BENIGN LIVER TUMORS
FROM DIAGNOSIS TO PROGNOSIS

Anne Julia Klompenhouwer

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Van diagnose tot prognose

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CHAPTER 1

General introduction and aims of the thesis

General introduction and aims of the thesis

The incidence of hepatic tumors has risen over the past decades due to the more widespread use of radiological imaging. The majority of these lesions are benign, but malignancy needs to be ruled out using extensive diagnostic work-up such as medical history, blood tests, imaging and sometimes liver biopsy. The differentiation between benign and malignant liver tumors is of great importance, as their management differs significantly.

Among the different types of benign liver lesions, management may also vary. Some have a completely benign course whereas others are at risk for complications and malignant degeneration. In this thesis, we focus on the management of four different types of benign liver tumors: hepatocellular adenoma, focal nodular hyperplasia, hepatic angiomyolipoma and biliary cystadenoma.

Hepatocellular adenoma

Hepatocellular adenoma (HCA) is a liver tumor that occurs mostly in females and is associated with the use of oral contraceptives and obesity (1-6). With cessation of oral contraceptives and weight loss regression of HCA may occur (7, 8). Although generally benign, HCA may be complicated by malignant transformation to hepatocellular carcinoma and hemorrhage (9, 10).

Microscopically, HCA consist of a proliferation of normal hepatocytes that are separated by dilated sinusoids. We can distinguish several HCA subtypes based on genetic and phenotype characteristics. These are HNF-1α inactivated (H-HCA), inflammatory (I-HCA), β-catenin-activated (β-HCA), β-catenin-activated inflammatory (β-IHCA), and recently, sonic hedgehog (sh-HCA) adenomas (11, 12). These subtypes can be differentiated based on imaging (contrast enhanced MRI), immunohistochemical staining and molecular diagnostics (13-15).

Due to the risk of complications, HCA may require invasive treatment. At this time, size is the most important reason for surgical resection as complications rarely occur in lesions smaller than 5cm. The first step in the management of HCA consists of cessation of oral contraceptives and weight loss if necessary. Subsequently, guidelines advocate surgical resection if the HCA still exceeds 5cm six months after implementation of these lifestyle changes (16). Additionally, surgical resection is advocated in all patients with β-(I)HCA and all males with HCA, as the risk of malignant transformation is higher in these patients (12, 16).

Focal nodular hyperplasia

Focal nodular hyperplasia (FNH) is typically an incidentally discovered, asymptomatic lesion. Comparable to HCA, FNH also has a strong female predominance, while a relation with oral contraceptives and female sex hormones appears to be absent (17, 18). FNH consist of nodules of normal hepatocytes, often surrounding a central scar containing a central artery. Complications such as malignant transformation do not occur in FNH and therefore treatment is not indicated when the diagnosis is well established (16).

Hepatic angiomyolipoma

Hepatic angiomyolipoma (HAML) is a rare mesenchymal liver tumor typically composed of blood vessels, smooth muscle cells and adipose cells, in varying proportions (19). Due to these various proportions of tissue components, HAML diagnosis on imaging is challenging (20). Although the majority of HAML are thought to be benign, a number of reports describe aspects of malignant behavior: growth, recurrence after surgical resection, metastasis and invasive growth patterns into the surrounding parenchyma (21). HAML can be histologically classified as classic (mixed) type with lipomatous, myomatous or angiomatous predominance or an epithelioid variant with the presence of 10-100% epithelioid cells.

Surgical resection is often advocated for HAML, although a conservative approach may also be justified in some cases. Being a very rare tumor, evidence is limited to single center reports with small sample sizes. Currently there are no clinical practice guidelines with an evidence-based consensus regarding the optimal management strategy for patients with suspected HAML.

Biliary cystadenomas

Biliary cystadenomas (BCA) are rare, multilocular cystic tumors arising from the liver. BCA are septated and contain mucinous or serous fluid (22). According to literature, up to 20% of BCA may transform to biliary cystadenocarcinoma (BCAC) (23, 24).

No clinical practice guidelines with an evidence-based consensus regarding the optimal management strategy for patients with suspected BCA are available. Many researchers advocate complete surgical resection due to the possible malignant transformation (25). However, other treatment strategies such as fenestration, marsupialization and drainage have been described as well (26).

Aim of the thesis

The overall aim of this thesis was to address the most challenging aspect in the management of the aforementioned benign liver tumors, namely to select those patients really needing surgery on the one hand and legitimize a wait and see policy in others.

As not every patient with a benign tumor is at risk of complications, treatment of these lesions by complex surgical procedures should be well balanced to prevent avoidable complications in case of a benign disease.
For HCA, guidelines advise cessation of oral contraceptives and surgical resection if the lesion is still >5cm at six months after diagnosis. **Chapter 2** describes a cohort study evaluating whether this six month interval is sufficient to expect regression to ≤5cm in large HCA. Continuing on this, in **chapter 3** we developed a model to predict regression of large size HCA. In patients with multiple HCA, guidelines advocate to base management decisions based on the size of the largest HCA. **Chapter 4** evaluates whether liver regeneration after resection causes growth of residual HCA. HCA requires special consideration in pregnant patients, due to the potential risk of hormone induced growth and hemorrhage. In **chapter 5** the PALM study is described, which is a prospective study investigating the management and incidence of growth in patients with HCA <5cm during pregnancy. **Chapter 6** focuses on the management of HCA in post-menopausal women, evaluating whether follow-up of HCA can be safely terminated after the occurrence of menopause. In **chapter 7** we focus on one of the complications: hemorrhage due to HCA. The outcome of acute management and risk of rebleeding in patients with massive hemorrhage are evaluated. Although surgical resection is the preferred treatment for HCA, transarterial embolization has been described as well. **Chapter 8** describes a retrospective study assessing the outcome of transarterial embolization in the management of HCA and its post-embolization effect. **Chapter 9** is a case report describing two patients who underwent resection of HCA with unusual pathological findings.

Due to the difference in risk of complications and management, differentiation between HCA and FNH is essential. In **chapter 10** we assessed the potential of point shear wave elastography to differentiate FNH from HCA. Growth of FNH may cause doubt about the initial diagnosis. **Chapter 11** is a retrospective cohort study addressing the implications of growth of FNH for clinical management.

Chapter 12 is a systematic review assessing the biological behavior and estimating the risk of HAML related mortality, finally recommending on a justifiable management strategy. In **chapter 13** we describe an international multicenter analysis characterizing clinical and radiological features associated with HAML.

Finally, **chapter 14** is a systematic review assessing the diagnostic work-up and necessity of complete surgical resection of BCA(C).

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CHAPTER 2

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 included, 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 χ^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. 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

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.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.

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.

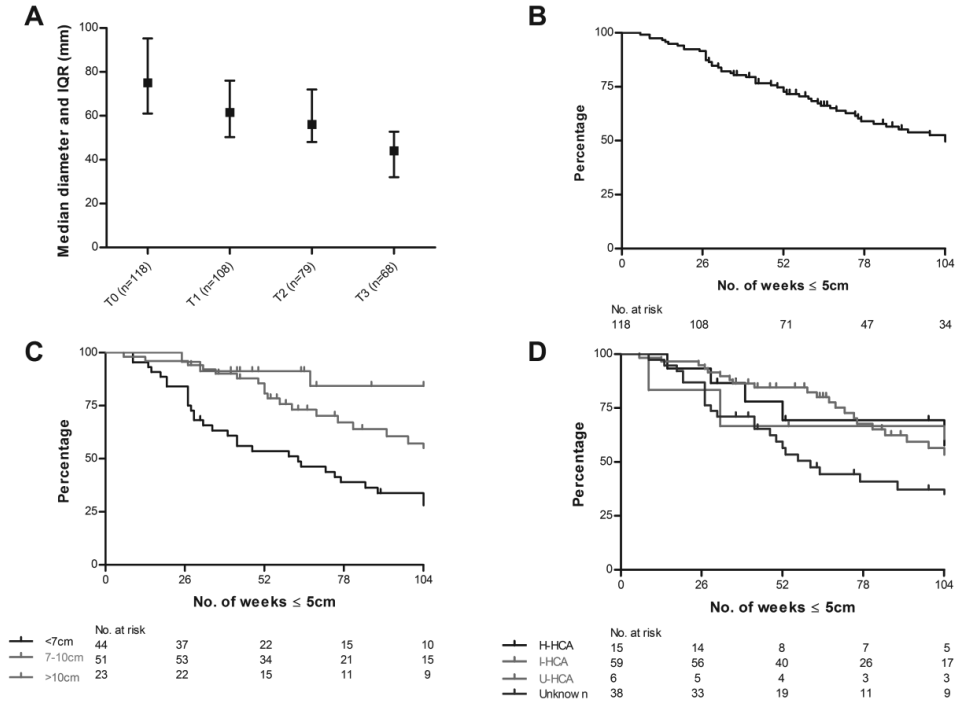

Figure 1. Diameter of HCA and regression to ≤ 5cm.

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.

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 ± 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.

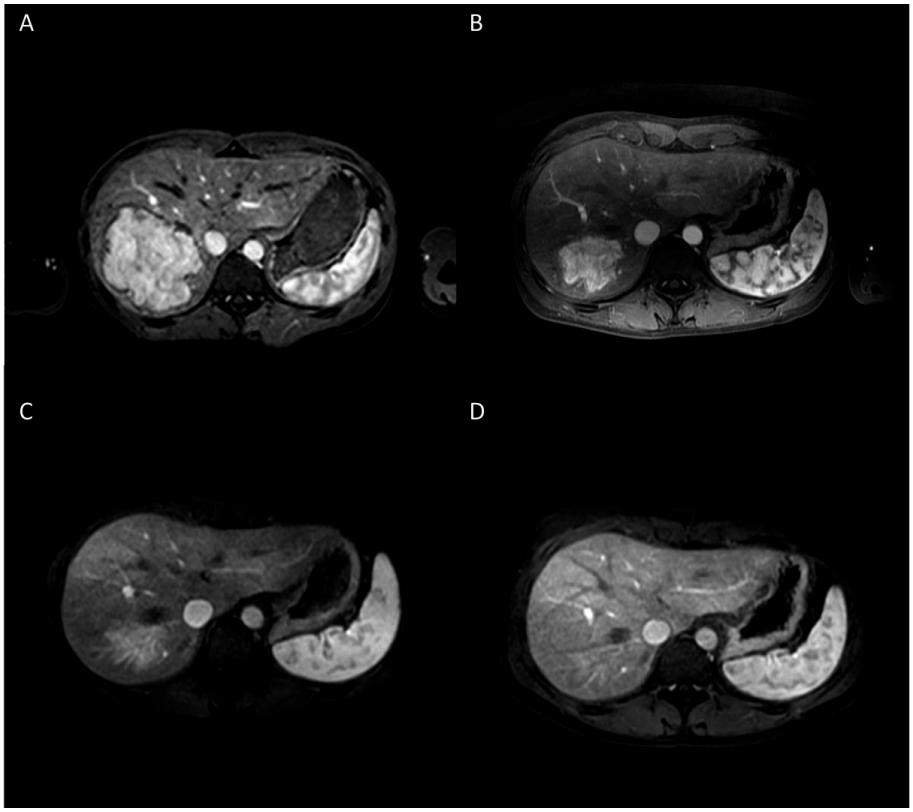

Figure 2. Example of a patient with HCA regression over time.

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.

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 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 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 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.

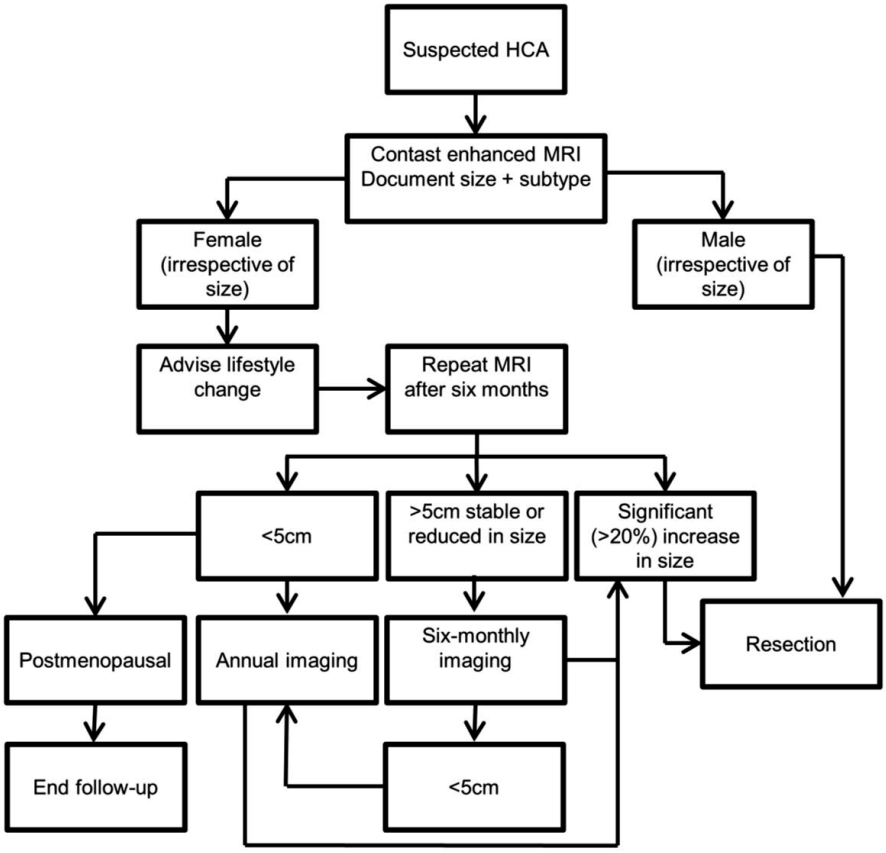

Figure 3. Flowchart for the management of benign liver tumors.

Adapted from EASL Clinical Practice Guidelines on the management of benign liver tumors(16).

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 3

Development and validation of a model to predict regression of large size hepatocellular adenoma

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ABSTRACT

Aim: Surgery is advocated in hepatocellular adenomas (HCA) >5cm that do not regress to <5cm after 6-12 months. The aim of this study was to develop a model for these patients, estimating the probability of HCA regression to <5cm at one and two years follow-up.

Methods: Data were derived from a multicenter retrospective cohort of female patients diagnosed with HCA >5cm at first follow-up. Potential predictors included age, BMI and HCA diameter at diagnosis (T0), HCA-subtype (H-HCA, I-HCA, U-HCA) and "T0-T1 regression-over-time" (percentage of regression between T0 and first follow-up (T1) divided by weeks between T0-T1). Cox proportional-hazards regression was used to develop a multivariable model with time to regression of HCA <5cm as outcome. Probabilities at one and two years follow-up were calculated.

Results: In total 180 female patients were included. Median HCA diameter at T0 was 82.0mm and at T1 65.0mm. Eighty-one patients (45%) reached the clinical endpoint of regression to <5cm after a median of 34 months. No complications occurred during follow-up. In multivariable analysis, the strongest predictors for regression to <5cm were HCA diameter at T0 (logtransformed, HR 0.05), T0-T1 regression-over-time (HR 2.15) and HCA subtype I-HCA (HR 2.93) and U-HCA (HR 2.40), compared to H-HCA (reference). The model yielded an internally validated c-index of 0.79.

