular interest is the fact that, in contrast to other HCAs, inflammatory HCA often harbors internal bile ducts, which could possibly explain the late isointensity in the hepatocyte phase. However, this phenomenon is difficult to discern from isointensity due to intrinsic hyperintensity prior to contrast.

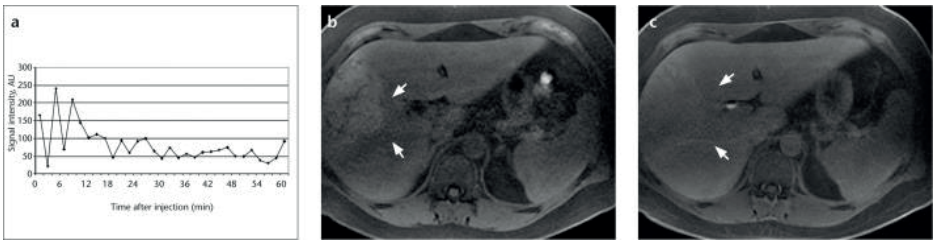

Figure 5a/b/c. Enhancement curve (a) of a known inflammatory HCA that was scanned every 2.5 min over a period of one hour following the injection of gadobenate dimeglumine. This approach allows an enhancement curve that shows no late enhancement to be reproduced. Comparison of axial fat-saturated T1-weighted sequences prior to contrast injection (b) and one hour after contrast injection (c) reveals that the lesion remains isointense (arrows) due to intrinsic hyperintensity

Implications for patient care

Though the use of either gadobenate dimeglumine or gadoxetate disodium may often be indicated, it can be difficult for a radiologist to choose between these two contrast agents. The major differences are listed in Table 3.

Table 3. Comparison of the hepatobiliary contrast agents gadobenate dimeglumine and gadoxetate disodium for liver imaging

	Gadobenate dimeglumine	Gadoxetate disodium
T1 effect	+++	+
Cost	+	++
Shortness of investigation	+	++(+)
Differentiation of HCA from FNH	+	+
Differentiation of benign liver lesions (hemangiomas)	++	– (+)
Hepatobiliary excretion	+	+++

FNH, focal nodular hyperplasia; HCA, hepatocellular adenoma.

As previously noted by others, gadoxetate disodium may be preferred over gadobenate dimeglumine when considering the rapid hepatobiliary phase at 20 minutes or earlier, whereas in patients scanned with gadobenate dimeglumine, the hepatobiliary phase is obtained with a second MRI performed a minimum of one hour after the initial MRI and injection of contrast agent (24). However, it is important to note that the early hepatobiliary excretion of gadoxetate disodium prevents the dynamic phase from being separated from the hepatobiliary phase, which results in the disturbing interpretation of the dynamic phase in cases of suspected hemangiomas. Additionally, when using gadoxetate disodium, a large number of liver lesions become hypointense in the late dynamic phase due to pseudo washout rather than actual washout (due to enhancement of the surrounding liver). Finally, radiologists

should be aware that because of this pseudo washout, the central scar in FNH may become hypointense instead of hyperintense in the late venous phase when using gadoxetate disodium.

Another argument in favor of gadobenate dimeglumine over gadoxetate disodium is the considerably lower cost of the former. This difference is most likely due to the wider range of possible indications for the use of gadobenate dimeglumine, which appears to be a useful, nonspecific gadolinium-based contrast agent. With the less pronounced T1-effect of gadoxetate disodium, this agent is indicated for specific liver imaging only.

In the case of an atypical liver lesion where the differential diagnosis is broad and includes hemangioma and malignant tumors, a nonspecific contrast agent or gadobenate dimeglumine is generally preferred over gadoxetate disodium. In cases where MRI is prescribed solely for the differentiation between FNH and HCA, gadoxetate disodium should be sufficient, which is often the case when preliminary external computed tomography or MRI data are present.

Although contrast-enhanced ultrasonography is beyond the scope of this review, it may be speculated that in the near future differentiation between the different HCA subtypes may also be possible with the use of contrast sonography (31).

Additionally, other markers may come to play a more prominent role in the differentiation of HCA from FNH or hepatocellular carcinomas (32, 33).

MALIGNANT DEGENERATION OF HEPATOCELLULAR ADENOMA

Malignant degeneration of HCA has been reported but seems to occur very rarely. In a recent meta-analysis, a total of 1635 HCA cases were examined and yielded an overall malignant transformation frequency of 4.2%. Most of those lesions were larger than 5 cm in diameter. However, the described overall frequency is most likely an overestimation due to the limited sample sizes of the studies and the fact that most studies only described resected HCA (29).

Furthermore, it is interesting that these so-called malignant degenerated HCAs show a pattern of a nodule within a nodule or two tumors lying adjacent to each other (28, 30). In this situation, it might be questioned whether these nodules have undergone the same cellular changes.

The malignant potential of HCA seems stronger in β-catenin-mutated HCA, which is also more prevalent in men (15). Bioulac-Sage et al. (15) reported the occurrence of six hepatocellular carcinomas in 128 proven cases of HCA, all of which were β-catenin-mutated HCAs, whether they were inflammatory or not. Management of these β-catenin-mutated HCAs can vary between hospitals from conservative to

aggressive (surgical). Additional experiences from more hospitals are needed to correlate the classification system with clinical management (28).

INTERNAL BLEEDING IN HEPATOCELLULAR ADENOMA

Rupture and bleeding have both been described in HCA; the hypervascular nature of these lesions may make them more prone to bleeding. In contrast, FNHs do not demonstrate rupture or bleeding despite their hypervascularity. In a meta-analysis, we determined a HCA rupture prevalence of 16% (3). However, the papers studied did show some evidence of selection bias, as patients with silent HCAs were underreported due to the lack of any indication for imaging in most of these patients. The meta-analysis showed that larger lesions (larger than 5 cm) are more often involved. There also seems to be no difference in the occurrence of internal bleeding depending on HCA subtype. After analyzing their own database, Bioulac-Sage et al. (15) also found no difference in the chance of macroscopic bleeding between HNF1A-mutated HCA and inflammatory HCA, the two most prevalent subtypes (Fig. 4).

As the actual risk of bleeding is reported to be higher in lesions larger than 5 cm, resection of these lesions may be warranted. Decisions related to the treatment of smaller HCAs are still complex and other characteristics, such as male gender, β-catenin positivity, and the wish to become pregnant, can also play an important role. Larger studies will need to be performed to evaluate whether there is a correlation between the subtype and bleeding and rupture. Pregnant patients with HCA deserve special attention, as maternal and fetal mortality rates are not negligible (34–36). We advocate that patients with a HCA larger than 5 cm be treated before becoming pregnant. In cases where a HCA larger than 5 cm is found during pregnancy, the data are currently too sparse to draw firm conclusions, and we believe that the management in these patients should be individualized (36, 37).

Hepatocellular adenoma in males

HCA mainly occurs in women in their second and third decades of life (5), and the incidence in men is very low. Most described cases of HCA in men occurred after the chronic intake of exogenous hormones (38) or in men with glycogen storage disease (39). Because of its low incidence, its diagnosis should be questioned, and we advise that a biopsy be performed more often when these tumors are found in men to exclude the presence of a premalignant or malignant liver tumor.

CONCLUSION

In summary, advances in recent years have greatly improved our understanding of HCA, leading to subtype recognition and better differentiation from FNH. Reports and conclusions drawn from MRI data in older studies, particularly prior to the introduction of glutamine synthetase staining, should therefore be evaluated with caution.

HCA diagnosis has been improved by additional experiences with atypical findings and potential pitfalls that may be encountered with MRI. The introduction of hepatobiliary contrast agents has helped even more, particularly in differentiating between HCA and FNA, and these agents have now become valuable tools in daily clinical practice. However, the radiologist must always be aware of possible errors in diagnosis due to differences between hepatobiliary and nonspecific gadolinium-based contrast agents. With respect to the treatment of HCA, a number of recommendations can be made; however, other questions require further studies.

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CHAPTER 9

The predictive value of GP73 in differentiating between solid benign and malignant liver tumours.

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ABSTRACT

Introduction

In the work up of primary solid liver lesions it is essential to differentiate correctly between benign and malignant tumors, such as hepatocellular adenoma (HCA) and hepatocellular carcinoma (HCC) respectively. A promising new marker to detect HCC is Golgi Protein 73 (GP73). Studies comparing patients with HCC and cirrhosis with normal controls suggested that GP73 is specific for patients with HCC; however, patients with other liver tumors were not included. We therefore studied the predictive value of GP73 in differentiating between solid benign and malignant liver tumors.

Materials and methods

This study included 264 patients: 88 patients with HCC, 88 with hepatocellular adenoma (HCA), and 88 with focal nodal hyperplasia (FNH). A blood sample was collected from each patient to measure GP73 levels using a quantitative ELISA assay and differences in outcome between subgroups were compared. The receiver operating characteristic (ROC) curve, sensitivity and specificity of GP73 were calculated and compared to alpha-fetoprotein (AFP) levels.

Results

When comparing malignant and benign liver tumors the area under ROC was 0.701 and 0.912 for GP73 and AFP respectively. Test characteristics revealed a sensitivity of 60% for GP73 and 65% for AFP; in addition the specificity was 77% for GP73 and 96% for AFP.

Conclusion: Although the literature suggests that GP73 is a valuable serum marker in patients with HCC, the serum concentration may also be increased in patients with solid benign liver tumors. Therefore, a GP73 assay is less suitable for discriminating between primary malignant and benign tumors of the liver.

INTRODUCTION

Over the past 15 years ultrasound examination of the liver has increased in frequency [1,2]. Ultrasonography can be used to detect solid liver lesions in asymptomatic patients [3]. Unfortunately, such a finding may cause distress when additional characterization is unable to differentiate between a benign liver tumor, such as hepatocellular adenoma (HCA) and focal nodular hyperplasia (FNH), and a malignant tumor such as hepatocellular carcinoma (HCC). Even when more refined imaging technologies are used, such as contrast-MRI or contrast-enhanced ultrasound, a definitive diagnosis may be difficult to establish in such solid 'incidentaloma' in the liver [4], eventually leading to a liver biopsy.