Conclusion: In patients diagnosed with HCA >5cm that still exceed 5cm at first follow-up, regression to <5cm can be predicted at one and two years follow-up using this model. Although external validation in an independent population is required, this model may aid in decision-making and potentially avoid unnecessary surgery.

INTRODUCTION

Hepatocellular adenoma (HCA) is a rare benign liver tumor which is usually discovered incidentally in women using estrogen containing oral contraceptives (OC). It has been associated with obesity, metabolic disorders and the intake of androgens. With cessation of OC and weight reduction regression of HCA may occur (1-3).

HCA can be subdivided based on genetic and phenotype characteristics, among which Hepatocyte Nuclear Factor 1α inactivated (H-HCA), inflammatory (I-HCA), β-catenin-activated (β-HCA), β-catenin-activated inflammatory (β-IHCA), and most recently, sonic hedgehog (sh-HCA) adenomas (4, 5) (table 1). HCA with no specific mutations are termed unclassified adenomas (U-HCA). These subtypes may be distinguished radiologically or based on immunohistochemical staining or molecular characterization. Contrast-enhanced MRI has the highest sensitivity and specificity for diagnosis of HCA and may also be used for subtype determination (6, 7). Liver biopsy can be performed in case of inconclusive imaging or when its result is expected to impact treatment decisions.

Table 1: HCA subtypes

H-HCA	Inactivating mutation of Hepatocyte Nuclear Factor 1α
I-HCA	JAK/STAT pathway activation, caused by mutations in different parts of the signaling pathway.
β-HCA	Mutation in either exon 3 or exon 7/8 of the CTNNB1 gene, causing activation of the β-catenin protein. At risk for malignant transformation.
β-IHCA	Both JAK/STAT pathway activation and a mutation in CTNNB1. At risk for malignant transformation.
sh-HCA	Activation of sonic hedgehog signaling pathway
U-HCA	Restgroup of HCA without distinctive underlying mutations or activations

The most common complication of HCA is hemorrhage, thought to occur mostly in I-HCA (8) and sh-HCA (5). A more rare complication is malignant transformation to hepatocellular carcinoma, occurring particularly in β-HCA or β-IHCA and in men with HCA (9, 10). Both complications seem to occur mostly in HCA exceeding 5cm (10, 11).

In the clinical practice guideline regarding the management of benign liver tumors, it is stated that a conservative approach with lifestyle adaption (cessation of OC, weight reduction) is justified in women with HCA (12). Resection of HCA is indicated in men, patients with β-HCA or β-IHCA, in case of significant growth and when HCA diameter exceeds 5cm 6 months after lifestyle changes. However, a recent study showed that the follow-up of potential regression could be prolonged to 12 months, and possibly even

longer for large HCA (13) as these lesions will regress over time and sometimes even disappear completely (figure 1).

The present study focuses on patients diagnosed with HCA >5cm that still exceed 5cm at first follow-up imaging. The aim of this study was to develop a clinical prediction model estimating the probability of HCA regression to <5cm at one and two years of follow-up, which can be used in timely selection of patients for surgery.

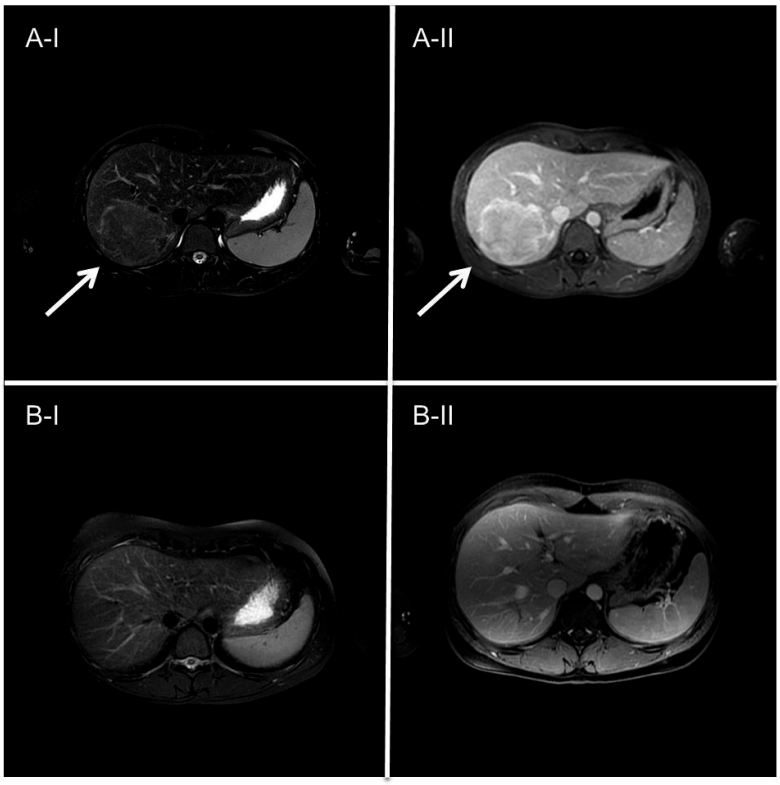

Figure 1. Example of regressing HCA

Example of a patient with a large HCA in the right hemiliver.
A: At diagnosis in 2013. A-I T2-W fatsaturated sequence. A-II T1-W fatsaturated sequence venous phase. B: Nearly complete regression 3 years after cessation of oral contraceptives. B-I T2-W fatsaturated sequence. B-II T1-W fatsaturated sequence venous phase.

METHODS

Design and study population

Patients were derived from a retrospective cohort of patients diagnosed with HCA in three tertiary referral centers in the Netherlands (the Erasmus MC University Medical Center in Rotterdam, the Amsterdam University Medical Centers (location Academic Medical Center) in Amsterdam and the University Medical Center in Groningen) between January 2000 and October 2017. HCA diagnosis was established by either contrast enhanced MRI, histological examination (biopsy or resection specimen) or both. Patients were included if they were female and had at least one HCA with a diameter >5cm at the moment of diagnosis (T0) as well as at first follow-up imaging (T1). The minimum follow-up time was six months. Men with HCA and all patients with histologically proven β-(I)HCA were excluded, as resection is recommended in these patients due to the higher risk of malignant transformation. Patients who underwent an intervention prior to first follow-up imaging and those who experienced hemorrhage before HCA diagnosis causing an unreliable radiological assessment of the diameter were also excluded. The study protocol was reviewed by the accredited institutional review board; informed consent was waived. All patients were treated according to the same treatment algorithm. At diagnosis, patients were presented at a multidisciplinary tumor board (MDTB) to establish a definitive diagnosis. When HCA was diagnosed, patients were urged to discontinue OC and other systemic hormonal agents and to reduce weight in case of a BMI >25 kg/m^2. First follow-up imaging was scheduled usually around 6 to 12 months after diagnosis. For each patient, all follow-up imaging was discussed at the MDTB, and management (continuing follow-up or intervention) were determined.

Electronic medical records were retrospectively reviewed to collect clinical data including sex, age at diagnosis, diagnostic work-up (imaging modality, biopsy), date and size of HCA at time of diagnosis (T0) and first follow-up (T1), date of last follow-up imaging, management (follow-up, intervention) and HCA-subtype (H-HCA, I-HCA, and U-HCA). As sh-HCA were not described until recently, these are not included as separate subtype in this study. HCA-subtype was determined based on typical contrast-enhanced MRI features (6, 7, 14), immunohistochemistry (15) or patho-molecular characterization. In all three centers, histologic specimens have been recently revised in order to determine HCA subtypes of older patients diagnosed before 2013. U-HCA were only considered to be unclassified based on patho-molecular characterization, when only imaging report was available the subtype remained undetermined (missing).

The clinical outcome was regression to <5cm and the date of the follow-up imaging where the HCA was seen to have regressed to <5cm for the first time was documented. In patients with multiple lesions, the size of the largest lesion was taken as the EASL guideline states

to base management decisions on the size of the largest lesion (16). A new variable was calculated to objectify the regression-over-time between T0 and T1. First, we calculated the regression coefficient between T0 and T1: (diameter HCA T0 – diameter HCA T1) / diameter HCA at T0. This was then standardized by dividing the regression coefficient by the number of weeks between T0 and T1. This results in a new variable called "T0T1 regression-over-time".

Statistical analysis

Continuous variables are summarized as median and interquartile range (IQR), categorical variables as frequency (n) and percentages (%). All statistical analyses were performed using IBM SPSS software version 21.0 (Chicago, Illinois) or R version 3.3.3.

Differences between groups were analyzed using χ^2 test for categorical variables. Correlation between variables was analyzed using Pearson product-moment correlation coefficient. Overall time-to-event analysis was performed using the Kaplan-Meier method and log-rank test with regression of the HCA to <5cm as outcome. Patients who were treated conservatively and failed to reach this clinical endpoint were censored at the time of last follow-up imaging, patients who underwent an intervention were censored at the last imaging before intervention.

To identify predictors of HCA regression to <5cm, a multivariable Cox proportional hazards model was developed. We only considered variables that were regarded as clinically relevant and that were easily accessible. These were age at diagnosis, body mass index (BMI), HCA diameter at T0, T0T1 regression-over-time and HCA-subtype. OC use was not considered as a predictor as almost all patients used OC. We used natural logarithmic transformation to correct for nonlinearity when indicated. Multiple imputation with 5 complete datasets (R, *mice* package, van Buuren 2017) was applied to account for missing data for BMI (14.7%) and HCA subtype (18.9%).

The inclusion of variables into the multivariable model was assessed using a stepwise backward selection method (R, *rms* package, Harrell 2017) based on the Akaike information criterion (AIC). We used an internal validation procedure with bootstrap resampling with 500 replications to correct the model performance for optimism, and to compute a shrinkage factor to correct for overfitting (17). Point estimates were reported as hazard ratios (HR) with 95% confidence intervals (95% CI). The overall performance in terms of discriminative ability of the prediction model was measured with Harrell's concordance index (C-statistic) and corrected for optimism. A C-statistic below 0.5 was considered as very poor, a C-statistic over 0.7 as good and a C-statistic over 0.8 as strong. All tests were two-sided and a p-value <.05 was considered as the level of significance.

The prediction model was developed and reported in accordance with the transparent reporting of a multivariable prediction model for individual prognosis or diagnosis (TRIPOD) guideline (Appendix A) (18). The following sensitivity analysis were performed to validate the model: one with only baseline characteristics, a second in patients with pathologically proven HCA and subtypes based on patho-molecular characterization only and a third in patients who were treated conservatively only (excluding those who underwent an intervention).

RESULTS

Clinical characteristics

A total of 180 patients met the inclusion criteria: 122 from Erasmus Medical Center Rotterdam, 30 from Amsterdam University Medical Centers Amsterdam and 28 from University Medical Center Groningen. They were all female patients diagnosed with HCA at a median age of 36 years and with a median BMI of 32.0 kg/m^2. Almost all (95.6%) used OC. All but one patient underwent contrast enhanced MRI and in 98 patients (54.4%) HCA was histologically proven. No statistically significant differences in diagnostic work-up were seen between the three participating centers. Over half of the study population (57.2%) had I-HCA, 15% U-HCA and 8.9% H-HCA (table 2).

Follow-up and primary endpoint

The median follow-up time was 24.0 months, median HCA diameter at diagnosis (T0) 82.0mm and at first follow-up imaging (T1) 65.0mm (table 2). Median time between diagnosis and first follow-up imaging was 6 months (IQR 5-8 months). Kaplan-Meier analysis showed 81 patients reaching the clinical endpoint of regression to <5cm (45%) after a median of 34 months since diagnosis (95% CI 25.8-42.2 months) (figure 2A). Subanalysis in patients who used OC showed no statistical significant difference in reaching the clinical endpoint between patients with BMI < or > 30 kg/m^2 (p=0.78, figure 2B). The majority of patients was treated conservatively (67.2%), the remaining 32.8% underwent an intervention (27.8% resection, 3.3% embolization and 1.7% radiofrequency ablation). Out of the 81 patients who reached the clinical endpoint of regression to <5cm 8 still underwent an intervention, all because of an active pregnancy wish or on patients own request. No statistically significant correlation was found between year of diagnosis and whether an intervention was performed (r=-0.145, p=0.053). No statistically significant differences in management were seen between the three participating centers (p=0.650). HCA was confirmed in all resection specimens. No growth of HCA or complications (hemorrhage or malignant transformation) occurred during the surveillance period.

Table 2: baseline characteristics

	Included patients with HCA N = 180
Female	180 (100%)
Median age at diagnosis (yr)	36 (29 – 45)
Median BMI (kg/m²)	32.0 (27.4 – 35.9)
Hormone usage	
Oral contraceptives	172 (95.6%)
Never	3 (1.7%)
Steroids as medication	1 (0.6%)
Unknown	4 (2.2%)
Median follow-up time (months)	24.0 (13.0 – 49.0)
Median time between diagnosis and first follow-up imaging (months)	6.0 (5.0 – 8.0)
Median diameter of HCA at diagnosis (mm)	82.0 (65.0 – 100.0)
Median diameter of HCA at first follow-up imaging (mm)	65.0 (56.0 – 80.0)
Diagnostic work-up	
Contrast enhanced MRI	179 (99.4%)
Histologically proven	98 (54.4%)
HCA subtype	
H-HCA	16 (8.9%)
I-HCA	103 (57.2%)
U-HCA	27 (15%)
Undetermined	34 (18.9%)
Management	
Conservative	121 (67.2%)
Resection	50 (27.8%)
Embolization	6 (3.3%)
Radiofrequent Ablation	3 (1.7%)

This table shows baseline characteristics of included patients. Values are given in median (IQR) or n (%). The HCA-subtypes are explained in the methods section.

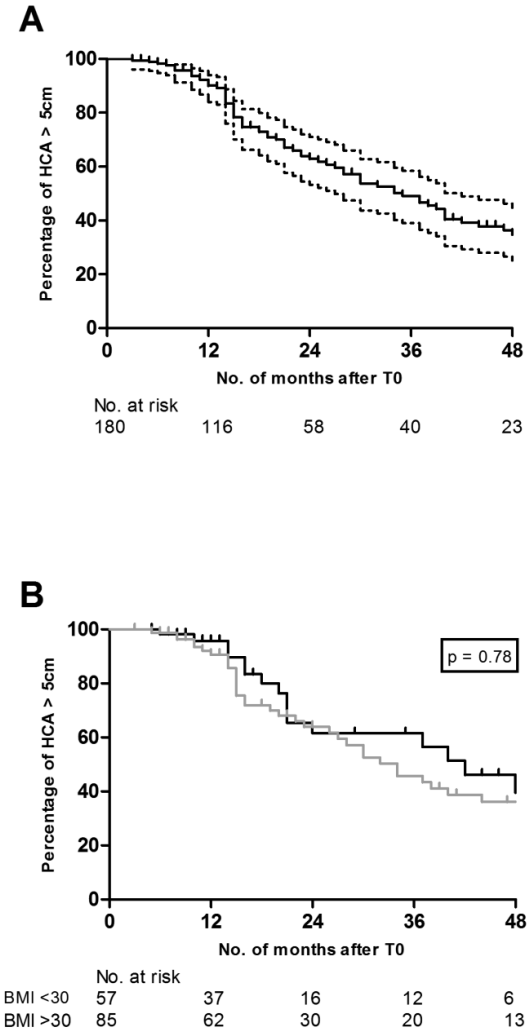

Figure 2. Kaplan Meier analysis

A. Kaplan-Meier curves for the event HCA regression to <5 cm in months after diagnosis (T0), median and 95% CI. B. Subanalysis based on BMI (< or > 30 kg/m2) in patients who used oral contraceptives.