Although in some cases it may be possible to differentiate between malignant and benign tumors using molecular markers, the low sensitivity of tests to detect HCC via serum alpha-fetoprotein (AFP) limits clinical decision making [5]. Therefore more accurate markers are needed. Golgi Protein 73 (GP73), also named Golgi phosphoprotein 2 (GOLPH2), was recently introduced as a potential new candidate to identify HCC. GP73 is a resident Golgi-specific membrane expressed by biliary epithelial cells and is enhanced in HCC cells [6].

Several studies have described GP73 as a HCC-specific marker. However, these studies mainly included patients with liver cirrhosis and/or healthy people as controls [7,8], and thus lack information on patients with other liver tumors such as HCA and FNH. We therefore determined whether GP73 can differentiate between solid benign and malignant liver tumors and whether GP73 has a predictive value if an unknown solid liver 'incidentaloma' is present.

MATERIALS AND METHODS

Study protocol was in conformity with the ethical guidelines of the 1975 Declaration of Helsinki and approved by the local Institutional Review Board and Ethical Committee from the Erasmus MC University. Oral informed consent was obtained from all patients, as was approved by the Institutional Review Board. The no-objection policy was approved as only one extra blood vial was collected during regular blood sampling and patients were informed prior to blood sampling. All patients visiting the out-patient department are informed before they visit the clinic that data generated from their visit can be used for scientific studies as we are an academic hospital. Patients can actively opt out when visiting the out-patient clinic. This no-objection procedure has been approved for all outpatient visits. The protocol was approved separately. Blood sampling and the purpose of it are discussed with the patients

during their visit at the outpatient clinic as, according to Dutch law, patients have to be informed for which purpose blood samples are taken.

Between July 2007 and October 2012 a total of 264 patients enrolled in this study: 88 patients with HCA, 88 patients with FNH and 88 patients with HCC.

Patients aged 18 years and older, with a proven diagnosis of hepatocellular carcinoma, hepatocellular adenoma or focal nodular dysplasia, were included. The diagnosis was based on histopathology (95 patients, 36%), and if histopathology was not available, on two imaging modalities (magnetic resonance imaging, computed tomography or contrast enhanced ultrasound). All patients had been discussed by our multidisciplinary tumor board committee. Patients were excluded if there was doubt about the diagnosis or if multiple types of tumor were present in the liver.

Data characteristics and a 10-ml blood sample were collected from each patient in the out-patient clinic of the Erasmus University Medical Center. Each blood sample was centrifuged and the serum aliquotted and stored at -80°C until tested.

Blood samples were blinded for analysis. Quantitative ELISA (Antibodies-online GmH, Germany, ABIN365730, intra-assay CV% less than 8%, inter-assay CV% less than 10%.) was performed to measure GP73 levels according to the manufacturer's instructions. A standard curve was run for each assay using six provided standards, measured in duplicate per ELISA.

The serum AFP level was also determined for each patient using the Elecsys AFP quantitative electrochemiluminescence immunoassay (Roche, Switzerland) and a value >10 µg/L was considered as an elevated level. The receiver operating characteristic (ROC) curve, sensitivity and specificity, and positive predictive value of GP73 were calculated and compared with those of AFP. To determine the optimal cut-off value for GP73, ROC was constructed using all possible cut-offs for each essay. The area under the curve (AUC) was constructed for both AFP and GP73, including 95% confidence intervals (CI). Approval was obtained from the medical ethics committee.

Our primary hypothesis was that GP73 was superior to the predictive value of AFP for the detection of HCC. A power analysis was conducted using a sensitivity of 85% for GP73 and 58% for AFP, with a specificity of 97% and 85%, respectively [9,10]. A minimum of 40 patients per group was needed for an alpha of 0.05 and a beta of 0.20. The number of patients was increased to the maximum number of wells on the ELISA plates (N=88).

Data analysis

Variables were compared using the t-test or a one-way ANOVA, whenever appropriate. Statistical significance was considered at a p-value < 0.05. All analyses were performed using the Statistical Package for the Social Sciences (SPSS) (IBM Corp. Released 2011. IBM SPSS Statistics for Windows, Version 20.0. Armonk, NY: IBM

Corp). Patient characteristics and treatment were compared using the t-test, the chi-square-test and the Fisher exact test whenever appropriate.

RESULTS

A total of 264 patients were enrolled in this study, including 88 patients with HCC, 88 with HCA, and 88 with FNH. The demographic and etiologic data of these patients are shown in Table 1. The percentage of males, age, body mass index (BMI), alanine aminotransferase (ALT), aspartate aminotransferase (AST) and gamma-glutamyl transferase (GGT) differed significantly between patients with HCC, HCA and FNH.

Table 1. Patient characteristics

	HCC N=88	HCA N=88	FNH N=88	P-value
Male gender (%)	62 (94%)	2 (3%)	2 (3%)	<0.001
Age (years)	63 (34-82)	40 (20-58)	38 (19-70)	<0.001
BMI (kg/m^2)	27 (17-39)	30 (19-62)	26 (16-37)	<0.001
AST (I/U)	82 (15-897)	30 (13-86)	26 (8-64)	<0.001
ALT (I/U)	61 (8-461)	33 (7-126)	26 (5-121)	<0.001
GGT (I/U)	304 (11-4570)	88 (8-802)	67 (11-372)	<0.001
Lesion size (mm)	65 (10-250)	61 (8-177)	54 (4-110)	0.178
HBV	16 (18%)	-	-	-
HCV	15 (17%)	-	-	-

Data are presented as median (range) unless other indicated
HCC: hepatocellular carcinoma, HCA: hepatocellular adenoma, FNH: focal nodular hyperplasia, BMI: body mass index, AST: aspartate aminotransferase, ALT: alanine aminotransferase, GGT HBV: hepatitis B virus, HCV: hepatitis C virus.

Table 2 shows the distribution of serum GP73 values (IU/l) in the different groups of patients. The mean serum concentration of GP73 was 47 IU/l in the HCC group, 21 IU/L in the HCA group and 17 IU/l in the FNH group (P<0.001). Within the HCC group, GP73 did not differ between patients with hepatitis compared with patients without hepatitis, at 47 and 48 IU/l respectively (p=0.51). The median serum concentration of AFP was 9184 Ug/L in the HCC group, 3 Ug/L in the HCA group and 3 Ug/L in the FNH group (P=0.001). The data are shown in Table 2 and Figure 1. In Figure 1 three outliers are depicted (1 HCA, 2 FNH). In the patient with the extreme (FNH) a biopsy was performed. In the two other patients the diagnosis was confirmed by two imaging modalities (magnetic resonance imaging, computed tomography or contrast enhanced ultrasound) in 2010. Follow-up did not reveal a HCC.

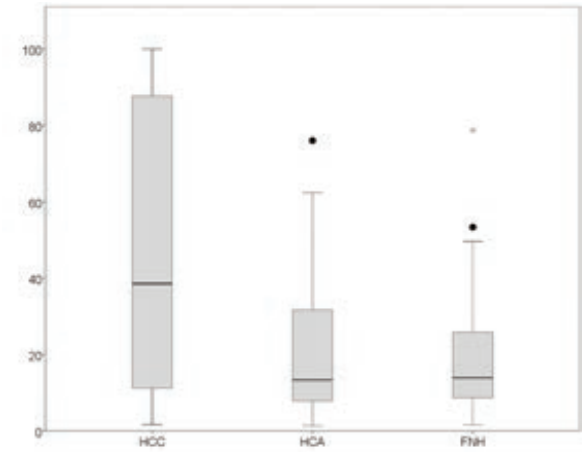

Figure 1. Boxplot GP73
Boxplots showing the serum GP73 levels in patients with hepatocellular carcinoma (HCC), hepatocellular adenoma (HCA) and focal nodular hyperplasia (FNH). • indicates the outliers and *the extreme.

Table 2. Biomarkers to differentiate between benign and malignant liver tumors

	HCC	HCA	FNH	P-value
GP73 (IU/ml)	39 (16-100)	13 (1.3-76)	14 (1.6-78)	<0.001
AFP (Ug/L)	9184 (1-212600)	3 (1-16)	3 (1-50)	0.001

Data are presented as median (range)
HCC: hepatocellular carcinoma, HCA: hepatocellular adenoma, FNH: focal nodular hyperplasia, GP73: Golgi Protein 73, AFP: alpha-fetoprotein

ROC curves

ROC curves were plotted to determine the optimal cut-off value for GP73 and to identify the sensitivity and specificity of GP73 and AFP in differentiating patients with malignant and benign solid liver tumors (HCC vs. HCA and FNH). The AUC for GP73 was 0.701 with a 95% CI of 0.625 to 0.776, and a sensitivity of 60% and specificity of 77 %, using a cut-off value of 29.2 IU/L. The positive predictive value (PPV) for GP73 was 56% and the accuracy of the test was 71%.

The AUC for AFP was 0.91 (95% CI of 0.871 to 0.943); with a cut-off value of 10 Ug/L, the sensitivity was 77% and the specificity was 96%. The PPV for AFP was 89% with an accuracy of 85%. Comparing the two ROC curves showed AFP to be superior to GP73 (p<0.001) (Fig. 2).

Using a cut-off value of 2.92 IU/L, GP73 was positive in 17 out of 31 AFP-negative HCC patients. AFP and GP73 were combined and were reported as positive if one out of two markers, AFP or GP73, was positive. The sensitivity and specificity of the combined marker was 84% and 73%, respectively (Table 3).