Construction of the prediction model

After stepwise backward selection based on the AIC, the final multivariable model was comprised of three variables. These were HCA diameter at T0 (logtransformed, HR 0.05), T0T1 regression-over-time (HR 2.15) and HCA-subtype (HR 1.00 (reference), 2.93 and 2.40 for H-HCA, I-HCA and U-HCA, resp.) (table 3). The predicted chance (%) of HCA regression to <5cm within 1 and 2 years after diagnosis can be determined by:

1 year after diagnosis: $P = [1 - (\exp(-\exp(B) \times 0.063))] \times 100\%$

2 years after diagnosis: $P = [1 - (\exp(-\exp(B) \times 0.306))] \times 100\%$

B = [(LN(HCA diameter T0) x -2.996) + (T0T1 regression-over-time x 0.736) + [0 if H-HCA; 1.091 if I-HCA; 0.878 if U-HCA] + 11.749] x 0.830.

The overall predictive ability for regression to <5cm, calculated with internally validated C-statistic (corrected for optimism), was 0.79 (95%CI 0.73-0.85).

Table 3: multivariable Cox proportional hazards model

	Hazard ratio (95% confidence interval)	**p-value**
Diameter of HCA at diagnosis (logtransformed, mm)	0.05 (0.02– 0.13)	<.001
T0T1 regression over time	2.15 (1.75 – 2.70)	<.001
HCA subtype		
H-HCA	1.00 (reference)	
I-HCA	2.93 (1.19 – 7.21)	0.02
U-HCA	2.40 (0.88 – 6.55)	0.09

This table shows the multivariable Cox proportional hazards analysis of selected factors.

Sensitivity analyses

Three sensitivity analyses were performed. In the first only baseline characteristics were used, so T0T1 regression over time was discarded. This resulted in a multivariable model with HCA diameter at T0 (logtransformed, HR 0.1) and HCA subtype (HR 1.00 (reference), 9.86 and 15.34 for H-HCA, I-HCA and U-HCA, resp.). The internally validated C-statistic (corrected for optimism) was 0.79 (95%CI 0.72-0.91). Sensitivity analysis in patients with pathologically proven HCA only (n = 98 of which 29 reached the clinical endpoint of regression to <5cm) provided us with a multivariable model comprised of the same three variables as the complete analysis with similar hazard ratios and a C-statistic of 0.77. The third analysis was performed in patients who were treated conservatively only (n = 121 of which 74 reached the clinical endpoint of regression to <5cm), also resulting in a

multivariable model comprised of the same three variables as the complete analysis and a C-statistic of 0.73.

Application of the prediction model

The final model was translated into a chance assessment tool. Predictors include diameter at diagnosis, diameter at first follow-up, dates of diagnosis and first follow-up and HCA-subtype (T0T1 regression-over-time will be calculated automatically). The chance assessment tool will provide the estimated chance of regression to <5cm at 1 and 2 years after diagnosis (figure 3).

The chance assessment tool is available via https://hcaprediction.shinyapps.io/calculator/.

Predictors		**Value**
Diameter at diagnosis	*[mm]*	70
Date diagnosis	*[dd-mm-yyyy]*	20-09-2017
Diameter at first follow-up	*[mm]*	60
Date first follow-up	*[dd-mm-yyyy]*	01-03-2018
Subtype	*[0=H-HCA | 1=I-HCA | 2=U-HCA]*	1

Predicted chance of regression to <5cm (%)	
1 year after diagnosis	**7**
2 years after diagnosis	**29**

Figure 3. Chance calculator

An example of a patient in the chance calculator. Patient had a 70mm inflammatory HCA at diagnosis that regressed to 60mm at first follow-up. The predicted chance of regression to <5cm is 7% one year after diagnosis and 29% two years after diagnosis. The chance calculator is available via https://hcaprediction.shinyapps.io/calculator/.

DISCUSSION

In this study of 180 female patients diagnosed with HCA >5cm in three tertiary referral centers in the Netherlands, we present a clinical chance assessment tool able to predict the probability of HCA regression to <5cm at one and two years after diagnosis. The model comprises three easily accessible variables: HCA diameter at diagnosis, T0T1 regression-over-time and HCA subtype. This study is the first to develop a prediction model from a clinical perspective for patients with HCA. The model can be used for patients diagnosed with HCA >5cm that still exceed 5cm at first follow-up imaging and estimates the chance of regression to <5cm at 1 and 2 years after diagnosis. It can be of aid to clinicians in

decisions pertaining to surgery or continued surveillance. Using this model resection can be reserved for patients with low probability of HCA regression, whereas unnecessary resection in patients with a high chance of HCA regression can be avoided. A considerable health benefit could be provided as HCA is associated with obesity and the complication risk following surgery is significantly increased in such patients (19, 20).

To identify factors predictive of HCA regression to <5cm, five variables were considered to be clinically relevant and easily accessible. Age at diagnosis and BMI turned out to be the least predictive and were therefore not included in the final model. Ideally, we would have wanted to add change in BMI as a potential predictive variable, as weight loss seems to be a factor to cause regression of HCA (2). In our series a subanalysis in patients who used OC showed no significant differences in reaching the clinical endpoint of <5cm between patients with BMI < or > 30 kg/m^2. Unfortunately, change in BMI was underreported in all centers. Our results show that the association between a high BMI at diagnosis and regression of HCA is minor if anything, future studies should focus on prospectively assessing the association between regression and weight loss. The finding that age is not a significant predictor surprised us, as it has been established that HCA regress after menopause (21). This may be attributed to the fact that the effect of cessation of OC causes the first regression while the effect of age will not be noticeable until a later stage of follow-up.

The model shows that size of the HCA at diagnosis and T0T1 regression-over-time, defining the regression of the HCA over time between diagnosis and first follow-up imaging, are associated with regression to <5cm. Larger HCA have a lower chance of reaching the clinical endpoint of <5cm, as do HCA that show little regression in the first follow-up period. Hemorrhage was only seen on diagnostic imaging and did not occur after establishment of HCA diagnosis and cessation of OC and no malignant transformation was seen during follow-up. This suggests that a significant decrease in size as such might be just as relevant to prevent bleeding as the decrease in size to <5cm. Currently there is no data supporting the concept that there is still a risk of bleeding in regressing HCA. Therefore, surgery might not be necessary even in HCA >5cm to prevent bleeding as the risk of bleeding in a HCA showing regression in size, apparently is very small. This study supports this concept in a large clinical series.

The results show that the chance of regression to <5cm is lower in H-HCA, as compared to I-HCA and U-HCA. However, given the low risk of complications in H-HCA, a conservative approach seems justified in confirmed H-HCA, independent of the chance of regression. Additionally, as this study might lead to a more conservative approach regarding HCA in general, subtype determination and biopsy within the diagnostic workup becomes increasingly important. In this study we deliberately excluded men with HCA and patients

with histologically proven β-(I)HCA, as resection is recommended in these cases given the higher risk of malignant transformation (9, 10). Additionally, early resection might be performed in sh-HCA given the apparent higher risk of bleeding (5). A conservative approach may only be justified when HCA-subtype is established in I-HCA and H-HCA, preferably with biopsy.

We performed three different sensitivity analyses in this study. In the first, the model was developed with baseline characteristics only to see whether the model may be used at diagnosis as well. This resulted in a model with HCA diameter at diagnosis and HCA-subtype only and a comparable C-statistic as compared to the complete analysis. We believe however that the model is of more interest to use after the first follow-up imaging, as a conservative approach with lifestyle changes and follow-up is advised in all patients with HCA, irrespective of the diameter. The second and third sensitivity analyses were performed in patients with pathologically proven HCA and those who were treated conservatively only. Both show a model comprised of the same variables as the model from the complete analysis with comparable HR's. We aimed to make the cohort for the complete analysis as large as reliably possible, in order to make a more accurate model.

A previous study performed in the corresponding center aimed to evaluate whether a 6-month interval as suggested in the EASL guideline is sufficient to expect HCA regression to <5 cm and showed that the cut-off point for the assessment of regression could be prolonged to at least 12 months (12, 13). Time-to-event analysis showed that HCA with larger baseline diameter take considerably longer to regress, as was seen in the current prediction model. The previous study did not find a statistically significant difference between HCA subtype and median time to regression, whereas in the current study HCA subtype is included in the prediction model. This difference might well be explained by the larger population in the current study (180 vs 118) or the statistical analysis (Kaplan-Meier versus multivariable Cox regression).
This study is subject to a few limitations. Patients were included between 2000 and 2017, a period in which the quality of imaging improved considerably and management might have changed. However, no correlation was found between year of diagnosis and whether an intervention was performed. Secondly, interval censoring may occur as imaging provides measurements at a set time point. Thirdly, we used two dimensional measurements to assess tumor regression. We are well aware that two dimensional measurements do not represent a volume decrease, however the long-term follow-up indicates a reliable outcome of these measurements. A fourth limitation might lie in the fact that all data was collected retrospectively and we had missing data for BMI and HCA subtype. Multiple imputation was used to account for the missing data.

Finally, the last and most important limitation is the lack of external validation in an independent study population. Although internal validation with bootstrapping techniques suggested a good model fit with minimal overfitting, external validation is preferred before implicating the model in the management of HCA. For external validation it is custom to have a dataset of at least 100 events, and as HCA is a rare tumor which used to be mostly resected, it will take more time before such a dataset for external validation is available.

In conclusion, it was demonstrated in a large clinical series that a multivariable model comprising of the three easily accessible variables HCA diameter at diagnosis, T0T1 regression-over-time and HCA subtype could be helpfull to assess the chance of HCA regression in female patients with non β-catenin mutated HCA >5cm at diagnosis that still exceed 5cm at first follow-up imaging if they adhere to life style adaptations, including cessation of OAC. The model may be of help to clinicians in making a well-informed management decision, reserving invasive treatment only for those patients with a with a high risk of complications and a low chance of HCA regression to <5cm. The model still requires external validation in an independent study population. Other investigators are invited to share their data in order to further improve the risk estimations of the current model.

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CHAPTER 4

A multicenter retrospective analysis on growth of residual hepatocellular adenoma after resection

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Submitted

ABSTRACT

Background: HCA is a benign liver tumor that may require resection in select cases. The aim of this study was to assess growth of residual HCA in the remnant liver and to advise on an evidence-based management strategy.

Method: This multicenter retrospective cohort study included all patients with HCA who underwent surgery of HCA and had residual HCA in the remnant liver. Growth was defined as an increase of >20% in transverse diameter (RECIST criteria). Data on patient and HCA characteristics, diagnostic work-up, treatment and follow-up were documented and analyzed.

Results: A total of 134 patients were included, one male. At diagnosis, median age was 38yrs (IQR 30.0-44.0) and median BMI 29.9kg/m^2 (IQR 24.6-33.3). After resection, median number of residual sites of HCA was 3 (IQR 2-6). Follow-up of residual HCA showed regression in 24.6%, stable HCA in 61.9% and growth of at least one lesion in 11.2%. Three patients (2.2%) developed new HCA that were not visible on imaging prior to surgery. Four patients (3%, one male) underwent an intervention as growth was progressive. No statistically significant differences in clinical characteristics were found between patients with growing residual or new HCA versus those with stable or regressing residual HCA.

Conclusion: In patients with multiple HCA who undergo resection, growth of residual HCA is not uncommon but interventions are rarely needed as most lesions stabilize and do not show progressive growth. Surveillance is indicated when residual HCA show growth 6-12 months after resection, enabling intervention in case of progressive growth.

INTRODUCTION

Hepatocellular adenoma (HCA) is a benign tumor of the liver which predominantly occurs in females. It is associated with the use of oral contraceptives (OC) or androgens, obesity and metabolic disorders such as glycogen storage disease (GSD) or hepatocyte nuclear factor 1a maturity onset diabetes of the young (HNF1A-MODY)(1-3). Regression often occurs after cessation of OC and weight reduction (4-6). Several molecular HCA subtypes have been described: HNF-1α inactivated (H-HCA), inflammatory (I-HCA), β-catenin-activated (β-HCA), β-catenin-activated inflammatory (β-IHCA), and recently sonic hedgehog (sh-HCA) adenomas (7, 8). When no specific mutations are found, the HCA are termed unclassified (U-HCA).

After HCA diagnosis, lifestyle adaption with cessation of OC and weight reduction is indicated, irrespective of HCA diameter (9). In case of growth or when the HCA fails to regress to less than 5cm, resection may be indicated (9, 10). Other indications for resection are HCA in men, patients with β-HCA or β-IHCA.

Up to half of all patients with HCA present with multinodular disease which appears to be associated with a higher BMI (11, 12). The risk of complications does not differ from those with solitary HCA, therefore it has been recommended to base management decisions in patients with multiple HCA on the size of the largest tumor (9). In patients with unilobular disease, a hemihepatecomy or segmental resection can be performed to resect all HCA. However, in patients with widespread HCA, residual HCA may remain in situ after resection. It is unclear whether these HCA may grow due to post resectional liver regeneration, as studies regarding the follow-up of residual HCA have been lacking from published literature. The aim of this study was to assess whether growth of residual HCA in the remnant liver occurs and to advise on an evidence-based management strategy..

METHODS

This study was a multicenter retrospective cohort study performed in five major hepatobiliary centers in the Netherlands and Belgium [Erasmus MC University Medical Center Rotterdam, Amsterdam University Medical Centers, Location Academic Medical Center and Location VU Medical Center, University Medical Center Groningen and University Hospital KU Leuven]. Eligible patients were those diagnosed with multiple (>1) HCA, based on either contrast-enhanced MRI, histological examination or both. Patients were included if they had undergone resection of one or more HCA and had residual sites of HCA in situ after resection. The minimum follow-up time after resection was six months.

The study protocol was reviewed by the accredited institutional review board; informed consent was waived.