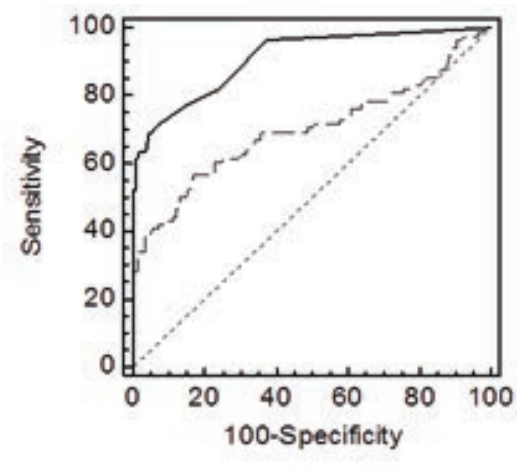

Figure 2. ROC-Curve GP73 and AFP
ROC curves comparing AFP (straight line) and GP73 (dashed line). Pairwise comparison of ROC curves (Hanley & McNeil, 1983) revealed a significant difference of P<0.0001

Table 3. Sensitivity and specificity of the biomarkers

	Sensitivity	Specificity
GP73	60%	77 %
AFP	77%	96%
AFP combined with GP73	84%	73%

GP73: Golgi Protein 73, AFP: alpha-fetoprotein

DISCUSSION

When a solid tumor of unknown origin is found in the liver an extensive diagnostic work-up is often necessary. In 10–40% of cases the final diagnosis remains unclear unless invasive techniques are used [4]. Therefore it is of utmost importance to find a serological marker, with a high sensitivity and specificity that is able to discriminate between benign and malignant solid liver tumors. Recent studies showed the potential of GP73 as a marker for HCC [7,10-12]. They suggested that GP73 might even be better than AFP [10]. This study showed the potential of GP73 to distinguish patients with HCC from patients with a solid benign liver tumor, HCA or FNH. However almost all serological data concerning GP73 and AFP in patients with HCC used patients with cirrhosis, hepatitis or no liver disease as controls [8,9,12-18].

It has been suggested that GP73 could be increased in liver disease due to viral causes (hepatitis B virus and hepatitis C virus) [19,20]. GP73 could even be associated with the progression of this liver disease [18]. However no significant difference was found between patients with HCC with hepatitis compared with patients without hepatitis. As there were no patients with hepatitis in the HCA or FNH group, this could not explain the elevated levels of GP73 in patients with HCA or FNH.

Riener et al. performed immunohistochemical staining of GP73 on tumor samples from HCC, as well as a small group of tumor samples of HCA and FNH, and found that GP73 is frequently expressed in samples of HCA and FNH [21]. In combination with our results, this suggests that GP73 is not a specific marker for HCC.

Two studies included serum from other focal liver lesions. Tian et al included 6 patients with FNH, in 3 out of 6 patients (50%) serum GP73 was elevated (14). Mao et al. suggested that GP73 might be a useful tool for discriminating benign from malignant liver tumors (9.) Although they only studied a small group of patients with benign liver tumors (hepatic cysts, FNH and hepatic cystadenoma), they also found an elevated serum GP73 in the group of patients with benign liver tumors [9]. Our study, which was conducted in a larger and better-defined group of patients with solid liver tumors, confirmed the significantly higher levels of GP73 in patients with HCC. However, analysis of AFP in our study population indicated that AFP is superior to GP73 for discriminating patients with HCC from patients with a solid benign liver tumor. The low number of patients with a false positive test result for AFP was particularly noticeable.

In recent studies evaluating the value of GP73, three types of assay were used: immunoblot assay, Western blot assay and ELISA. It has been suggested that GP73-specific antibodies might interfere with the ELISA analysis [7,22], as five studies that used ELISA found no significant elevation of GP73 when comparing the serum levels of HCC patients with their controls [14,15,17,23]. Immunoblot assay is too labor-intensive for large patient numbers [12], therefore ELISA is preferred. As we found a significant difference in GP73 between malignant and benign solid liver tumors, we believe that the use of ELISA is no longer an obstacle for the performance of large-scale studies.

Although the ELISA GP73 test is suitable we do not believe that further testing and development for unknown solid tumors in the liver will lead to better results in patients with a solid liver tumor of unknown origin. We do not expect GP73 to complement the results of AFP, as we studied a large and unique group of patients with benign and malignant solid liver tumors. Therefore imaging will continue to have an important place, next to AFP, in distinguishing benign from malignant liver tumors. If GP73 is further developed and analyzed to determine whether it is able to distinguish patients with hepatitis and cirrhosis from patients with HCC, one should take into account that GP73 is also elevated in benign liver tumors.

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CHAPTER 10

The Management of Pregnancy in Women with Hepatocellular Adenoma: A Plea for an Individualized Approach.

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ABSTRACT

Because of the risk of hormone induced growth and spontaneous rupture of hepatocellular adenoma (HCA) during pregnancy, special considerations are required. Due to the scarcity of cases there is no evidence-based algorithm for the evaluation and management of HCA during pregnancy. We think it should be questioned if it is justified to discourage pregnancy in all women with HCA. The biological behavior of this benign lesion might be less threatening than presumed and a negative advice concerning pregnancy has great impact on the lives of these young female patients. The balance between the pros and cons of hepatic adenomas and pregnancy should be reconsidered. In our centre pregnancy in women with a HCA up to 5 cm is no longer discouraged in close consultation with the patient, her partner and members of the liver expert team.

A strong association between hepatocellular adenoma (HCA) and the use of oral contraceptives (OC) was first described in 1973(1). The hypothesis that there is a relation between steroids and HCA has been supported by many authors but is still not understood.(2-4) Due to the increased levels of endogeneous hormone production, which may cause hormone induced growth and rupture, HCA requires special attention during pregnancy.(5, 6) Patients with a growing or ruptured HCA mostly present themselves with persistent or acute severe pain localized in the upper right quadrant and in the epigastric region. In the literature, the maternal and fetal mortality risks of ruptured HCA during pregnancy has been reported to be 44 and 38% respectively.(7) However, all these cases were published in the 1970s or 1980s, in which there might have been a delay in diagnosis as the entity of ruptured HCA was not well known and less advanced imaging methods were used.

In the recent years the widespread use of highly advanced image modalities has probably decreased the delay in the diagnosis of HCA and the associated maternal and fetal mortality significantly. Because of the unpredictable behavior of HCA during the increased levels of endogeneous hormones we used to advise women with a large HCA or a growing and hormone sensitive HCA to avoid pregnancy, as most other experts in this field do.(6, 8) Even if HCA were incidental findings previous to a pregnancy without having caused any complications, women were still advised not to get pregnant as long as the HCA is present. Because of the overall agreed advice to avoid pregnancy in patients with HCA, the diagnosis of HCA has severe impact on the lives of these young fertile women.

As to date there are limited data about the behavior of HCA during pregnancy and labor.

From the international literature between 1966 and 2003, Cobey et al. retrieved 26 cases of women presenting with HCA during pregnancy or early postpartum and proposed an algorithm for their diagnosis and management(7). Presentation was acute and often dramatic with rupture of the adenoma in 16 women, and frequently with a delay in establishing the correct diagnosis, with high maternal and fetal mortality (44% and 38%, respectively). The hormone induced growth and risk of rupture seemed to by the highest during the third trimester of pregnancy, most probably because of the cumulating level of oestrogens and an increase in hyperdynamic circulation combined with an increase in vascularity of the liver.(7) An aggressive approach towards resection of HCA was advocated, especially for those greater than 5 cm. Small adenomas were supposed to be managed by observation(7). It is important to realize that most of these reports were published in a time period during which this disease entity was relatively unknown and treatment in an emergency setting was less advanced.

In our hospital, we monitored 12 women with one or more documented HCA's during a total of 17 pregnancies. In four cases, HCA grew during pregnancy, requiring a Caesarean section in one patient (two pregnancies) and RFA in one patient during the first trimester of pregnancy because of significant growth of the adenoma. All pregnancies had an uneventful course with a successful maternal and fetal outcome(9). We concluded not to discourage all women with HCA from pregnancy. In our tertiary referral centre, we closely observe pregnant women with a HCA smaller than 5 cm in a clinical trial(10). In this study, the size of the lesion is an exclusion criterion when exceeding 5 cm, but the number of HCA's present in the liver is not. Three studies investigated the association between the risk of rupture and the number of HCA's(11). This risk did not differ between single and multiple HCA's(12-14). In our previous study, the number of HCA's in the women observed during pregnancy varied between 1 and more than 10 HCA's. We concluded that only in women with large tumours and a complicated pregnancy previously, pregnancy should be discouraged(9).

Furthermore, in our opinion, none of the subgroups from the molecular and pathological subtype classification of the Bordeaux group legitimizes objection against pregnancy. Although the number of cases described in literature is small no difference has been demonstrated in the risk of bleeding between the two major subgroups, the inflammatory and the hepatocyte nuclear factor 1α-inactivated HCA's(15, 16).

If women have large tumours or have experienced complications of HCA in previous pregnancies, an intervention (surgery, RFA, embolisation) should be recommended before pregnancy. Moreover, in 2006 we reported a series of 48 patients in which 44% of HCA were discovered after the patient had sustained at least one pregnancy(17).

Intervention during pregnancy may be associated with greater risk for both mother and child. The Society of American Gastrointestinal and Endoscopic Surgeons (SAGES) provided guidelines for diagnosis, treatment, and use of laparoscopy for surgical problems during pregnancy.(18, 19) In one in 635 pregnancies a non-obstetric operation, in particular appendectomy, cholecystectomy and adnexal procedures, is required during pregnancy (20). These guidelines suggest that the laparoscopic approach should be preferred in stead of laparotomy in most abdominal operations.