Data collection

Electronic medical records were reviewed for baseline patient characteristics: sex, age at diagnosis, BMI, comorbidity and use of hormonal medication were documented. Lesion characteristics documented were size of the largest HCA at diagnosis, number of lesions and size of the residual HCA before and after resection. Histopathological assessment of resection specimen was performed at each participating center. HCA subtypes were determined based on histology of the resection specimen or of a liver biopsy. When no material for pathology was present, subtype differentiation was based on contrast-enhanced MRI. Subtypes included were H-HCA, I-HCA, β-HCA, β-IHCA and U-HCA; sh-HCA were not included as immunohistochemical staining and molecular diagnosis was not yet implemented at the time of diagnosis of most patients. Indication for resection, type of resection (hemihepatectomy [at least 3 segments] or segmental resection [2 segments or less]) and the number of resected HCA were documented. Complications after surgery were documented and graded according to the Clavien-Dindo Classification, with grade I and II complications classified as minor complications and grade III or higher as major complications. Follow-up of the residual HCA was based on imaging (MRI or CT), two dimensional measurements were performed and the largest transversal diameter was documented. Tumor growth was defined as an increase of >20% and HCA regression as a decrease of >30% as per RECIST criteria for response evaluation in solid tumors (13). The three largest residual tumors were assessed as index lesions for growth or regression. If at least one HCA showed an increase of >20% this was scored as growth [regardless the behavior of the other two], if at least two HCAs showed a decrease of >30% and the other remained stable, this was scored as regression.

Statistical analysis

Continuous variables were summarized as median and interquartile range(IQR), categorical variables as frequencies (n) and percentages. Differences between groups were analyzed using Mann–Whitney U test for continuous variables and $\chi 2$ test for categorical variables. All statistical analyses were performed with SPSS software version 24.0 (IBM, Armonk, New York, USA).

RESULTS

Clinical characteristics

A total of 134 patients were included: 48 from Erasmus MC University Medical Center Rotterdam, 30 from Amsterdam University Medical Centers, Location Academic Medical

Center and 6 from Location VU Medical Center, 35 from University Medical Center Groningen and 15 from University Hospital KU Leuven. Patients were diagnosed between 1992 and 2018 [1989-1999: n = 5, 2000-2004: n = 15, 2005-2009: n = 42, 2010-2014: n = 51, 2015-2018: n = 21]. Baseline characteristics are summarized in Table 1.

Table 1: baseline characteristics

	N (%) or median (IQR)
Sex	
Female	133 (99.3)
Male	1 (0.7)
Age at diagnosis (yr)	38 (30.0 – 44.0)
BMI (kg/m²)	29.9 (24.6 – 33.3)
HCA related Comorbidity	
Diabetes mellitus	17 (12.7)
Glycogen storage disease	3 (2.2)
Maturity onset diabetes of the young	2 (1.5)
Hormone usage	
Oral contraceptives	116 (86.6)
None	6 (4.5)
Steroids or other hormonal medication	2 (1.5)
Unknown	10 (7.5)
Diameter of largest HCA at diagnosis (mm)	89 (69.5 – 110.0)
Number of HCA at diagnosis	
2 – 5	53 (39.6)
6 – 10	48 (35.8)
> 10	33 (24.6)
Diagnostic work-up	
Contrast enhanced MRI	123 (91.8)
Biopsy	48 (35.8)
HCA subtype	
H-HCA	18 (13.5)
I-HCA	69 (51.5)
B-HCA	2 (1.5)
B-IHCA	3 (2.2)
U-HCA	10 (7.5)
H-HCA + I-HCA	2 (1.5)
Undetermined	30 (22.4)

Surgical resection

All patients underwent surgical resection of HCA, median time between diagnosis and surgery was 5 months (IQR 2.0 – 12.0). The most common indication for resection was size of the HCA >50mm (46.3%), followed by atypical imaging characteristics or suspected hepatocellular carcinoma (HCC) (20.1%), hemorrhage (11.9%) and symptoms (10.4%). Other indications were growth of HCA (6.7%) after cessation of OC, an active pregnancy wish (3.0%), confirmed β-HCA on biopsy (0.7%) and male sex (0.7%). The majority was treated with a segmental resection (61.2%) or hemihepatectomy (32.8%), median hospital stay after resection was 9 days (IQR 7.0 – 10.0). No major postoperative complications occurred, 18.7% of the patients suffered minor postoperative complications.

Follow-up

Median total follow-up time was 49 months (IQR 27.0 – 78.0) and the median number of residual HCA was 3 (IQR 2 – 6). Follow-up of residual HCA showed regression in 24.6%, stable lesions in 61.9%, growth in 11.2% and in 2.2% new lesions were observed that were not visible on imaging prior to resection (Figure 1A). No hemorrhage occurred in the residual HCA during follow-up.

No statistically significant differences between patients whose residual HCA showed growth or who developed new lesions versus those whose residual HCA were stable or showed regression were found for BMI, age at diagnosis, number of residual HCA, HCA-related comorbidity, HCA subtype and resection type (Table 2 and Figure 1B and 1C).

Among the 18 patients with growing or new lesions, 14 were treated conservatively. In these patients, growth was diagnosed at the first follow-up imaging after resection, all lesions remained <50 mm and the majority stabilized within two years of follow-up. Four patients (3%, three females and one male) underwent intervention for growing residual HCA. Of the females, one underwent transarterial embolization (TAE), the second underwent two re-resections and the third patient underwent both re-resection as well as TAE for growing residual HCA (Figure 2). None of these patients had HCA-related comorbidity and no dysplasia or transformation to HCC was found in these patients. The only male patient included in this study, known with non-cirrhotic portal hypertension and diagnosed with β-IHCA, underwent radiofrequency ablation (RFA) for a new HCA and is currently on the waiting list for liver transplantation because of multiple new and progressively growing HCA as depicted by contrast-enhanced MRI. Histopathologic examination of the resection specimen confirmed HCA in all four patients. In one patient with a residual HCA of 15mm, follow-up after resection showed complete regression after resection as depicted by contrast-enhanced MRI. She then restarted her OC and the residual HCA grew to 60mm. After cessation of OC, the residual HCA regressed completely again (Figure 2).

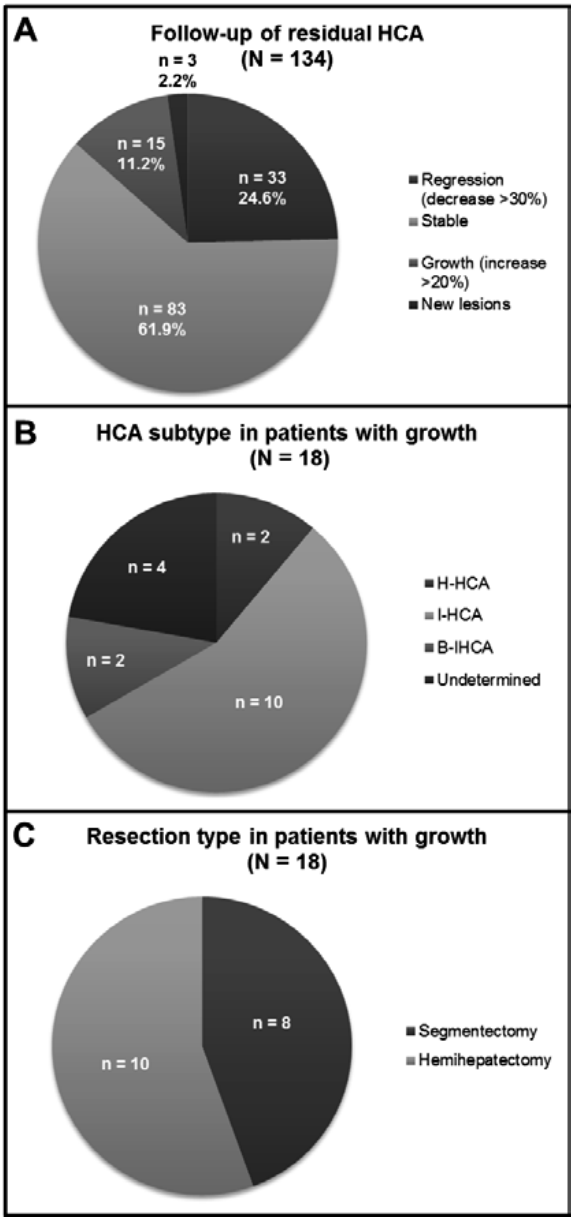

Figure 1. Follow-up of residual HCA and characteristics of growing or new lesions

A: Follow-up of residual HCA in remnant liver showing regression in 24.6%, stable lesions in 61.9%, growth in 11.2% and new lesions in 2.2%. B: HCA subtype distribution in 18 patients with growing or new lesions with 2 H-HCA, 10 I-HCA, 2 B-IHCA and 4 undetermined. C: Resection type distribution in 18 patients with growing or new lesions with 8 patients who underwent segmental resection and 10 who underwent hemihepatectomy.

Table 2: growing or new lesions vs stable or regressing lesions

	Growing/new lesions n = 18	**Stable/regressing lesions n = 116**	**p-value**
	N (%) or median (IQR)	**N (%) or median (IQR)**	
BMI (kg/m²)	26.4 (23.7 – 30.5)	30.1 (24.8 – 33.6)	0.169
Age at diagnosis (yr)	41 (33.3 – 44.3)	38 (29.3 – 44.0)	0.579
Number of residual HCA	2 (1.5 – 5)	3 (2 - 6)	0.339
HCA related Comorbidity			0.136
Diabetes mellitus	0 (0)	17 (14.7)	
GSD	0 (0)	3 (2.6)	
MODY-3	0 (0)	2 (1.7)	
HCA subtype			0.291
H-HCA	2 (10.5)	16 (14.0)	
I-HCA	10 (55.6)	59 (50.9)	
B-HCA	0 (0)	2 (1.7)	
B-IHCA	2 (10.5)	1 (0.9)	
U-HCA	0 (0)	10 (8.6)	
H-HCA + I-HCA	0 (0)	2 (1.7)	
Undetermined	4 (22.2)	26 (22.4)	
Resection type			0.098
Segment resection	8 (44.4)	74 (63.8)	
Hemihepatectomy	10 (55.6)	34 (29.3)	
Enucleation	0 (0)	8 (6.9)	

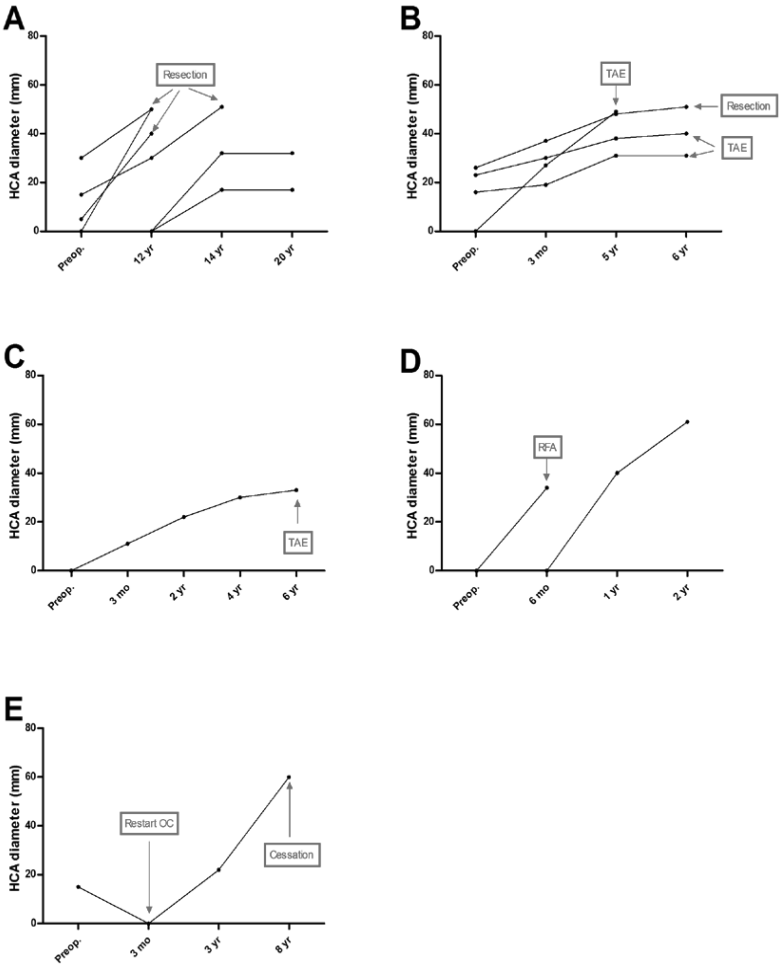

Figure 2. Cases with growing residual HCA

HCA = hepatocellular adenoma. Preop = preoperative. Yr = number of years postoperative. Mo = number of months postoperative. OC = oral contraceptive. TAE = transarterial embolization.
A: Female patient with multiple residual HCA who underwent a re-resection 12 and 14 years after the first resection due to progressively growing residual HCA. B: Female patient with multiple residual HCA who underwent transarterial embolization and re-resection 5 and 6 years after the first resection due to progressively growing residual HCA. C: Female patient with single residual HCA who underwent transarterial embolization 5 years after resection due to progressively growing HCA. D: Male patient with β-catenin mutated HCA, who underwent radiofrequency ablation six months after resection due to one new HCA. Patient still has multiple new growing lesions and is currently on the waiting list for liver transplantation. E: Female patient with single residual HCA that showed complete regression after resection. When OC was restarted the lesion showed progressive growth. It regressed again after cessation of OC.

DISCUSSION

Hepatocellular adenoma may require resection in selected cases. In patients with many and widespread HCA, residual sites of HCA may remain in situ after resection. It is unclear whether these lesions may grow due to liver regeneration after resection. This retrospective cohort study including 134 patients evaluated whether in patients with multiple HCA, growth of residual HCA in the remnant liver occurs and to advise on an evidence-based management strategy.

In this study, growth of residual HCA was observed in 11.2% of patients, whereas in 2.2% new tumors were observed that were not visible on imaging prior to resection. The majority of patients who had growing residual HCA lesions were managed conservatively, whilst growth or new HCA were a reason for intervention in 3.0% of patients. Reasons for intervention in cases with growing residual lesions were either an increase to >50mm or progressive growth without stabilization of the lesion. Patients either underwent RFA, re-resection, TAE or a combination of these modalities. One of these patients was a male with non-cirrhotic portal hypertension, the remaining three females did not have HCA-related comorbidity. The male patient with growing and new HCA is still under surveillance because of progressive growth and is now on the waiting list to undergo liver transplantation, as this is a male patient with β-catenin mutated HCA. Although this patient was diagnosed with HCA based on histopathological examination, the new and progressively growing lesions suggest that the diagnosis might also be well-differentiated HCC. It may be very difficult to distinguish HCA from well-differentiated HCC, even for expert pathologists (14-16).

In one patient with a residual HCA, follow-up after resection showed complete regression after resection as depicted by contrast-enhanced MRI. After she restarted her OC, the residual HCA showed growth. Complete regression occurred again after cessation of OC. This confirms that patients with HCA have a lifelong contraindication for the use of OC, even if no residual HCA are present after resection or complete regression of residual HCA has occurred.

Regression of residual HCA occurred in 24.8% of the patients. This could either still be a result of cessation of OC or a result of weight loss or menopause, but might also be explained by the altered tumor regulation of the tissue environment in the liver with resection of the largest lesion(s). It has been established that this tumor regulation of the tissue environment plays a role in tumor formation and tumor growth in HCC[17]. Unfortunately, the latter is still unexplored territory in the field of HCA. An interesting group to study regarding tumor environment of HCA would be patients with multiple tumors, who have undergone TAE or RFA for the largest tumor, since liver regeneration due to increased portal flow plays no role in these patients.

In this cohort, factors predictive of growth of residual HCA could not be identified. BMI, age at diagnosis, resection type, number of residual HCA, HCA-related comorbidity and HCA subtype were considered to be clinically relevant, but no statistically significant differences were found between patients whose residual HCA showed growth or who developed new lesions versus those whose residual HCA were stable or showed regression. Ideally, change in BMI would have been added as a potential predictive variable, as weight loss seems to be related to regression of HCA and therefore weight gain may induce growth (18). Unfortunately, in the participating centers, change in BMI was underreported. Surprisingly, growth did not occur more often in patients who underwent hemihepatectomy versus those who underwent segmental resection or enucleation. As liver regeneration increases with a larger resected hepatic volume, a difference would have been expected. Additionally, no differences were found for HCA subtype while there has been a difference found for HCA regression in different subtypes (19). The lack of statistically significant differences may well be attributed to the relatively small sample size.

The current study is the first to rapport on the management of residual HCA after hepatic resection in a large, multicenter cohort with long follow-up. The multicenter design offers advantages of an increase of statistical power and generalizability of the results. Inevitably, it is also subject to some limitations. First of all, the retrospective study design is inherent to bias. Secondly, patients were included during a long period with five patients diagnosed even before 2000. All of these patients were diagnosed at an early age and followed for at least 20 years. Additionally, all HCA were proven on histopathological examination. A third limitation might lie in the fact that transversal measurements were used to assess tumor growth or regression as per RECIST criteria, and that the exact regeneration volume of the remnant liver was not measured. However, the authors believe that using transversal measurements reflects clinical practice. Finally, one might be that only one male patient was included. However, in the participating centers, males usually undergo treatment for all HCA given the higher risk of malignant transformation and therefore do not have any residual HCA after resection.

In conclusion, this study shows that growth of residual HCA is not uncommon and interventions are rarely needed as most lesions stabilize and do not show progressive growth. Follow-up of residual HCA should be performed at least once after surgery. When residual HCA show growth at the first follow-up after resection, patients should be kept under surveillance until the lesions stabilize. An intervention such as TAE or RFA for small HCA or re-resection in large or atypical residual HCA may be considered in case of progressive growth and if lifestyle advices (cessation of OC and weight loss) fail to cause stabilization or regression.

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CHAPTER 5

Hepatocellular adenoma during pregnancy: a prospective study on growth of the liver lesions

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ABSTRACT

Background & Aims: The presence of hepatocellular adenoma (HCA) in pregnant women requires special consideration, as it has been reported to carry the risk of growth and clinically significant haemorrhage. In this prospective study we assessed aspects of growth of HCA <5cm during pregnancy.

Methods: This was a multicentre prospective cohort study in pregnant women with suspected HCA <5cm on imaging. Definitive HCA diagnosis was established with MRI with hepatobiliary contrast agents (LCE-MRI), preferably before pregnancy. If at study inclusion a definitive diagnosis was lacking, LCE-MRI was performed after giving birth. Growth of the adenoma (defined as an increase of >20%) was closely monitored with ultrasound-examinations throughout pregnancy.

Results: Eighteen of 66 included women were excluded from analysis because postpartum LCE-MRI did not confirm the diagnosis HCA and showed the lesion to be focal nodular hyperplasia (FNH). The remaining 48 with a LCE-MRI confirmed HCA were followed during 51 pregnancies. Median age was 30 years (IQR 27-33) and BMI 31.9 kg/m^2 (IQR 26.3-36.6). Growth of HCA was seen in 13 of the pregnancies (25.5%); the median increase was 14mm (IQR 8-19). One woman whose HCA grew to >70mm successfully underwent transarterial embolization at week 26 of pregnancy to prevent further growth. The other 50 pregnancies proceeded without complications.

Conclusion: This study suggests that a HCA <5cm bears minimal risk for a pregnant woman and none for the child. As in a quarter of cases the HCA increased in size during pregnancy, we recommend close monitoring with ultrasound-examinations, enabling intervention if needed. In light of the large proportion of misdiagnosed HCA, LCE-MRI should be performed to prevent unnecessary anxiety in women with a benign liver lesion.

INTRODUCTION

Hepatocellular adenoma (HCA) occurs particularly among reproductive women and is associated with the use of oestrogen-containing oral contraceptives, androgens intake, obesity, and metabolic disorders (1, 2). The tumour may regress upon cessation of oestrogen-containing oral contraceptives and weight reduction (3, 4).

Several HCA subtypes can be distinguished radiologically on contrast-enhanced MRI with hepatobiliary contrast agents (LCE-MRI) (5, 6)) or on the basis of immunohistochemical staining or molecular characterization. They include hepatocyte nuclear factor 1α inactivated (H-HCA), inflammatory (I-HCA), β-catenin-activated (β-HCA), β-catenin-activated inflammatory (β-IHCA), and sonic hedgehog (sh-HCA) adenomas (7, 8). If specific mutations are not found, the HCA is labelled unclassified (U-HCA). Resection of a HCA >5cm is usually advocated if it does not regress to <5cm within 12 months, because the risk of complications is thought to be higher in HCA >5cm (9-12).

In pregnant women, HCA requires special attention because of the risk of hormone induced growth and rupture, which may threaten the life of both mother and child. Cobey and Salem reported that the mortality risk of ruptured HCA > 6.5cm during pregnancy was 44% for the mothers and 38% for the fetuses (13). However, almost all cases included in this review dated from the 1970s and 1980s. In 2011, our research group proposed close monitoring of pregnant women with small HCA instead of intervention, and suggested that women with small HCA should not be discouraged to fall pregnant. This suggestion was based on a study in which we monitored twelve women with documented HCA (5 with HCA >5cm and 7 with HCA <5cm) during a total of 17 pregnancies (14, 15). All pregnancies in this study had an uneventful course without adverse maternal or fetal outcomes.

Data on the behaviour of HCA during pregnancy and the safety aspects is still very limited. In this prospective study we assessed the effect of pregnancy on the biological behaviour of HCA, including the incidence of growth, the occurrence of complications and HCA-related interventions during pregnancy. To select a group of women with an a priori low risk of complications, we set the maximum HCA size at 5cm, as the relevant European guidelines state that HCA <5cm carry low risk of haemorrhage (11).

PATIENTS AND METHODS

Study design and population

This was a multicentre prospective cohort study performed in the Netherlands, the coordinating centre being a tertiary referral centre for focal liver lesions. The study protocol

was reviewed and approved by the institutional review board, has been published before and was registered in the Dutch trial register (NTR3034) (16).

We included pregnant women over 18 years of age who had been diagnosed with single or multiple HCA – the largest not exceeding 5cm at the moment of inclusion. Pregnancy had to be confirmed by an obstetrician or midwife with ultrasound (US) examination. Patients were included regardless of parity. The definite HCA diagnosis was based on LCE-MRI or biopsy, preferably performed before pregnancy because contrast agents might be toxic during pregnancy. When the lesion was found incidentally during pregnancy, LCE-MRI was performed postpartum. Exclusion criteria for the per protocol analysis were lack of LCE-MRI or biopsy, no confirmation of the diagnosis HCA on LCE-MRI, and termination of the pregnancy. Inclusion was open from November 2011 until January 2019.

Study procedures

Eligible women were invited to participate by their treating physicians at the outpatient clinic. Those who were interested were sent a letter explaining the study aims and were contacted by telephone to provide additional information and answer questions. Those who provided written informed consent completed a questionnaire registering: date of birth, length and weight, use of hormonal supplements before pregnancy (including possible fertility treatment), co-morbidities, parity and estimated date of delivery.

The participants were scheduled for repetitive ultrasound (US) examination at 14 (+/- 3), 20, 26, 32, 38 weeks of gestation and 6-12 weeks postpartum at their preferred hospital. The US examinations were performed by either a radiologist or a hepatologist with experience in hepatic US, who assessed HCA number, location, size, possible growth or regression, as well as US characteristics. Lesion growth was defined as an increase of >20% in transversal diameter and lesion regression as a decrease of >30% as per the response evaluation in solid tumours (RECIST) criteria (17). In case of suspected growth or atypical US characteristics during pregnancy, a conventional MRI (without contrast agents) was performed; the coordinating centre was consulted; and the hepatobiliary multidisciplinary tumour board considered the optimum treatment.

The US reports of participants in the various centers were collected at the coordinating center. Apart from HCA size at all time points, data on complications during pregnancy, vaginal or caesarean delivery, diagnostic work-up, size of HCA at the time of diagnosis and prior to pregnancy were recorded. In addition, the HCA-subtype was documented on the basis of immunohistochemistry or pathomolecular characterization when biopsy material was available, or on LCE-MRI features (5, 6, 18). If haemorrhage had occurred before pregnancy (causing a less reliable subtype determination based on LCE-MRI) or the subtype was not determined otherwise, the subtype remained undetermined ('missing').

The biological behaviour of HCA prior to pregnancy was assessed, that is: regression, growth or stable prior, also according to the RECIST criteria. The percentage of regression was calculated as followed: (diameter HCA at diagnosis – diameter HCA at last follow-up prior to pregnancy) / diameter HCA at diagnosis.

Statistical analysis

Continuous variables are summarized as mean ± standard deviation (SD) or as median and interquartile range (IQR). Categorical variables are presented as frequency (n) and percentages. Comparative analysis was performed with the Mann–Whitney U test for continuous variables and the $\chi 2$ test for categorical variables. All statistical analysis was performed with SPSS software version 21.0 (IBM, Armonk, New York, USA).

RESULTS

Inclusion and baseline characteristics

A total of 74 pregnant women diagnosed as having a HCA <5cm at the moment of inclusion were scheduled for close monitoring with US during pregnancy. Four refrained from follow-up, however, and four had a miscarriage, leaving 66 who completed follow-up. Eighteen women (18/66; 27%) were excluded from the analysis because LCE-MRI performed after delivery showed the lesion to be focal nodular hyperplasia (FNH) instead of HCA. Ultimately, 51 datasets were analysed as one woman participated twice and one women participated three times. Baseline characteristics are depicted in table 1.

In 40 pregnancies (40/51; 78%) the diagnosis HCA was confirmed with LCE-MRI prior to pregnancy. Three patients underwent diagnostic biopsy confirming HCA diagnosis. The median HCA diameter at diagnosis was 33mm (IQR 20 – 59); at the last imaging prior to pregnancy it was 23mm (IQR 19 – 39). In 26 cases, regression had occurred after cessation of oral contraceptives prior to pregnancy; in 14 cases the HCA size had remained stable. The other 11 HCA were found incidentally during pregnancy.

Follow-up during pregnancy

In 18% of the 51pregnancies, all five scheduled US examinations had been performed; in 54% four examinations; in 22% three examinations; and in 6% two. Reasons for missing examinations were inclusion later than 14 weeks of pregnancy, delivery prior to 38 weeks and patient non-compliance. Postpartum US was performed in 70% and showed either stable or regressing lesions.

In 27 of the 51 pregnancies (53%), the lesions remained stable; in 11 (22%) they had regressed, and in 13 (25%) they had grown. In all 13 cases, growth had occurred between

Table 1: baseline characteristics

	N (%) or median (IQR)
Age at inclusion (yr) (n = 51)	30 (27 – 33)
BMI (kg/m²) (n = 51)	31.9 (26.3 – 36.6)
Diameter HCA at diagnosis (mm) (n = 48)	33 (20 – 59)
Diabetes (n = 48)	
Diabetes mellitus	3 (6.3)
Gestational diabetes	3 (6.3)
No	42 (88.8)
HCA subtype (n = 48)	
H-HCA	2 (4.2)
I-HCA	16 (33.3)
U-HCA	12 (25.0)
Undetermined	18 (37.5)
Hormone usage before pregnancy (n = 48)	
Oral contraceptives	43 (89.6)
None	4 (8.3)
Other	1 (2.1)
Prior pregnancy (n = 51)	
Yes	12 (23.5)
No	39 (76.5)
Fertility treatment (n = 51)	
Yes	3 (5.9)
No	48 (94.1)
Delivery (n = 51)	
Vaginal	45 (88.2)
Caesarean	6 (11.8)

Age, BMI and prior pregnancy are given per pregnancy (n = 51); the occurrences of diabetes and hormone usage before pregnancy are given per patient (n = 48).

14 and 32 weeks of gestation; the median growth was 14mm (IQR 8 – 19) (Figure 1). During pregnancy, HCA imaging indicating bleeding was not found and lesion characteristics did not differ between the subgroup of women with stable or regressing HCA and the subgroup of women with growing lesions (p = 0.249, table 2). Additionally, there were no statistically significant differences between these subgroups for age at inclusion, BMI at inclusion, HCA diameter at diagnosis, HCA subtype and the percentage of HCA regression before pregnancy (table 2). One woman underwent a transarterial embolization of the HCA during pregnancy because it had grown from 49mm at week 14 to a maximum of 76mm at week 20 (figure 2). She had been diagnosed with HCA two years prior to pregnancy;

at baseline it measured 93mm and it regressed to 49mm within one year after cessation of oral contraceptives (figure 3). After the embolization, the lesion regressed to 65mm at week 26 and 51mm at week 32. Labour was induced at 38 weeks, proceeded without complications, and resulted in the birth of a healthy child. The other 50 pregnancies proceeded without complications. Six patients had a caesarean delivery, all because of reasons unrelated to HCA. The remaing deliveries were vaginal with an uneventful course.

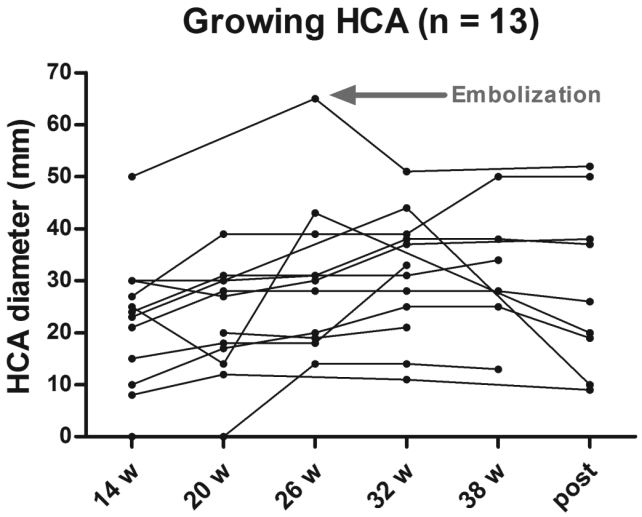

Figure 1. Growing HCA during pregnancy.

This figure shows the 13 growing HCA during pregnancy at each time point: 14 (+/-3), 20, 26, 32, 38 weeks of gestation and 6-12 weeks postpartum).