The maternal and fetal outcomes following abdominal surgery in pregnancy improved over last decade but the exact risk of HCA-related interventions during pregnancy to both mother and fetus is unknown(21). Abdominal surgery may be more difficult during pregnancy in the late second and third trimester because of the limited wideness in the upper abdomen due to the enlarged uterus and risk of steatotic changes of the liver in these patients. General anaesthesia seems to

have the least risk in the 2nd trimester of pregnancy(5). The role of RFA during pregnancy is not yet been studied extensively. In our previous study we described a RFA procedure during the first trimester of pregnancy(9) and a pregnant patient with a HCA which was treated by RFA during her second trimester of pregnancy (18th week of gestation) was reported by Fujita et al.(22). After systematically reviewing the literature Wilson et al. suggested that angioembolisation and formal resection in case of haemorrhage of HCA during pregnancy is safe for both the mother and the fetus with good clinical outcomes(23). We believe that selective arterial embolisation should only be used as a live-saving treatment in those cases where RFA or surgery are inadequate or too risky to control the bleeding adenoma. ,The increased risk of radiation exposure to the fetus, especially before 26 weeks of gestation(24, 25), should be avoided if possible.

Because HCA might have the tendency to rupture during delivery, some authors suggest a caesarean section (C-section). In our study three C-sections (two patients) were performed, without complications. In one case the C-section was performed in consultation with the patient because of marked growth and an unknown risk of rupture of the HCA's. In the other C-section was due to decelerations on the cardiotocography (9). All other patients had a normal delivery without complications. Therefore, in our opinion patients with HCA may deliver vaginally if there are no complicating factors, like perinatal problems.

In conclusion, it seems to be justified that a pregnancy should be discouraged in patients with a large HCA (> 5 cm) or those who experienced complications of the lesion in previous pregnancies (figure 1). In those case a surgical resection, RFA or embolisation should be recommended before pregnancy. In our centre we do not discourage pregnancy in women with a HCA <5cm (figure 2) if they accept the risk of interventions in case of growth of the adenoma. Close guidance of these women and monitoring of the hepatic adenoma by liver ultrasound every 6 weeks during pregnancy is strongly advocated .(10, 26)

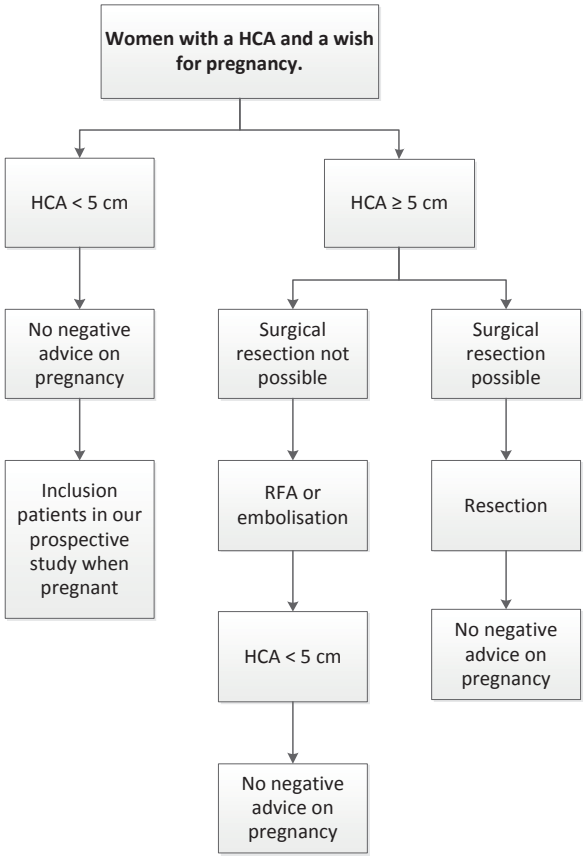

Figure 1. Flowchart for women with a HCA and a wish for pregnancy

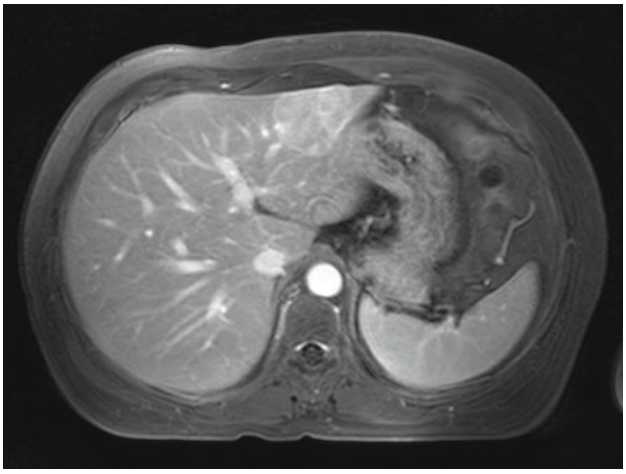

Figure 2. An example of a woman with a HCA of 4.2 cm in segment 2/3 in which pregnancy will not be discouraged.

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CHAPTER 11

Pregnancy and liver adenoma management: PALM-study.

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ABSTRACT

Background

Hepatocellular adenoma (HCA) in pregnant women requires special considerations because of the risk of hormone induced growth and spontaneous rupture, which may threaten the life of both mother and child. Due to scarcity of cases there is no evidence-based algorithm for the evaluation and management of HCA during pregnancy. Most experts advocate that women with HCA should not get pregnant or advise surgical resection before pregnancy. Whether it is justified to deny a young woman a pregnancy, as the biological behavior may be less threatening than presumed depends on the incidence of HCA growth and the subsequent clinical events during pregnancy.

We aim to investigate the management and outcome of HCA during pregnancy and labor based on a prospectively acquired online database in the Netherlands.

Methods/design

The Pregnancy And Liver adenoma Management (PALM) - study is a multicentre prospective study in three cohorts of pregnant patients. In total 50 pregnant patients, ≥ 18 years of age with a radiologically and/or histologically proven diagnosis of HCA will be included in the study. Radiological diagnosis of HCA will be based on contrast enhanced MRI. Lesions at inclusion must not exceed 5 cm. The study group will be compared to a healthy control group of 63 pregnant patients and a group of 63 pregnant patients with diabetes mellitus without HCA. During their pregnancy HCA patients will be closely monitored by means of repetitive ultrasound (US) at 14, 20, 26, 32 and 38 weeks of gestation and 6 and 12 weeks postpartum. Both control groups will undergo US of the liver at 14 weeks of gestation to exclude HCA lesions in the liver. All groups will be asked to fill out quality of life related questionnaires.

Discussion

The study will obtain information about the behaviour of HCA during pregnancy, the clinical consequences for mother and child and the impact of having a HCA during pregnancy on the health related quality of life of these young women. As a result of this study we will propose a decision-making model for the management of HCA during pregnancy.

Trial registration
Dutch trial register: NTR3034

BACKGROUND

Hepatocellular adenoma (HCA) is rare benign tumor of the liver that occurs particularly in women during their reproductive years. The incidence is not exactly known. Studies performed years ago show an estimate incidence of 1-1.3 per 1,000,000 in women who have never used oral contraceptives (OC), compared to 30-40 per 1,000,000 in long-term users. [1,2] The association of HCA with the use of OC was first described in 1973. [3] In subsequent years many authors have supported the hypothesis of an association between OC and HCA. [4–8] The mechanism by which estrogen or other steroids contributes to the development of HCA is still not understood and studies are rare. Symptomatic patients with HCA present with right upper quadrant abdominal pain or discomfort secondary to bleeding within the HCA, elevated liver enzymes and symptoms of life treating hemorrhage into the peritoneal cavity. However, most patients with HCA are asymptomatic and present as an incidental finding during ultrasonographic examination of the abdomen for unrelated reasons or are noted during laparoscopic cholecystectomy. Despite its benign nature, the diagnosis of HCA has a great impact of the lives of these, mostly, young women because HCA can be complicated by hormone induces growth and rupture. Besides that malignant transformation of HCA into hepatocellular carcinoma has been reported with an overall frequency of 4.2%. [9]

Regardless of the exact etiology and risk factors all female patients should be advised to stop OC's and other hormone medication such as hormone replacement therapy, since regression of HCA may occur when steroids are withdrawn [10–13] and observation should be the first choice of treatment for most patients with HCA. Because of the risk for spontaneous rupture most authors believe that surgical resection is required if the diameter exceeds 5 cm after 6 months of follow-up without OC use, if the lesion does not show adequate regression after discontinuation of OC or if rebleeding occurs. [14–17] Surgical resection is also indicated if there is diagnostic doubt e.g. whether a tumor is malignant. [18,19]

HCA in pregnant women requires special considerations because of the risk of hormone induced growth and spontaneous rupture, due to increased levels of steroid hormones during pregnancy that may threaten the life of both mother and child. Most experts advocate that women with HCA should not get pregnant or advise surgical resection before pregnancy. [2,18] Cobey et al. reported a maternal and fetal mortality risk of ruptured HCA during pregnancy of 44% and 38%, respectively. [20] However, all these cases were published in the 1970s and 1980s and nowadays the introduction and widespread use of highly advantage imaging modalities have probably decreased the doctors' delay in the diagnosis of HCA. We recently proposed not to discourage all women with HCA from pregnancy, based on

a study in which we monitored twelve women with documented HCA during a total of 17 pregnancies. In 4 cases HCA's grew during pregnancy, requiring a Caesarean section in 1 patient (2 pregnancies) and radiofrequency ablation (RFA) in 1 case during the first trimester of pregnancy. All pregnancies had an uneventful course with a successful maternal and fetal outcome. [21] However, there is no evidence-based algorithm for the evaluation and management of HCA during pregnancy and labor, due to scarcity of cases. The conclusion not to discourage all women with HCA from pregnancy has, however, to be proven in a large multicentre study in which we will closely monitor pregnant patient with a HCA in a prospectively acquired database to give more insight in the behaviour of HCA during pregnancy.

METHODS/DESIGN

Study objective

In this study we will investigate the management and outcome of HCA during pregnancy and labor based on a prospectively acquired online database in the Netherlands.

Main objective of the PALM-study

– To investigate the incidence of HCA growth during pregnancy and labor.