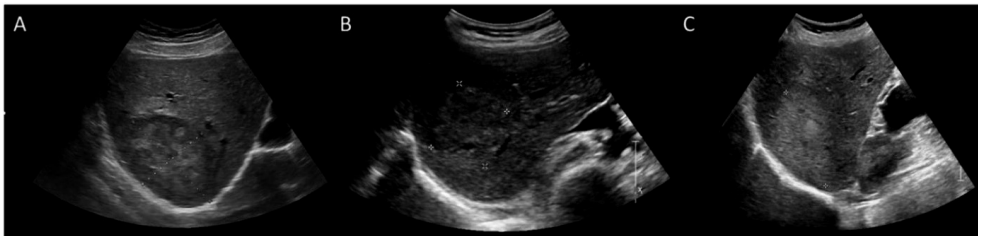

Figure 2. Ultrasound of growing HCA.

Patient who was diagnosed with a single HCA of 93mm (A: ultrasound at diagnosis). After cessation of oral contraceptives the lesion regressed to 49mm (B: last ultrasound prior to pregnancy). During pregnancy the lesion showed growth again to a maximum of 76mm (C: ultrasound showing growth during pregnancy). A transarterial embolization was performed.

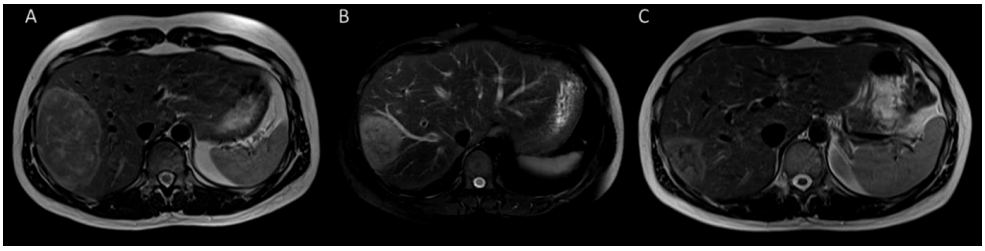

Figure 3. MRI of growing HCA.

T2 weighted MRI images of a patient with a larger single HCA. A: Diagnostic contrast enhanced MRI showing a large HCA (93mm) in the right hemiliver. B: MRI performed during pregnancy due to suspected growth, showing a 65mm lesion. As the lesion grew significantly early on in the pregnancy, transarterial embolization was performed. C: MRI postpartum, showing a 52mm lesion with central necrosis after transarterial embolization.

Table 2: Comparison of growing vs stable/regressing lesions during pregnancy

	Stable or regression (n = 38)	Growth (n = 13)	p-value
Age at inclusion (yr)	29 (27 – 32.3)	32 (29 – 34)	.155
BMI (kg/m²)	32.3 (26.1 – 37.6)	29.0 (27.1 – 35.9)	.603
Diameter HCA at diagnosis (mm)	35.0 (20.0 – 64.0)	30.0 (20.0 – 60.0)	.952
HCA subtype			.540
H-HCA	2 (5.3)	0 (0)	
I-HCA	12 (31.6)	4 (30.8)	
U-HCA	12 (31.6)	2 (15.4)	
Undetermined	12 (31.6)	7 (53.8)	
Percentage regression of HCA before pregnancy	35.9 (13.9 – 59.5)	47.3 (0 – 73.0)	.858
Biological behavior HCA prior to pregnancy			.249
Regression	23 (60.5)	8 (61.5)	
Stable	5 (13.2)	4 (30.8)	
Unknown	9 (23.7)	2 (15.4)	

Comparative analysis was performed with Mann–Whitney U test for continuous variables and $\chi 2$ test for categorical variables. The percentage of regression of HCA before pregnancy was calculated as followed: (diameter HCA at diagnosis – diameter HCA at last follow-up prior to pregnancy) / diameter HCA at diagnosis. The biological behavior of HCA prior to pregnancy was assessed and it was documented whether the lesion regressed or was stable by the RECIST criteria.

Three patients underwent fertility treatment in order to become pregnant, of which two underwent in vitro fertilisation (IVF) and one ovulation induction (OI). Although no ultrasounds were performed during the fertility therapy and therefore possible growth

of HCA was not monitored, no clinically relevant haemorrhage occurred during the treatment. During pregnancy, the HCA showed growth in 1 patient and in the other 2 remained stable.

Two women were enrolled in the study more than once, respectively two and three times. Both underwent LCE-MRI after each pregnancy. The former had a lesion of 20mm that remained stable during the first pregnancy, and then regressed in the two years until the second pregnancy to a point where it was not visible anymore. During the second pregnancy it grew to a maximum of 13mm. The latter had a lesion that remained stable at 43mm during the first and stable at 25mm during the second pregnancy, while it regressed during the third pregnancy.

Five women had been diagnosed with haemorrhagic HCA prior to pregnancy; all were hemodynamically stable and intervention was judged unnecessary. In two, the HCA had grown during pregnancy; in the other three it had remained stable. Neither of the lesions showed signs of haemorrhage during pregnancy.

None of the 18 women who were diagnosed with FNH postpartum had undergone LCE-MRI prior to pregnancy in the hospital where they were diagnosed. Suspicion of HCA had been raised during conventional MRI (without hepatobiliary contrast agent) in 12, ultrasound in 4, CT in 1 and contrast-enhanced ultrasound in 1.

DISCUSSION

In this study of 51 pregnancies in 48 patients with HCA <5cm, we investigated the incidence of growth, occurrence of complications and HCA-related interventions during pregnancy. HCA growth was defined as an increase of more than 20%, as per the RECIST criteria, and this was seen in one quarter of cases. The median growth was 14mm. Neither tumour rupture nor haemorrhage was observed during the study period, and factors predictive of growth could not be identified.

To the best of our knowledge, this is the first prospective study investigating the biological behaviour of small HCA during pregnancy. Until recently, the general consensus had been that women with HCA should be discouraged to become pregnant without prior treatment – in view of the risk of clinically relevant or even life-threatening haemorrhage. Furthermore, in a paper studying mainly cases from the 1970s and 1980s it was reported that during pregnancy the mortality risk of ruptured HCA may be as high as 44% for the mother and 38% for the foetus (13). It should be noted that this was at a time in which the

entity of HCA was less well-known and embolization for acute haemorrhage was not yet available in all hospitals.

Only in one case, in which rapid growth of the lesion during pregnancy was seen, an intervention was performed to reduce the risk of haemorrhage. This patient had previously been diagnosed with a large HCA that had regressed to <5cm within one year after cessation of oral contraceptives. The intervention consisted of transarterial embolization; labour was uncomplicated and resulted in the birth of a healthy child. As other complications did not occur in this cohort, we suggest that women with HCA <5cm who wish to conceive should not be discouraged or advised to refrain from this. Still, in view of the fact that growth occurred in one quarter of cases, shared decision making with the patient and close monitoring during pregnancy is indicated. We surmised that HCA that regress after cessation of oral contraceptives are more sensitive to hormones – and therefore are more prone to grow during pregnancy. However, we could not confirm this with the present data, probably because of the lack of statistical power resulting from the small sample size. Likewise, we could not detect differences between HCA subtypes, although previous studies have shown different probabilities of HCA regression and risk of complication for various HCA subtypes (8, 19). Surprisingly, almost one quarter of HCA in this study regressed during pregnancy. The exact mechanism and reason for this regression remains unclear.

In this study, only three patients underwent fertility treatment in order to become pregnant. No ultrasounds were performed during fertility treatment as these patients were only included after conception, but fertility treatment was described as uneventful. Indeed, it would be interesting to monitor the size of HCA during fertility treatment and its hormonal stimulation and to relate the outcome with changes during pregnancy. Future studies should aim to investigate the effect of fertility treatment on HCA.

Two women in this study were enrolled more than once: one during two pregnancies and one during three pregnancies. HCA behaviour was not the same in the consecutive pregnancies. An additional nine women already had had an uneventful pregnancy before being diagnosed with HCA, and all had uneventful pregnancies again during this study. By way of comparison, in a previous study almost half of the women with HCA already had carried at least one pregnancy to term before diagnosis (20).

The median BMI at inclusion of patients enrolled in this study is fairly high. A relationship between HCA and obesity has been well established in literature (4, 21-23). Additionally, the median BMI in this study is comparable to the median of previous study populations (12, 19). Another notable result in the baseline characteristics is the high fraction of patients with U-HCA, especially as compared to previously published studies (7). Previous

studies regarding the distribution of HCA subtypes have particularly been performed in cohorts of patients with larger adenomas. An analysis regarding HCA subtype related to the size has yet to be performed, but subtype distribution might differ between large and small HCA. Additionally, biopsy is less often performed when the lesion is small.

In the present study, a large proportion of suspected HCA actually proved to be FNH. Such a misdiagnosis could have a major impact on the life of the young woman, as FNH is not associated with hormone-induced growth and the lesion need not be closely monitored during pregnancy. Therefore, we highly recommend performing LCE-MRI if HCA is suspected and the woman wishes to conceive.

Regrettably, we had to deviate from the original study protocol of the PALM study as published in 2012 (16). This included two control groups for the purpose of assessing quality of life aspects: one of healthy pregnant women without HCA and one of pregnant women with diabetes mellitus. Because we could not find women without HCA willing to undergo regular liver US during pregnancy, these two control groups were discarded. Venipuncture, as described in the protocol, was discarded as well, because the majority of participants were unwilling to undergo venipuncture.

This study is subject to some limitations. First, only 18% of participants underwent the US-examination at every planned follow-up moment. The incomplete data do not, however, affect the primary study outcome. Second, the study cohort was too small to identify with a high degree of certainty risk factors for rupture, bleeding or growth. Nevertheless, considering the rareness of this liver tumour, the sample size was relatively large. Future studies should aim at identifying risk factors for pregnancy-related haemorrhage or growth of HCA. Lesion characteristics such as HCA subtype or HCA response after cessation of oral contraceptives might affect the risk of rupture or bleeding (8).

International collaborations are needed to create a study cohort large enough to identify subgroups at risk of pregnancy-related complications. Taking biopsies could help to identify HCA subtypes and define women at risk who may benefit from follow up and those who do not require follow-up due to the very low risk of complications. To gain more insight in the accuracy of diagnostics, the biological behaviour of HCA during pregnancies, and the risk of growth and haemorrhage of HCA during pregnancy, we initiated the EuroPALM registry, which enables to create a large international cohort.
In conclusion, this study indicates that in well-diagnosed patients a HCA smaller than 5cm during pregnancy seems to bear minimal risk for the mother and no risk for the foetus. As it cannot be excluded that a HCA will grow during pregnancy, we recommend close monitoring with ultrasound-examinations.

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CHAPTER 6

Evidence of good prognosis of hepatocellular adenoma in post-menopausal women

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ABSTRACT

Objective: Hepatocellular adenoma (HCA) is a rare benign liver tumor, which typically develops in women in their reproductive phase and is associated with the use of oral contraceptives. The aim of this study was to evaluate whether follow-up of HCA can be safely terminated after the occurrence of menopause. Secondary, we studied the impact of the diagnosis HCA on health-related quality of life (HRQoL).

Design: This was a cross-sectional cohort study, including 48 post-menopausal women with HCA. Patients underwent ultrasound examination and the size of HCA was compared to size at the last follow-up imaging (CT, MRI or ultrasound). HRQoL was evaluated by the Liver Disease Symptom Index 2.0 and Short-Form 12.

Results: Median time since last follow-up was 60,5 months. In 44 patients 43,5% of the lesions were undetectable, 32,6% were stable in size and 19,6% became smaller. Mean diameter of HCA was 17,2mm compared to 35,9mm at last follow-up ($p < .001$). There was a positive correlation between difference in size and time since last follow-up ($p < .001$). No significant effect of HCA-subtype on difference in size was found. Regarding HRQoL, study patients scored significantly lower on the Mental Component Summary score compared to the general female Dutch population.

Conclusion: HCA-diameter becomes significantly smaller after the occurrence of menopause and as time progresses this regression increases**.** This suggests that routine follow-up of HCA <5cm in post-menopausal women after subsequent follow-up is not required. Notably we found that patient's mental HRQoL was inferior to that of the general population.

INTRODUCTION

Hepatocellular adenoma (HCA) is a rare benign liver tumor, predominantly occurring in woman in their reproductive phase. HCA appears to be more common in women using estrogen-containing oral contraceptives (OC); this association was first described in seven patients in 1973 and supported by many authors in the years that followed (1-4). The incidence is low, estimated to be 30-40 per million per year in long-term (>2 years) OC users and 1 per million in non-users or women with less than two years of OC-use (5). With cessation of OC, regression of HCA may occur (6, 7). Other conditions that may be associated with the occurrence of HCA are obesity, the intake of alcohol or androgens, glycogen storage disease type I and maturity-onset diabetes of the young type 3 (8-11).

HCA can be divided into four subgroups based on genetic and phenotype characteristics: HNF1A-mutated or steatotic adenomas (H-HCA, account for 30-40% of total HCAs), inflammatory adenomas (I-HCA, 40-50%), β-catenin-activated adenomas (β-HCA, 10-15%) and unclassified adenomas (5-10%). Distinction of subtypes is possible both immunohistochemically and radiologically (11-13). Despite its benign character, HCA can be complicated by growth and rupture. Malignant transformation to Hepatocellular Carcinoma (HCC) has been reported up to 4%, occurring mostly in β-HCA (14, 15). Bleeding occurs mostly in younger patients with a median age ranging from 28 to 38 years (16), as opposed to HCC which is seen in a median age ranging from 48 to 68 years (17).

Patients may worry about these complications, especially when at first imaging a suspicion of malignant origin of the lesion(s) is expressed to the patient. Even though this gets revoked after additional diagnostics are performed, it may have an impact on patients' health related quality of life.

If symptoms are present they are a result of tumor growth or bleeding and consist of right upper quadrant abdominal pain or discomfort and rarely symptoms of hemorrhage into the peritoneal cavity. In rare cases of large subcapsular HCA, patients may present with chronic abdominal pain and/or a palpable liver mass(18). However, as most patients are asymptomatic, the presence of HCA is more often discovered during radiologic imaging of the abdomen for unrelated reasons. The diagnosis HCA is made based on contrast enhanced ultrasound (CEUS), contrast-enhanced (CE) computed tomography (CT) or CE magnetic resonance imaging (MRI) (19, 20). In some cases imaging is inconclusive. A liver biopsy could be considered if its result will have an impact on treatment decisions, especially in suspected β-HCA due to the higher risk of malignant transformation.

Recently the European Association for Study of the Liver introduced a guideline on benign liver tumors (21). After a certain diagnosis most often based on CE-MRI, conservative

management entails OC discontinuation and weight loss, intermittent follow-up by radiological imaging and possibly advice regarding pregnancy. The most important indication for surgery is size of the lesion (>5cm) after at least six months of interruption of OC use. Size is associated with a risk of complications like rupture or malignant transformation: events seldom seen in lesions <5 cm (22, 23).