Secondary objectives of the PALM-study

– To investigate in which trimester of pregnancy growth of HCA occurs;
– To investigate the degree of growth of HCA during pregnancy;
– To investigate whether there is regression of HCA postpartum;
– To investigate the HCA-related interventions during pregnancy and labor;
– To investigate the incidence of bleeding of HCA during pregnancy and labor;
– To investigate liver-related clinical signs during pregnancy;
– To investigate elevated liver enzymes during pregnancy;
– To evaluate the health related quality of life of pregnant patients with HCA;
– To investigate whether there is a difference between health related quality of life of pregnant patients with HCA and pregnant patients with other comorbidity that have an indication for pregnancy care at the obstetrician in secondary care and healthy pregnant patients.

STUDY DESIGN

The PALM-study is a multi-centre prospective study in three cohorts of pregnant women. The study started on November 1 2011 and inclusion of patients will be a

period of minimal 3 to maximal 5 years. In total 50 pregnant patients with HCA < 5 cm will be included in the study. These patients will be compared to a healthy control group consisting of 63 pregnant patients without HCA and a group consisting of 63 pregnant patients with diabetes mellitus (DM). Approval of the Medical Ethics Committee of Erasmus Medical Centre was obtained, NL36058.078.11.

Patient selection

Study group

Properly Dutch speaking, pregnant patients, 18 years of age or older with a radiologically and/or histologically proven diagnosis of HCA can be included in the study. Radiological diagnosis of HCA will be based on contrast enhanced magnetic resonance imaging (MRI) and if available in combination with (contrast enhanced) ultrasonography (US). Lesions must not exceed 5 cm. In the first weeks of pregnancy patients will be referred to the obstetrician for pregnancy care. Baseline starts at 14 (+/−3) weeks of gestation. At this day and every 6 weeks patients will undergo US of the HCA lesion at the radiologist. Before US of the HCA lesions patients will be asked to fill out generic health related quality of life questionnaires (12-item Short Form SF 12 and EuroQol questionnaire EQ-5d), a generic anxiety questionnaire (State-Trait Anxiety Inventory STAI-6) and the Impact of Event Scale (IES) questionnaire for thoughts and feelings about HCA around the US. One week afterwards the study group will be asked to fill out the STAI-6 en IES again. At 14 and 32 weeks of pregnancy patients will undergo venapunction.

Control group 1 (healthy pregnant patients without HCA)

Properly Dutch speaking, healthy pregnant patients, 18 years of age or older without HCA.

In the Netherlands, pregnant women will start pregnancy care with an independently practicing midwife early in pregnancy at the primary care level. [22] The midwife is responsible for the pregnant women as long as the pregnancy, labor or postpartum period is normal. [23] In case of complication, the midwife will refer the women to the obstetrician in secondary care. [22,23] Women with a high risk profile based on their medical or obstetric history will be cared for the obstetrician from the start of pregnancy. [22,23]

Patients presenting at the practicing midwife will be asked to participate in the study. Thereafter, the patients will be included in the study by the study investigator. Patients will undergo US of the liver at 14 (+/-3) weeks of gestation to exclude HCA lesions in the liver. At this day and every other 6 weeks patients will be asked to fill out the SF-12 and EQ-5d questionnaire. At 14 and 32 weeks of pregnancy

patients will undergo venapunction. In case of an uncomplicated pregnancy, the patient remains under the care of her practicing midwife during her pregnancy and postpartum.

Control group 2 (pregnant patients with Diabetes Mellitus)
Properly Dutch speaking, pregnant patients, 18 years of age or older with Diabetes Mellitus, can be included in the study. These patients have an indication for pregnancy care at the obstetrician in secondary care. Patients will undergo US of the liver at 14 (+/-3) weeks of gestation to exclude HCA lesions in the liver. At this day and every other 6 weeks patients will be asked to fill out the SF-12 and EQ-5d questionnaire. At 14 and 32 weeks of pregnancy patients will undergo venapunction.

For all groups informed consent is mandatory. A patient can always withdraw her consent at anytime during the study where after she is referred for the present standard of care.

Hypothesis

Pregnancy may be allowed in case of one or more known HCA < 5 cm (without previous intervention), because a HCA < 5 cm will not disturb the course of pregnancy.
Disrupted course of pregnancy:
- interventions during pregnancy (radiological and/or surgical intervention).
- Decreased quality of life and/or anxiety in patients during pregnancy related to the presence of HCA in the liver and possible growth during pregnancy.

Retrospective cohort study

We have previously reported that more than half of the HCA are discovered after the patient has sustained at least one pregnancy and none of these patients have reported problems during their pregnancies. [19] As mentioned above, recently we described a small but unique series of 12 women with documented HCA who were closely monitored during a total of 17 pregnancies between 2000 and 2009. In 4 cases HCA's grew during pregnancy, requiring a Caesarean section in 1 patient (2 pregnancies) and RFA in 1 case during the first trimester of pregnancy to treat a hormone sensitive HCA, thereby excluding potential growth later on in pregnancy. No intervention was performed in the other 14 cases. All pregnancies had an uneventful course with a successful maternal and fetal outcome and we concluded that a "wait and see" management may be advocated in pregnant women presenting with HCA. In women with large tumours or in whom HCA had complicated previous pregnancies, surgical resection may be recommend. [21] However, additional data from different centres for the risk of hormone induces growth and rupture of HCA during pregnancy is needed.

Interventions

During their pregnancy HCA patients will be closely monitored by means of repetitive US (and MRI in case of growth of the lesion) at 14 (+/−3) and 20 and 26 and 32 and 38 weeks of gestation and 6 and 12 weeks postpartum. At the same days both control groups will be asked to fill out the SF-12 and EQ-5d questionnaire at 14 (+/−3) and 20 and 26 and 32 and 38 weeks of gestation and at 6 and 12 weeks postpartum. (Fig. 1) The study group will be asked to fill out the SF-12, EQ-5d, STAI-6 and IES questionnaires before and one week after US of the HCA lesion(s). Both control groups will undergo US of the liver at 14 (+/−3) weeks of gestation to exclude HCA lesions in the liver. At 14 and 32 weeks of pregnancy all patient groups will undergo venapunction.

Online database

We established a website which allows hepatologists, surgeons and gyneacologists to submit clinical data in an online database. Each centre will have a code to log in and patients will be consecutively assessed a unique number. Registration of a new patient includes entry of the following data: date of birth, weight, height, date of hospital admission, symptoms at presentation, known risk factors for HCA such as glycogenosis and familial polyposis, [24] previous pregnancies, previous use of OC or other hormone medication including hormone replacement therapy, course of HCA after discontinuation of OC, size of HCA before pregnancy, size of HCA during pregnancy (14 (+/−3) and 20 and 26 and 32 and 38 weeks), course of HCA postpartum (6 and 12 weeks postpartum), complications and management during pregnancy, gestation time, way of delivery (vaginally, Caesarean section), maternal and fetal outcome, complications and management after delivery. Only authorized users can gain action to the online database of his or her patients. The database offers excess to the registered data on anytime and anywhere. The coordinating investigator will monitor whether all required fields are completed.

Follow-up

Follow-up of patients takes place at 6 and 12 weeks postpartum postpartum by means of US (and MRI in case of growth) to document the size of HCA postpartum (table 1). Both control groups will be asked to fill out the SF-12 and EQ-5d questionnaires at these days. The study group will be asked to fill out the SF-12, EQ-5d, STAI-6 and IES questionnaires before and one week after US of the HCA lesion(s).

Table 1. Follow-up PALM-study

Pregnancy			
Weeks	Ultrasonography	Venapunction	Questionnaires
14	S, C1, C2	S, C1, C2	S, C1, C2 *
20	S		S, C1, C2 *
26	S		S, C1, C2 *
32	S	S, C1, C2	S, C1, C2 *
38	S		S, C1, C2 *
Post-partum			
Weeks	Ultrasonography	Venapunction	Questionnaires
6	S		S, C1, C2 *
12	S		S, C1, C2 *

S, study group; C1 control group 1 (healthy pregnant patients without HCA); C2, control group 2 (pregnant patients with Diabetes Mellitus)
*The study group will be asked to fill out the SF-12, EQ-5d, STAI-6 and IES questionnaires before and one week after US of the HCA lesion(s). Both control groups will be asked to fill out the SF-12 and EQ-5d questionnaire

Outcome measures

Primary outcome: Biological behaviour and clinical consequences of HCA < 5 cm during pregnancy. Growth is measured by repetitive US (and MRI in case of growth) at 14 (+/−3) and 20 and 26 and 32 and 38 weeks of gestation.

Secondary outcome: General health and pain scales as a measure for quality of life and anxiety related questionnaires for thoughts and feelings of adenomas around US. Other secondary outcomes are complications due to growth of the HCA during pregnancy possibly followed by interventions during pregnancy, incidence of hemorrhage and rupture of the HCA, incidence of liver-related clinical signs during pregnancy (itch, icterus), incidence of elevated liver enzymes during pregnancy.

Power calculation

In our previous study we measured growth of HCA in 4 out of 17 pregnancies (24%) or in 3 out of 12 women (25%). On a yearly basis approximately 50 new patients with HCA are seen at the outpatient clinic of the Erasmus University Medical Centre. The expectation is that 5% (5) of these women get pregnant. The expectation is that a total of 50 pregnant HCA patients from different tertiary referral centres in the Netherlands can be included in the study during a period of 3 to maximum 5 years.

A difference of 0.5 Cohen's D in health-related quality of life is a relevant difference. [25] We calculated that for this purpose 63 patients in both control groups have to be enrolled. A two-sample t test was performed with a two-sided significant level of 0.05 and a power of 0.80.

Access to personal data

Medical data with which the identity of a patient could be traced will be replaced by a code number. The coordinating investigator is the only one who has the key to the code numbers and knows which code number stands for which patient. The principal investigator has only access to the coding system of his or her patients and will never be able to open the database from other centres. Only members of the investigating team and members of the medical ethical committee of the participating centres will have access to the medical data. All data will be collected in a prospectively acquired database by the principal investigators and managed by the coordinating investigator.

DISCUSSION

Once the diagnosis of HCA has been established, patients will be advised to discontinue OC. Expert opinions are very variable regarding treatment and follow up in complex situations were multiple factors play a role in determining the management strategy, like pregnancy. [18]

As to date there are limited data about the behavior of HCA during pregnancy and labor and therefore we cannot identify precisely those at risk for complications. However, in 2006 we reported a series of 48 patients of which in 44 % HCA were discovered after the patient had sustained at least one pregnancy. [19] None of these patients have reported problems during their pregnancies. Likely, only a small subgroup of patients may experience complications and to date pregnancy might be discouraged in too many patients caused by unnecessary intervention before pregnancy. We hypothesize that pregnancy may be allowed in case of one or more known HCA < 5 cm (without previous intervention), because HCA < 5 cm will not disturb the course of pregnancy. Close monitoring during pregnancy by means of repetitive US (and MRI in case of growth) should be carried out to rule out rapid growth of the lesion. The risk of rupture seems the highest during the third trimester of pregnancy. [20] Most likely due to the cumulating level of estrogens and an increase in hyperdynamic circulation combined with an increase in vascularity of the liver with growth of the adenoma. [20] Symptoms and the level of liver enzymes will be registered to find out if there is a relation between symptoms, elevated liver enzymes and growth of the HCA during pregnancy. Patients will be followed-up postpartum to investigate if there is a risk of HCA complications after delivery.

The Society of American Gastrointestinal and Endoscopic Surgeons (SAGES) provided guidelines for diagnosis, treatment, and use of laparoscopy for surgical problems during pregnancy. [26] However, these guidelines are mostly based on case reports

and retrospective studies and therefore graded at a low level of evidence. The SAGES suggest that MRI without the use of intraveneous gadolinium, and US is considered safe and can be used at any stage of pregnancy (Level IIIB and Level IIA respectively). [26] Data regarding safety of CEUS during pregnancy is scare and yet uncertain. However, Hua et al. reported an animal study in which SonoVue may affect the placenta. [27] Therefore, we will not use CEUS for patient follow-up during pregnancy.

One should be aware of the potential risks as an intervention may still be indicated during pregnancy. In approximately one in 635 pregnancies a non-obstetric operation during pregnancy is required, especially appendectomy, cholecystectomy and adnexal precedures. [28] However, it is conceivable that more non-obstetric operations might be required due to the risk of hormone induced growth and spontaneous rupture of HCA during pregnancy. Despite maternal and fetal outcomes following abdominal disease and surgery in pregnancy improved over the past years, the exact risk of HCA-related interventions during pregnancy to both mother and fetus is unknown. [29] We do know that changes in physiology and abdominal anatomy characteristics of pregnancy make abdominal surgery more difficult. [29] The least risk of general anaesthesia is in the 2nd trimester of pregnancy. [30]

Based on a systematic review of the literature, Wilson et al. suggested angioembolisation and formal resection in case of haemorrhage of HCA during pregnancy and suggested this strategy to be safe for both the mother and the fetus with good clinical outcomes. [31] The role of RFA during pregnancy is not well studied. In our previous study we described a RFA procedure during the first trimester of pregnancy [21] and Fujita et al. reported a pregnant patient with a HCA that was treated by RFA during her second trimester of pregnancy (18th week of gestation). [32]

The influence on the course of pregnancy, since a woman is aware of having a HCA, is also unknown. Patients can get horrified when confronted with the new diagnosis of a hepatic mass [20] and it is conceivable that women can be anxiety during pregnancy due to the presence of HCA in the liver and the possible growth during pregnancy. Therefore, quality of life will be an important measurement for future management of HCA during pregnancy. It is conceivable that frequent monitoring by means of US may comfort the patients or can be frightening. All patient groups will be asked to fill out the SF-12 and EQ-5d questionnaires every 6 weeks. HCA patients will be asked to fill out the STAI-6 and IES questionnaires before the US of the liver lesions and one week after US to investigate anxiety related to HCA and US during pregnancy.

Our main point of interest is whether it is justified to deny a young woman with a HCA < 5 cm a pregnancy. With this study we hope to obtain information about the behaviour of HCA during pregnancy and the impact of HCA during pregnancy on the life of these young women. Furthermore we hope to propose a decision-making model for the management of HCA during pregnancy.

Abbreviations

HCA, Hepatocellular Adenoma; US, Ultrasound; OC, Oral Contraceptives; DM, Diabetes Mellitus; MRI, Magnetic Resonance Imaging; SF 12, 12-item Short Form; EQ-5d, EuroQol questionnaire 5d; STAI-6, State-Trait Anxiety Inventory; IES, Impact of Event Scale; SAGES, Society of American Gastrointestinal and Endoscopic Surgens; CEUS, Contrast Enhanced Ultrasound; RFA, Radiofrequency ablation

Competing interests

The authors declare that they have no competing interests.

Authors' contribution

SMA and MEEB are responsible for the drafting of the manuscript and study design, these authors contributed equally to this work. JJB, HJDK, RADM, EAPS, EWS, TT and JNMIJ are responsible for the study design and revision of the manuscript. All authors have read and approved the manuscript.

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CHAPTER 12

Summary in English and Dutch

SUMMARY

In this thesis, the diagnosis and treatment of benign solid liver lesions is being studied. Among these, focal nodular hyperplasia (FNH) and hepatocellular adenoma (HCA) may offer the most challenge in management in daily clinical practice. In this thesis the characteristics of these lesions are described in more detail.

In **chapter 2**, the biological behaviour of FNH is studied and the relevance of change in size is analysed. In 162 patients with FNH, multiple imaging was performed with at least a six-months interval. In case there was suspicion of growth as defined by a 20% increase in size of the lesion according to the RECIST criteria, imaging was reviewed by two independent radiologists. Out of 162 patients diagnosed with FNH, 19 had an increase in size ranging from 21% to 200%. We found that FNH lesions may grow significantly without causing any symptoms and conclude that an increase in size does not have to have implications but it is it is of imminent importance to diagnose these lesions with certainty.

In **chapter 3** patients with HCA registered in our database were analysed over time, and compared with the presentation of patients with single and multiple lesions. Three groups were defined, including patients with a single adenoma, multiple adenomas and liver adenomatosis (>10 lesions). Through this study, it was observed that multiple HCAs are more frequently diagnosed in our institute than would be expected from the literature. Out of 458 patients diagnosed with hepatocellular adenoma, 121(26%) presented with a single adenoma, 223(53%) with multiple adenomas and 82 (20%) with liver adenomatosis. The comparison between the three groups revealed a significant correlation between multiple adenoma/liver adenomatosis and BMI. The highest median BMI was found in the Inflammatory-HCA group. This result may support the hypothesis that HCA may be associated with obesity.

In females, a conservative approach for HCA is justified and consists of cessation of oral contraceptive and weight reduction. If, after a period of six months, radiological imaging shows the HCA to remain stable in size or to regress to a diameter <5cm, annual imaging is indicated. Significant growth (>20% according to RECIST criteria) or a HCA diameter >5cm after six months is stated as an indication for resection. However, in cases of multiple HCA or large lesions located in the central part of the liver, resection may be challenging and a more balanced approach is warranted as liver resections may have a perioperative complication rate of up to 20% [17, 18] increasing significantly with the presence of steatosis. [19] In **chapter 4** the natural behaviour of large hepatocellular adenomas >5 cm which were under surveillance for more than 6 months was assessed. In our cohort, the median number of weeks for the event "regression to ≤5cm" to occur was 81 weeks, in which no clinical events such as bleeding occurred. We therefore suggest that it may be warranted in cases

of multiple HCA or large lesions located in the central part of the liver to offer close surveillance with transversal imaging and follow a watchful waiting strategy.

In **chapter 5** we describe the characteristics of HCA and FNH as depicted by a new imaging technique, the contrast-enhanced ultrasound (CEUS). We determined the presence of specific features and defined a model for prediction using age and the presence of a central scar, central artery, and enhancement pattern. With the formula 1/(1+e(0.778+(0.36 *Age) + (central scar) (central artery) (enhancement mixed) the probability of a lesion being an HCA can be predicted correctly in 0.854 cases using CEUS.

In **chapter 6** we compared the diagnostic performance of CEUS with MRI using gadobenate dimeglumine (CEMRI) for the diagnosis of HCA and FNH. A hundred and nineteen patients had undergone a CEUS as well as CEMRI in the prospective work-up. The final diagnosis was considered definitive when the outcome of CEUS and CEMRI were concordant. Histopathological assessment followed in cases of discrepancy. The sensitivity and specificity of CEUS was 83% and 72% respectively. CEUS and CEMRI were comparable for diagnosis of FNH and HCA with characteristic findings. However, in case of disconcordance between both imaging techniques there CEMRI demonstrated and advantage. Therefore, we recommend the use of CEMRI in all patients in which a HCA or FNH is suspected.

The role of CEMRI in HCA is further reviewed in **chapter 7 and chapter 8** where we focus on the possibility of distinguishing between the different subtypes of HCA. It is important to diagnose H-HCA (HNF1A-mutated HCA) correctly as this steatotic subtype has a particular benign course, allowing a conservative approach. H-HCA is characterised by a large percentage of aberrant fat. The inflammatory subtype, Inflammatory HCA (I-HCA), is hyperintense on T2-weighted images and has a persistent enhancement on delayed imaging in the venous phase. MRI-features of β-catenin-HCA are being described as heterogeneous I-HCA lesions with a high specificity on imaging but with a significant risk on false negative diagnosis.

To support the differentiation between benign liver tumours including HCA and FNH from the malignant liver tumour hepatocellular carcinoma (HCC) we focused on biomarkers, as described in **chapter 9**. Golgi protein 73 is a resident Golgi-specific membrane expressed by biliary epithelial cells and enhanced in HCC. We included 264 patients with HCC, HCA and FNH and found that although GP73 is a valuable marker in HCC, the serum marker may also be raised in HCA and FNH. Therefore, it cannot be used to discriminate HCC from the benign liver tumours HCA and FNH.