A remaining unanswered question is when it would be safe to stop surveillance. In 2012, a Dutch research group proposed a standardized decision-making model for the management of HCA. In their model they suggested that it would be safe to stop surveillance of post-menopausal patients who were maintained in subsequent follow-up and showed regression of HCA to <5 cm (24). However, this was based on expert opinions and has not yet been studied. It is important to test this hypothesis in order to minimize healthcare costs and to stop follow-up in patients in whom this is not strictly necessary. Also, if we can prove that post-menopausal age reduces the risk of complications we can reassure patients, which may have a positive impact on their health-related quality of life (HRQoL).

The primary aim of this study was to evaluate whether in post-menopausal women the size of HCA had decreased. In other words; can the follow-up of HCA indeed safely be terminated after the occurrence of menopause or is it necessary to keep patients under life-long surveillance to monitor potential growth or malignant transformation of the HCA? Secondary, we studied the impact of the diagnosis of HCA on patients' HRQoL.

PATIENTS AND METHODS

Study design and population

This study was a cross-sectional cohort study performed in a tertiary referral center for focal liver lesions. The study protocol was reviewed and approved by the local medical ethical committee.

With the availability of a large HCA cohort including 450 patients we selected all females over 50 years of age with imaging (CEUS or contrast-enhanced MRI) or biopsy proven HCA who were diagnosed before August 2014 (>1 year since diagnosis) and did not undergo surgery or otherwise ablative intervention for solitary adenoma. Patients with multiple adenomas who underwent intervention and had remaining HCA after treatment were also included. At inclusion patients had to be post-menopausal, defined as occurrence of amenorrhea for at least 12 months (25). Excluded from the study were patients diagnosed with severe liver disease of any origin or on medication that is known to be associated with induction of HCA (OC, estrogen containing medication otherwise, androgens). Patients

diagnosed with severe other illnesses and unable to visit our hospital were excluded, except when they underwent an US in the past year in another hospital and we were able to request the radiology reports. These patients were sent questionnaires by mail to assess baseline characteristics.

Additionally, we retrospectively reviewed the medical records of all female patients who did have treatment in the form of resection or ablative intervention otherwise for HCA after the age of 50 years.

Study procedures

All selected patients were addressed with a letter containing information on background and study aims. The researchers contacted the patients by telephone to answer any questions about the study and to assess whether they fulfilled the in- and exclusion criteria. When informed consent was given they were scheduled for a onetime appointment in the outpatient clinic.

The electronic patient records were reviewed to assess baseline characteristics including age, age at first diagnosis, total follow-up time, number of times patients underwent follow-up imaging, time since last follow-up, number of lesions, size of the largest lesion at first diagnosis, size of the largest lesion at last follow-up, management and whether the HCA was detectable with US at some point in follow-up.

Interview and ultrasound

The appointment consisted of two parts: an interview and an US of the liver. After receiving written consent of participation patients had an interview with one of the investigators in which they assessed the remaining patient characteristics including medical history, use of medication and hormonal status. Height was measured in cm in a standing position with shoes removed, using a wall-mounted stadiometer. Weight was measured in kg with shoes removed on an electronic scale. Height and weight were used to calculate BMI; categorization of BMI was done using the World Health Organization (WHO) international classification for BMI: underweight (<18.50 kg/m^2), normal (18.50–24.99 kg/m^2), overweight (25.00–29.99 kg/m^2), and obese (>30.00 kg/m^2) (26).

After reviewing previous imaging ranging from CEUS to (CE) CT or MRI, patients underwent an US of the liver performed by a hepatologist with 8 years of experience in liver US, using the Philips EPIQ 7 ultrasound system. The diameter of the largest lesion was measured in mm at the study ultrasound (T2) and compared to the diameter at last known follow-up imaging (T1) as measured by CE-CT, CE-MRI or CEUS. Lesion growth was defined as an increase of >20%, lesion regression was defined as a decrease of >30% as per RECIST criteria for response evaluation in solid tumors (27).

When HCA subtype had not yet been established by MRI or biopsy in patients, previous available MRI imaging was reviewed by a radiologist with 10 years of experience in abdominal imaging. Differentiation of the four subtypes (H-HCA, I-HCA, β-HCA and unclassified HCA) was based on typical features as previously published (13).

Questionnaires

To objectify the impact on HRQoL we asked patients to complete two questionnaires; the Liver Disease Symptom Index 2.0 (LDSI 2.0) and the Short Form 12 version 2 (SF-12). The LDSI 2.0 is a disease specific questionnaire that measures nine possible liver disease-specific symptoms and the hindrance that patients experienced from having these symptoms in the past week. It consists of 2 sections; the first is a symptom index with 18 items including itch, joint pain, pain in the right upper abdomen, fatigue, worry about family situation, decreased appetite, depression, fear of complications and jaundice. The second section includes six items considered to be important by the Dutch Liver Patient Association (NLV): memory problems, personality change, difficulty in financial affairs, daily time management, decreased sexual interest and decreased sexual activity. The items have a 5-level response option on a Likert-type scale ranging from 1 (severe symptoms) to 5 (no symptoms); higher scores mean better situations. The LDSI 2.0 is a validated questionnaire with good feasibility and good test-retest reliability. Weighed kappa's range from 0.32 to 0.99, with 19 of a total of 24 items showing weighed kappa's >0.63 (28).

The SF-12 (a subset of the SF-36) is a generic health status measure with good reliability and validity providing measures of physical and mental health in the past four weeks (29). The questionnaire consists of 12 items from eight health domains: physical functioning, role-physical, bodily pain, general health, vitality, social functioning, role-emotional and mental health. The scores in these domains contribute to the Physical Component Summary (PCS) and Mental Component Summary (MCS) scores; with higher scores meaning better HRQoL. The SF-12 is also a part of the annual health survey performed by the Central Bureau of Statistics in the Netherlands, including over 15.000 Dutch residents. The scores of our study population were compared to the results of the general female Dutch population in the age groups 50-55 years and 55-65 years, as scored in the annual health survey in 2013 (30). Also, a subgroup analysis between patients with- and without comorbidity was performed. Both HRQoL questionnaires were summed using standard scoring procedures.

Statistical analysis

Statistical analyses was performed using IBM SPSS software version 21.0 (Chicago, Illinois). Continuous variables were summarized as mean and standard error of the mean (SEM) 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 investigated using Student T-test or Mann-Whitney U test for continuous variables and χ^2 test for binary variables. Correlation between variables was analyzed using Pearson product-moment correlation coefficient. Analyses of variance was conducted using one-way ANOVA. A p value of <.05 was considered as the level of significance.

RESULTS

We selected 128 patients from the database based on gender and age, of which 39 did not fulfill the inclusion criteria. Eighty nine patients were approached to participate, of which 27 did not want to participate (main reasons were length of the journey and unable to take a day off work) and 6 patients were untraceable. Forty eight patients were enrolled in our study (response rate of 59%). Forty four visited our hospital and from four patients we requested radiology reports from their treatment hospitals (fig. 1).

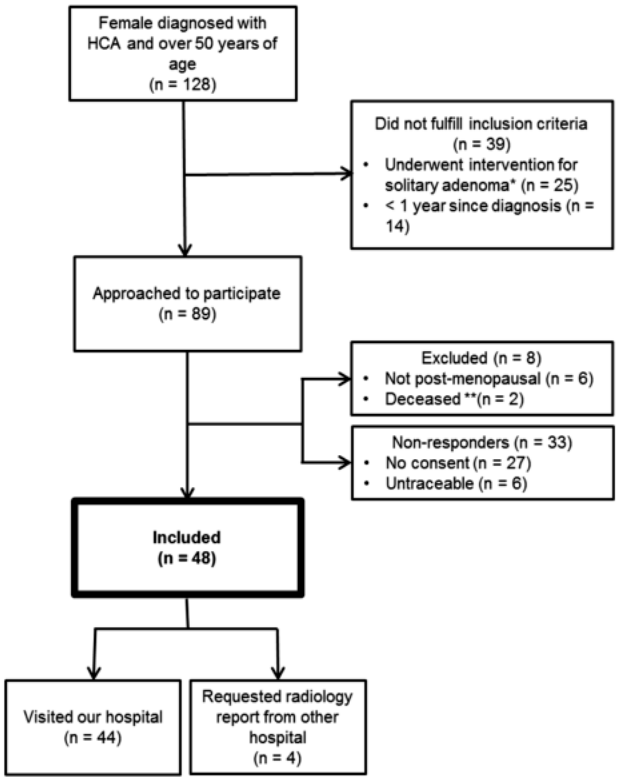

Figure 1. Patient enrollment.

*Interventions: surgery, radio frequent ablation, embolisation. **One patient deceased as a result of decompensated livercirrosis, the second as a result of non-liver related disease.

Patients and clinical characteristics

The clinical characteristics of the study population are described in table 1. We compared the mean or median baseline characteristics of the included patients to the age, age at first diagnosis, total follow-up time, number of HCA lesions at first diagnosis, size of the largest HCA at first diagnosis, size of the largest HCA at last follow-up and management (conservative versus intervention) of the non-responders and found no statistical significant differences.

The median age in our population was 55,5 years (IQR 54,0 – 60,8). The median total follow-up time was 22,0 months (IQR 7,3 – 41,5) in which patients underwent imaging 5 times (IQR 3 – 6,7) on average. In all patients who underwent regular US at some point in FU (86,4%) the HCA was detectable, 6 patients never underwent US. The median time since last follow-up 60,5 months (IQR 24,0 – 86,8) and the most used imaging modality at last follow-up was MRI (58,3%). Most of the patients were treated conservatively with watchful waiting, 7 patients underwent intervention in case of multiple adenomas. Patients were post-menopausal with a median time since menopause of 5,0 years (IQR 3,0 – 10,0); all patients except one had a history of long term (>2 years) OC use. Mean BMI was 29,5 kg/m^2. Cardiovascular disorders, musculoskeletal disorders and diabetes mellitus were the most prevalent comorbidities; 11 patients did not have any comorbidity at all.

Table 1: Description of clinical characteristics

	Included patients (n = 48)
Age (yr)*	55,5 (54,0 – 60,8)
Age at first diagnosis (yr)	49,5 (47,0 – 52,0)
Total follow-up time (mo)	22,0 (7,3 – 41,5)
No. of times imaging FU	5 (3 – 6,7)
HCA detectable with US at some point in follow-up	
Yes	38 (86,4 %)
Never underwent US	6 (13,6 %)
Time since last follow-up (mo)	60,5 (24,0 – 86,8)
No. of HCA lesions at first diagnosis	3,0 (2,0 – 10,0)
1 – 5	32 (66,7 %)
6 – 10	8 (16,7 %)
>10	8 (16,7 %)
Size largest HCA at first diagnosis (mm)	47,2 (± 3,5)
Size largest HCA at last follow-up (mm)	35,9 (± 3,5)
Management	
Conservative	40 (83,3 %)
Intervention in case of multiple HCA	8 (16,7 %)
History of long term (>2 years) OC-use	46 (95,8 %)
OC use (yr)	25,0 (20,0 – 30,0)
Time since menopause (yr)	5,0 (3,0 – 10,0)
BMI (kg/m^2)	29,5 (± 0,8)
Normal (18.50–24.99)	11 (22,9 %)
Overweight (25.00–29.99)	18 (37,5 %)
Obese (>30.00 kg/m^2)	19 (39,6 %)
Comorbidity	
None	11 (22,9 %)
Cardiovascular	18 (37,5 %)
Diabetes Mellitus	13 (27,1 %)
Musculoskeletal	12 (25,0 %)
Smoking	8 (16,7 %)
Yes, daily	5 (10,4 %)
Yes, periodically	24 (50,1 %)
No	19 (39,6 %)

Results are given in Median (IQR), Mean (± SD) or n (percentage). Yr = years, mo = months.

Ultrasound and HCA

The US of two of our participants could not be interpreted properly as a result of previous interventions; the first due to scarring after hemihepatectomy and the second underwent embolization of a haemangioma situated right next to the adenoma. These patients are excluded from analyses regarding the HCA. Two out of four patients from whom we requested radiology reports of other hospitals did not undergo an ultrasound in the past year; and were also excluded from further analyses (fig. 2). In four of the remaining 44 patients the first US was inconclusive and an additional contrast US was performed, confirming the lesions to be adenomas.

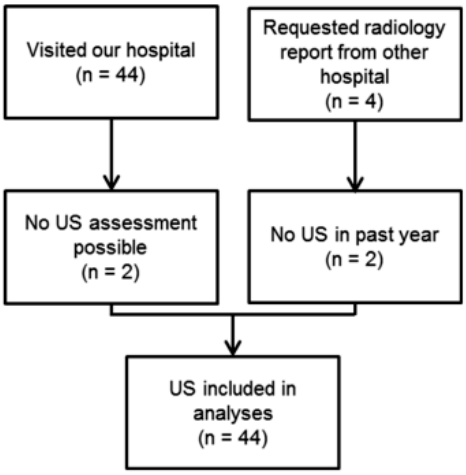

Figure 2. Ultrasounds included in analyses.

US = ultrasound.

The majority of the patients had HCA <100mm at first diagnosis (T0), one patient had a lesion >100mm. At last follow-up imaging (T1), after a median follow-up time of 22 months, most patients had HCA <50mm, eight patients still had a lesion of 51 – 100mm and one patient had a lesion >100mm. The study US (T2) showed no lesions to be >100mm, three patients had a lesion of 51 -100mm (fig. 3a). Two out of these three lesions showed regression, one lesion was stable (80mm). The lesion that was >100mm at T1 was not detectable anymore at T2.

In the majority of patients (20 out of 44) the HCA was not detectable anymore. In 9 patients the lesions became smaller and in 15 patients the HCA were stable in size (fig. 3b). 16 out of the 20 undetectable lesions were detectable with US at some point in follow-up, the remaining 4 never had an US before.

We found a significantly smaller diameter of the HCA at T2 compared to T1 (mean of 17,2mm compared to 35,9mm, p <.001) (fig. 3c).

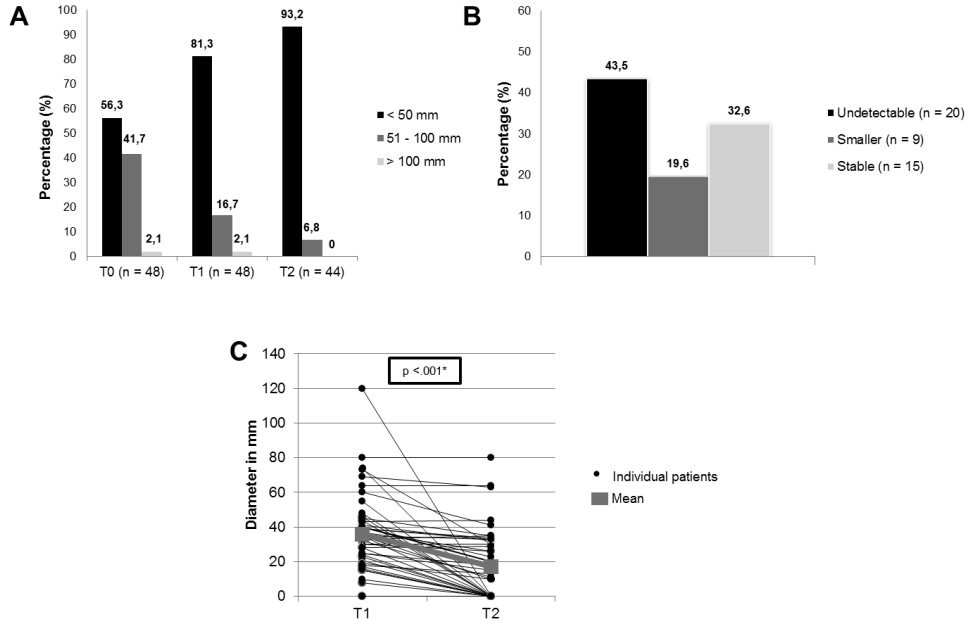

Figure 3. Results ultrasound.