In **chapter 10** we describe the evaluation and management of HCA < 5 cm during pregnancy. As HCA may increase in size due to hormones there is a tendency in the literature to discourage pregnancy in women with liver adenoma. We stated that the biological behaviour of HCA might be less threatening than presumed. As described

in chapter 10 we speculate that there is no need to discourage pregnancy in women with a HCA < 5cm. However, due to the scarcity of cases, there is as yet, no evidence-based algorithm for the management of HCA during pregnancy. We initiated a study to demonstrate the safety of pregnancy in women with a liver adenoma< 5cm. In **chapter 11** this protocol on Pregnancy and Liver Adenoma Management (PALM) is described.

NEDERLANDSE SAMENVATTING

Hoofdstuk 2 beschrijft het biologische gedrag van de goedaardige levertumor focale nodulaire hyperplasie (FNH). FNH wordt tegenwoordig steeds vaker gediagnostiseerd doordat de kwaliteit en toegankelijkheid van beeldvorming sterk is toegenomen. Als er een afwijking in de lever gevonden is, wordt beeldvorming vaak herhaald om het goedaardige karakter van de afwijking te bevestigen. Patiënten, maar soms ook artsen, kunnen twijfelen aan de diagnose FNH en het goedaardige karakter van de afwijking als er groei van de afwijking wordt gezien. In hoofdstuk 2 hebben we onderzocht hoe vaak FNH's groeien. Er waren gegevens van 162 patiënten beschikbaar met een radiologisch of pathologisch bewezen en meerdere momenten van beeldvorming met een minimaal interval van 6 maanden. Bij vermeende groei van het FNH werden de opnames opnieuw door twee onafhankelijke radiologen beoordeeld. We definieerden groei als een toename van de diameter van tenminste 20%, zoals beschreven in de RECIST-criteria voor solide levertumoren. Bij deze162 patiënten bleken er 19 FNH's te groeien, met een toename van de diameter met 21 tot 200%. Het klinsche beloop bij deze patiënten was zonder problemen. We concluderen dat FNH's kunnen groeien maar dat groei van een FNH geen invloed heeft op het welzijn van de patiënt en het beleid expectatief kan zijn. Indien de diagnose met zekerheid is gesteld aan de hand van criteria bij radiologisch onderzoek, is follow-up of resectie, niet geïndiceerd.

In **hoofdstuk 3** vergelijken we 3 verschillende patiëntengroepen met een hepatocellulair adenoom (HCA): patiënten met een solitair HCA, patiënten met multipele HCA (tussen de 2 en 9 adenomen) en patiënten met meer dan 10 HCA (het vroegere leveradenomatosis). In de literatuur staat beschreven dat HCA meestal solitair zijn en dat de minderheid van de patiënten meerdere HCA heeft. Multipele HCA bleken echter veel vaker te worden gediagnostiseerd dan in de literatuur wordt aangegeven. Van de 458 patiënten die werden gediagnosticeerd met een HCA, hadden 121(26%) patiënten een solitair HCA, 235 patiënten (51%) multipele adenomen en 102 patiënten (22%) meer dan 10 HCA. In de vergelijking tussen de drie groepen bleek er een correlatie tussen het voorkomen van multipele adenomen en de Body Mass Index (BMI). Daarnaast bleek in deze groepen een zeer hoog percentage inflammatoire adenomen (I-HCA) voor te komen, een adenoom met verhoogd risico op bloeding. Wij concluderen dat multipele en inflammatoire adenomen vaker voorkomen bij patiënten met obesitas.

Bij vrouwen met een HCA wordt in eerste instantie een conservatief beleid gevoerd door gebruik van orale anticonceptiva te ontraden en te adviseren om gewicht te reduceren, mocht er sprake zijn van overgewicht. Als na 6 maanden het HCA kleiner is geworden dan 5 cm wordt vervolgonderzoek met jaarlijkse beeldvorming geadvi-

seerd. Significante groei van het HCA (>20%), of een persisterende grootte boven 5 cm, is reden om een resectie te adviseren. Bij multipele adenomen kan dit beleid ingewikkeld zijn door de risico's van leverresectie, zeker bij patiënten met een sterk overgewicht. In **hoofdstuk 4** beschrijven we het biologische gedrag van HCA groter dan 5 cm bij wie een conservatief beleid gevolgd is voor meer dan 6 maanden. We zien dat de HCA ook na een half jaar nog kleiner dan 5 cm kunnen worden en dat de mediane tijd tot regressie 81 weken is. Patiënten hebben in de vervolgperiode geen complicaties gehad. Wij concluderen dat er veilig gekozen kan worden om patiënten niet chirurgisch te behandelen maar poliklinisch te vervolgen indien er sprake is van multipele adenomen of grote afwijkingen centraal in de lever die gerelateerd kunnen zijn aan grote operatierisico's.

In **hoofdstuk 5** beschrijven we de karakteristieken van HCA en FNH bij gebruik van contrast-echografie (CEUS). CEUS is een nieuwe opkomende techniek om leverafwijkingen in beeld te brengen en te diagnosticeren. We bestudeerden hoe vaak de beschreven karakteristieken van HCA en FNH op de contrast-echo daadwerkelijk zichtbaar zijn en wat de voorspellende waarde is hiervan. We creëerden een betrouwbaar model: 1/(1+e(0.778+(0.36 *Age) + (central scar) (central artery) (enhancement mixed) waarbij we gebruik hebben gemaakt van de leeftijd, het al dan niet aanwezig zijn van de 'central scar' en de manier van aankleuren door het contrast. Met het model dat wij presenteren kan aangegeven worden of er sprake is van een HCA of een FNH.

In **hoofdstuk 6** wordt de diagnostische kwaliteit van CEUS vergeleken met de contrast-MRI (CEMRI) voor het stellen van de diagnose HCA en FNH. 182 patiënten hebben zowel een CEUS als een CEMRI ondergaan. De definitieve diagnose was zeker indien de uitkomst van de CEUS en CEMRI unaniem waren. Indien er sprake was van discrepantie volgde bespreking in de leverwerkgroep met meerdere radiologen en werd de beeldvorming opnieuw beoordeeld. Bij twijfel werd er gekozen voor een biopt. De sensitiviteit en specificiteit van de CEUS waren respectievelijk 83 en 72%. Zowel de CEUS als de CEMRI waren goed in staat om de diagnose HCA of FNH te stellen echter in het geval van discrepantie bleek de CEMRI vaker in staat de juiste diagnose te stellen. Wij adviseren daarom om in de diagnostische work-up altijd ook een CEMRI te verrichten.

De rol van de CEMRI in relatie tot subtypering van adenomen werd bestudeerd **hoofdstuk 7**. Er wordt een overzicht gegeven van de mogelijkheden van de CEMRI bij HCA voor nadere subtypering. Dit is belangrijk omdat er bij het H-HCA (L-FABP negatief HCA subtype) mogelijk gekozen kan worden voor een conservatiever beleid in de toekomst en vervolgonderzoek niet meer geïndiceerd zal zijn. Deze H-HCA kunnen gekarakteriseerd worden door de verhoogde aanwezigheid van vet in de afwijking. Ook het subtype met een verhoogd risico, het zogenaamde inflammatoire

adenoom (I-HCA) kan worden gediagnosticeerd met deze techniek. De typering van mogelijk premaligne adenomen (B-HCA) met CEMRI kan op dit moment nog niet met voldoende accuratesse worden verricht.

Een andere mogelijkheid om de benigne solide levertumoren te onderscheiden van het maligne hepatocellulair carcinoom wordt onderzocht in **hoofdstuk 8**. Hier onderzoeken we het Golgi eiwit 73 (GP-73); eerdere studies hebben aangetoond dat serum niveaus significant hoger zijn in patiënten met hepatocellulair carcinoom (HCC) in vergelijking met gezonde individuen. In 264 patiënten met HCC, HCA of FNH vergelijken wij of GP73 onderscheid kan maken tussen deze solide levertumoren. Hieruit blijkt dat hoewel GP73 een waardevolle marker kan zijn voor het HCC het ook verhoogd kan zijn bij de goedaardige solide levertumoren als HCA en FNH. Hierdoor is het niet geschikt om te gebruiken in de differentiatie tussen deze drie tumoren.

In **hoofdstuk 10 en 11** beschrijven we de modificaties die zijn opgetreden in het advies over zwangerschap aan vrouwen met een HCA. In **hoofdstuk 10** worden data gepresenteerd die onderbouwen dat het hebben van een HCA gedurende de zwangerschap minder gevaarlijk is dan voorheen werd verondersteld. In het Erasmus MC wordt zwangerschap bij vrouwen met een HCA < 5cm niet langer ontraden en wordt tijdens zwangerschap zorgvuldig met echografisch onderzoek vervolgd hoe het beloop is. Een wetenschappelijk onderbouwd advies aan vrouwen met een HCA en zwangerschap is op dit moment echter nog niet mogelijk door het lage aantal observaties dat we hebben uitgevoerd. Om hier een beter advies over te kunnen geven wordt momenteel de PALM-studie (Pregnancy and Liver adenoma Management) uitgevoerd. Een multicenter, prospectieve studie die nader wordt beschreven in **hoofdstuk 11.**

CHAPTER 13

General discussion, recommendations and future perspectives

Compared to men, women more commonly present with the benign liver tumours; HCA and FNH. The pathophysiology of this gender difference is not completely understood. Oestrogen levels are thought to be associated with benign liver tumours, although the potential mechanisms are unclear (1-3). Data on increased hormone receptors or increased exogenous oestrogen levels due to oral contraceptives (OC) are inconsistent. In women with HCA, the use of OCs is reported in up to 80% of cases (4). OC use among women without HCA (15-44) is clearly lower, with a reported percentage of 25.9%(5). With the increasing widespread use of OC and the availability of more sensitive imaging techniques a rising incidence of HCA is reported from 0.001-0.004% up to 0.04% (6-8). Concurrent with this rising incidence, an increase of multiple HCAs has been observed. Our findings as well as reports from other studies indicate that these patients may be characterised by being overweight. Moreover, it has been suggested that the increase in adipose tissue may be responsible for the production of excessive oestrogens (9, 10). Obese patients have higher oestrogen levels compared to non-obese individuals (10, 11). In case of obesity, weight loss in addition to cessation of OC-use, might be strongly recommended in the future management of women with HCA (12).

FNH has a different response to the use of oral contraceptives and being overweight when compared with HCA and therefore it is important to distinguishing both lesions from one another. In the clear majority of hepatic tumours, high-quality contrast enhanced transversal imaging allows accurate diagnosis (13, 14). MRI with the use of a liver specific contrast-agent has a specificity of nearly 100%, although its sensitivity is lower, especially in lesions smaller than 3 cm in diameter. When the diagnosis on MRI is uncertain, addition of contrast-enhanced ultrasound (CEUS) may provide a higher diagnostic accuracy (15, 16). If there is still doubt about the diagnosis, a biopsy of the lesion may be performed. When imaging does indicate heterogeneity in the tumour nodule, the absence of malignancy must be confirmed, as it is critical that HCA is differentiated from HCC. A biopsy may be the next step. However, it should be noted that this procedure may be complicated by bleeding in up to 3/1000 patients and that there may be a sample error. Thus, the differential diagnosis of HCC must be kept in mind if there is a discrepancy between pathology results of needle biopsies and if the level of suspicion is raised by imaging findings. Furthermore, a positive Beta-catenin staining may be difficult to demonstrate in needle biopsies and the absence in the needle biopsy may differ from the findings in the resected specimen (17).

In the molecular and pathological subtype classification system of Bordeaux, β-catenin has been introduced as an important biomarker for I-HCA at risk. This classification directs clinical management. MRI with the use of a liver specific contrast has been demonstrated to be correlated with the pathological classification as well

and MRI imaging is nowadays the modality of choice to characterise HCA subtypes in the first instance (14). If the diagnosis is unclear, a percutaneous biopsy may be used as mentioned above (18-20). In addition, treatment decisions are based on gender, size and pattern of progression and heterogeneity, and immunohistological staining on β-catenin. Today, mutation analysis is not used routinely, but it must be explored as to its utility in identifying patients at risk of acquiring multiple inflammatory adenomas. (22)

If HCA has been diagnosed, the patient's life style should be addressed. It is recommended the patient stops the use of OC and for imaging to be repeated after 6 months. After cessation of OCs, 79% of patients show regression of HCAs, sometimes with complete disappearance of the lesion on imaging (14, 23). If HCA shows regression, but the diameter still exceeds the limit of 5 cm, longer observation instead of resection might be considered. We state that the risk of complications like bleeding may be negligible in adenomas showing regression and the risk of clinical relevant haemorrhage is overestimated. However, further follow up is recommended to monitor the progress of HCA. Future studies will help determine whether annual follow-up is indicated for all subtypes and all sizes.

Although research on benign liver lesions has shown great progress in recent years there are still a number of issues to be addressed in order to better understand and manage these lesions. Directions for further research could include the influence of life style changes, including cessation of OC with or without reduction of body weight, the impact of pregnancy, the clinical relevance of β-catenin staining, and the relevance of molecular subtyping on the scheme and length of surveillance. With a multicenter approach these questions may be answered in the following years leading to a more evidence-based management of young women with the incidental finding of a benign liver lesion.

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LIST OF PUBLICATIONS

Publications (this thesis):

M.E.E. Bröker, S.M. van Aalten, J.J. Busschbach, H.J .de Koning, R.A. de Man, E.A. Steegers, E.W. Steyerberg, T. Terkivatan, J.N.M. IJzermans. Pregnancy and liver adenoma management: PALM-study. BMC Gastroenterol. 2012 Jun 29;12:82.

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M.E.E. Bröker, A. J. Klompenhouwer, M.G.J. Thomeer, R.A. de Man, J.N.M. IJzermans. Retrospective cohort study on timing of resection of hepatocellular adenoma, Accepted BJS

M.E.E. Bröker, A.J. Klompenhouwer, M.P. Gaspersz, A.M.E. Alleleyn, R.S. Dwarkasing, I.C. Pieters, R.A. de Man, J.N.M. IJzermans, Growth of FNH is not a reason for surgical intervention. Accepted World Journal of Surgery

M.E.E. Bröker, P. Taimr, M. Doukas, R.A. De Man, M. de Vries, J.N.M. IJzermans , R.S. Dwarkasing. Is contrast enhanced ultrasound comparable to MRI with liver specific contrast agent for the diagnosis of hepatocellular adenoma and focal nodular hyperplasia. Submitted

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Z.S. Lalmahomed, **M.E.E. Bröker**, N.A. van Huizen, R.R.J. Coebergh van den Braak, L.J.M Dekker, D. Rizopoulos, C. Verhoef, E.W. Steyerberg, T.M. Luider, J.N.M. IJzermans . Hydroxylated collagen peptide in urine as biomarker for detecting colorectal liver metastases. Am J Cancer Res 2016;6(2):321-330.

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P.Y. Hernanda, A. Pedroza-Gonzalez, L.J.W. van der Laan, **M.E.E. Bröker**, M.J. Hoogduijn, J.N.M. IJzermans, M.J. Bruno, H.L.A. Janssen, M.P. Peppelenbosch, Q. Pan.Tumor promotion through the mesenchymal stem cell compartment in human hepatocellular carcinoma. Carcinogenesis. 2013 Oct;34(10)

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1. PhD Training

General Courses	Year	Workload (ECTS)
Basiscursus Regelgeving en Organisatie (BROK), Erasmus MC, Rotterdam	2011	1
Research Integrity, Erasmus MC, Rotterdam	2012	0.6
Biostatistics for clinicians, Erasmus MC, Rotterdam	2012	1
Basic introduction course SPSS, Erasmus MC, Rotterdam	2012	0.8
Molmed English biomedical writing and communication	2012	2.0
Methodologie van patiëntgebonden onderzoek en voorbereiding van subsidieaanvragen), Erasmus MC, Rotterdam	2012	0.3

In-depth Courses	Year	Workload (ECTS)
AASLD Transplant Surgery Workshop: Management of Rare Liver Tumors	2013	1

Presentations at (inter)national conferences	Year	Workload (ECTS)
Oral presentations		
The use of contrast-enhanced ultrasonography and MRI for the distinction between focal nodular hyperplasia and hepatocellular adenoma - ESSR, Lille	2012	1
The value of Golgi Protein 73 as a marker to differentiate between solid benign and malignant liver tumours - Belgische gastroweek, Antwerpen	2013	1
The value of Golgi Protein 73 as a marker to differentiate between solid benign and malignant liver tumours – NVGE, Antwerpen	2013	1
The value of Golgi Protein 73 as a marker to differentiate between solid benign and malignant liver tumours – ESSR, Istanbul	2013	1
The additional use of contrast-enhanced ultrasonography for the distinction between hepatocellular adenoma and focal nodular hyperplasia – AASLD, Washington	2013	1
The clinical significance of collagen peptides in urine – SEOHS, Maastricht	2013	1
HCA en zwangerschap - Landelijke leverwerkgroep, Utrecht	2013	1
FNH en Adenoom – EASL Richtlijn Benigne levertumoren NVGE Veldhoven	2013	1
	2016	1
Poster presentations		
The value of Golgi Protein 73 as a marker to differentiate between solid benign and malignant liver tumours – ESMO, Barcelona	2013	0.5

The value of Golgi Protein 73 as a marker to differentiate between solid benign and malignant liver tumours – AASLD, Washington	2013	0.5
Multiple adenoma and liver adenomatosis: incidence, patient characteristics and management– AASLD, Washington	2013	0.5
Attendance at (inter)national Conferences	**Year**	**Workload (ECTS)**
Chirurgendagen, Nederlandse Vereniging voor Heelkunde –Veldhoven (6x)	2011-2017	6
360 degrees in liver metastases, Rome	2012	0.5
Symposium Experimenteel Onderzoek Heelkundige Specialismen	2012	0.5

2. Teaching

Supervising Practicals and Excursions, Tutoring	**Year**	**Workload (ECTS)**
Examination Basic Life Support (EHBO) first year medical students	2011-2013	1
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Mirelle Elmira Elizabeth Bröker werd op 29 augustus 1985 geboren in Utrecht. Ze groeide op in Groenekan, Westervoort en Velp, alwaar ze een heerlijke jeugd heeft gehad. In 2004 slaagde zij voor haar Atheneum-eindexamen aan Arentheem College in Arnhem. Na 1 jaar Farmacie te hebben gestudeerd aan de Universiteit van Utrecht werd zij decentraal toegelaten tot de studie geneeskunde aan de Erasmus Universiteit Rotterdam.

Tijdens haar geneeskunde studie werkte ze in het medisch studententeam in het Erasmus MC op de afdeling transplantatiechirurgie. In haar vierde jaar deed ze haar keuze-onderzoek op de afdeling Heelkunde in het Sint Franciscus Gasthuis en het Erasmus MC (Prof. dr. J.N.M. IJzermans, Dr. W.W. Vrijland). Haar oudste-co-schap chirurgie volgde zij in het Reinier de Graaf Groep te Delft (Dr. M. van der Elst) alwaar zij in 2009 de wetenschapsprijs van de Reinier de Graaf groep behaalde.

Na het cum-laude behalen van haar arts-examen heeft zij 4 maanden als ANIOS Chirurgie op de afdeling Chirurgie gewerkt in het Erasmus MC (Prof. dr. J.N.M. IJzermans) alvorens te beginnen aan haar promotieonderzoek op de afdeling hepatobiliaire chirurgie (Prof. dr. J.N.M. IJzermans). Vanaf november 2013 heeft zij als ANIOS gewerkt op de afdeling chirurgie van het IJsselland (Opleider: Dr. I. Dawson).

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