N = 44. HCA = Hepatocellular Adenoma. T0 = diagnosis. T1 = last follow-up. T2 = study. (A) Diameter of HCA. (B) Lesion characteristics at ultrasound. Percentage of undetectable, smaller or stable lesions in comparison to size at last follow-up. (C) Diameter of HCA in individual patients. Black dots: size of HCA of individual patients. Blue squares: mean diameter of HCA in mm (T1 = 38,8 T2 = 17,3). Red lines: SE of mean.*Paired samples t-test showed statistically significant difference in size (p<.001).

We calculated difference in size of the HCA as diameter in mm at T1 minus diameter in mm at T2 and found a significant positive correlation (r = .553, p < .001) between time since last follow-up and the difference in size (figure 4). No significant correlation was found between difference in size and BMI (r = .206, p = .179) and difference in size and the amount of years since menopause(r = -.035, p = .821) . A subgroup analyses for difference in size between patients who were postmenopausal ≤5 years and >5 years did not result in a significant difference (p = .53) as well.

The majority (52,3 %) of patients had unclassified HCA, 20,5 % had I-HCA, 11,4% H-HCA and 11,4% β-HCA. In two patients we did not have previous MRI imaging and the subtype remained unknown. All molecular subgroups were represented in the 8 patients whose

HCA remained stable in size (table 2). A one-way ANOVA was conducted to compare the effect of subtype on difference in size of the HCA between T1 and T2. The assumption of homogeneity of variance was violated; therefore we performed a Welch and Brown-Forsythe test which did not result in a statistically significant effect (p = .274 and p = .087, resp.).

During the US we noticed that steatosis was present in most of the patients (n = 31), of which 26 had severe steatosis.

Table 2: Subtypes of HCA

Subtype	Frequency	Percentage	Difference in size (M ± SD)	US characteristic	Frequency
H-HCA	5	11,4	6,20 ± 7,46	Undetectable	-
				Regression	4
				Stable size	1
I-HCA	9	20,5	42,67 ± 37,07	Undetectable	6
				Regression	2
				Stable size	1
B-HCA	5	11,4	10,40 ± 8,74	Undetectable	1
				Regression	3
				Stable size	1
Unclassified HCA	23	52,3	13,48 ± 13,49	Undetectable	13
				Regression	6
				Stable size	4

Subtypes of HCA in 42 patients based on biopsy or CE-MRI, in two patients subtype remained unknown. Difference in size between last follow-up and study ultrasound and study ultrasound characteristics.

Patients >50 years who were treated for HCA

8 female patients underwent resection for HCA after the age of 50 years. Median age at resection was 53 years (51-54,8). Mean HCA diameter at diagnosis was 74,5mm (SEM 5,9) and at resection 77,9mm (SEM 4,9). Median time between diagnosis and resection was 9,5 months (IQR 3-41,3). Two patients were post-menopausal at the time of resection, one was not and of the remaining 5 this is unknown. In patients who were followed <6 months prior to treatment the indications for resection were one suspected HCC, one patient with breast cancer, one who stopped OAC 2 years prior to diagnosis had HCA >50mm and one high risk HCA based on biopsy (inflammatory with beta-catenin activation). In the group that was followed >6 months indications were lack of regression to <50mm, heamorrhage, symptoms and one growing HCA(table 3). Pathology reports all showed HCA without any signs of malignant degeneration.

Table 3: Clinical characteristics of treated patients

	n = 8
Age at resection (yr)	53 (51 – 54,8)
Diameter at diagnosis (mm)	74,5 (± 5,9)
Diameter at resection (mm)	77,9 (± 4,9)
Postmenopausal at resection	
Yes	2
No	1
Unknown	5
Follow-up <6 months	
1. 100mm – subtype unknown	Suspected HCC
2. 78mm – Inflammatory	Patient with breastcancer
3. 74mm – Inflammatory	Stop OAC 2 years prior to diagnosis, no regression after 3 months
4. 70mm – Inflammatory + Beta-catenin	High risk adenoma
Follow-up >6 months	
1. 76mm – Inflammatory	Regression to 63mm after two years
2. 70mm – Subtype unknown	Multiple lesions, haemorrhage in one. No regression after 6 months.
3. 73mm – Subype unknown	Left lateral HCA causing symptoms: hiccups and abdominal discomfort
4. 90mm – Steatotic	In 47 months growth from 70 to 90mm.

HRQoL

Figure 5 shows the mean scores of symptoms patients in our population experienced as measured by the LDSI 2.0. Each item is scored from 1 to 5, with higher scores accounting for a better situation. The item joint pain scored the lowest.

Figure 6a shows a non-significant mean difference for the PCS in our study population (M = 48,1 SEM = 1,31) compared to the general female Dutch population in the age of 50-55 (M = 48,3 SEM = 0,3) and the age of 55-65 (M = 49,9 SEM 0,4)(30). For the MCS, the study population (M = 43,3 SEM = 1,67) scored significantly lower in comparison to the annual health survey results in the age group 50-55 (M = 52,8 SEM 0,4) and 55-65 (M = 52,9 SEM 0,4) ($p < .001$) (30). A subgroup analyses between patients with comorbidity and without comorbidity (fig. 6b) showed a significant difference for the PCS ($p < .001$) and no significant difference for the MCS.

DISCUSSION

To the best of our knowledge, we present the results of the first study assessing the follow-up of HCA in post-menopausal women. We provide a group of women with a mean diameter of HCA at last follow-up of 35,9mm, who were recalled after a median time since last follow-up of 60,5 months. With a mean HCA diameter of 17,2mm the lesions had become significantly smaller. Most of the lesions were undetectable or remained stable in size. At the time of last follow-up the majority of lesions was <50mm, 9 were >50mm. Out of these 9 lesions one was >100mm: this patient had been lost to follow-up for ten years and when we saw her again the lesion was not detectable anymore. Out of the other eight patients, three HCA were still >50mm at time of the study. In two of these patients the lesions showed regression, the other one had a stable 80mm H-HCA. Because of the stable disease and low chance of complications in H-HCA we decided to keep this patient under surveillance and not perform surgery yet.

We found a strong positive correlation between time since last follow-up and difference in size; meaning that as time progresses the HCA become increasingly smaller**.** No new HCA were found and none of the lesions showed growth or signs of malignant transformation. All HCA-subtypes were represented in the seven lesions that remained the same size and an analysis of variance showed that the effect of subtype on difference in size of the HCA was not significant. We should notice that the percentage of patients without specific characteristics on MRI (unclassified HCA) was far greater in our study population compared to the previous described distribution (52,3 % versus 5-10 %). In the future it might be helpful to examine the effect of subtype on difference in size in a larger cohort.

Retrospective review of the medical records of 8 female patients who did have a resection or ablative intervention otherwise for HCA after the age of 50 years showed that this were patients with larger HCA (mean 74,5mm at diagnosis). Indications varied greatly from suspected HCC, high risk adenoma, symptoms, growth and lack of regression to <50mm.

Our results suggest that it is indeed safe to stop surveillance in post-menopausal patients with HCA <50mm that showed regression in subsequent follow-up. We also saw a trend of regression in the lesions >50mm, however given the higher risk of complications in tumors >50mm it is advisable to consider keeping the patients with large HCA in follow-up, especially in I-HCA and β-HCA.

As the disease is relatively rare (estimated incidence 30-40 per million per year in long-term OC users and 1 per million in non-users), we believe that with follow-up ultrasounds in 44 patients we provide an adequate estimation of the natural course of HCA in post-menopausal women. Regarding the characteristics of our study population it was noticed

that the mean BMI was 29,5 kg/m^2, compared to a mean BMI between 25,7-26,4 kg/m^2 in the general female population of the same age (50-70 years) in the Netherlands (31). The incidence of cardiovascular diseases, diabetes mellitus and musculoskeletal disorders was also higher in our study population, which may well be correlated with the higher BMI (32).

A relation between obesity and higher incidence of HCA has been described among others by Bunchorntavakul et al.(9) and Bioulac Sage et al (33). Lifestyle changes such as weight loss are thought to have a positive effect on regression of the lesion. In this study we did not find a statistically significant correlation between BMI and difference in size. However, we did not document changes in BMI over the years.

The role of estrogen and estrogen containing OC in the development or growth of HCA has often been described. However, the results of this study did not show a correlation between decrease in size and the amount of years since menopause. This might be explained by the fact that we did not have the exact moment of menopause based on hormone levels, we based the amount of years since menopause on the age patients remembered their final menstrual period to be on. The largest decline in estrogen levels is seen in the first 3-4 years after the final menstrual period, after that period the levels stabilize(34). Therefore one might assume that the largest decrease in size of the HCA would occur in the first few years after menopause. To assess if determining a cut point in time from onset of menopause was the key in seeing a relationship between difference in size and time since menopause, we performed a subgroup analyses for difference in size between patients who were postmenopausal ≤5 years and >5 years. This also did not result in a statistical significant difference. In 2013, Nault et al. proposed that estrogens play the biggest role in the development of H-HCA: a dysregulation in the estrogen metabolism and accumulation of 17β-estradiol in the hepatocyte is thought to cause proliferation of hepatocytes (11). In 2009, Carswell et all described a case regarding a 75-year-old female who showed increase of HCA after prescription of Raloxifene (oral selective estrogen receptor modulator) (35). These two articles contribute to the hypothesis that the lower estrogen levels indeed have a role in regression of HCA in post-menopausal women.

The second question in this research was whether the HRQoL in patients with HCA is impaired. The most severe symptom on the LDSI 2.0 was joint pain with a score of 3,73 on a scale from 1 (severe symptoms) to 5 (no symptoms). This symptom does not typically occur in patients with HCA and as the questionnaire does not differentiate in etiology, it is questionable whether HCA is the cause of the occurrence of joint pain. More likely it is explained by a higher incidence of arthrosis in a study population with higher BMI.

The generic health status was measured with the SF-12 questionnaire. We compared the scores of our study population to the scores of the general female Dutch population as measured by the 2013 annual health survey. We did not find a significant difference in PCS. However, the MCS in our study population was significantly lower compared to the age groups of 50-55 and 55-65 years. As well as with the LDSI, the SF-12 does not distinguish in underlying diseases. Therefore we performed a subgroup analysis comparing the scores of patients with comorbidity to the scores of patients without comorbidity, resulting in a significantly lower score in patients with comorbidity for the PCS but not for the MCS. This shows that HCA is not the determinant in a lower PCS. HCA might be the determinant in the lower MCS, however this does not rule out the influence of the higher average BMI and related co-morbidities on the impaired mental HRQoL. A possible explanation for the impaired mental HRQoL related to HCA is the fear of complications, but it might also be explained by anticipated excitement and anxiety surrounding the hospital visit and the result of the ultrasound, as the questionnaire only provides the health measures for the past four weeks.

We acknowledge that our study has some limitations. First, with a response rate of 59% we might be subject to a non-response bias. Comparison of the baseline characteristics of enrolled patients and the known characteristics of the non-responders did not result in any significant differences, making the occurrence of a non-response bias unlikely. Secondly, for ethical considerations we chose ultrasound as the least invasive method of follow-up for this study and compared the diameter of the HCA to the diameter at last follow-up measured in different modalities (contrast US, contrast enhanced CT and contrast enhanced MRI). Although in a clinical situation the modality of follow-up also varies often based on the preference of both clinician and patient, the fact that the majority of our patients had steatosis makes the interpretation of US findings even more difficult(36). By reviewing all available previous imaging prior to the US we knew the location of the lesions and therefore were able to get more accurate results. Also, 16/20 HCA that were undetectable at study US were detectable with US at some point in follow-up. Therefore the disappearance of the HCA could be conclusive.

In conclusion, this study showed that HCAs become significantly smaller in diameter after the occurrence of menopause in female patients. Especially HCA < 50mm demonstrated regression after subsequent follow-up of at least six months. Therefore, routine surveillance of HCA in post-menopausal patients with HCA <50mm that showed regression in subsequent follow-up is not required. Physical HRQoL does not seem to be impaired in patients diagnosed with HCA. Mental HRQoL might be, although it remains uncertain if HCA is the determinant.

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CHAPTER 7

Management and outcome of hepatocellular adenoma with massive bleeding at presentation

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ABSTRACT

Aim: To evaluate outcome of acute management and risk of rebleeding in patients with massive hemorrhage due to hepatocellular adenoma (HCA).

Methods: This retrospective cohort study included all consecutive patients who presented to our hospital with massive hemorrhage (grade II or III) due to ruptured HCA and were admitted for observation and/or intervention between 1999-2016. The diagnosis of HCA was based on radiological findings from contrast-enhanced magnetic resonance imaging (MRI) or pathological findings from biopsy or resection of the HCA. Hemorrhage was diagnosed based on findings from computed tomography or MRI. Medical records were reviewed for demographic features, clinical presentation, tumor features, initial and subsequent management, short- and long-term complications and patient and lesion follow-up.

Results: All patients were female (n = 23). Treatment in the acute phase consisted of embolization (n = 9, 39.1%), conservative therapy (n = 13, 56.5%), and other intervention (n = 1, 4.3%). Median hemoglobin level decreased significantly more on days 0-3 in the intervention group than in the patients initially treated conservatively (0.9 mmol/L vs 2.4 mmol/L respectively, p = 0.006). In total, 4 patients suffered severe short-term complications, which included hypovolemic shock, acute liver failure and abscess formation. After a median follow-up of 36 months, tumor regression in non-surgically treated patients occurred with a median reduction of 76 mm down to 25 mm. Four patients underwent secondary (elective) treatment (i.e. tumor resection) to address HCA size of >5 cm and/or desire for future pregnancy. One case of rebleeding was documented (4.3%). None of the patients experienced long-term complications (mean follow-up time: 36 mo).

Conclusion: With a 4.3% risk of rebleeding, secondary (elective) treatment of HCA after massive hemorrhage may only be considered in patients with persistent HCA >5 cm.

INTRODUCTION

Hepatocellular adenoma (HCA) is a rare benign liver tumor that occurs mostly in women in their reproductive phase. An association with the estrogen-containing oral contraceptive (OC) was first described in 1973 (1, 2). Currently, the estimated annual incidences are 30-40 per million in long-term (>2 years) OC users and 1 per million in non-users or women with less than 2 years of OC use (3).

HCAs are most often asymptomatic and discovered during radiologic imaging of the abdomen for unrelated reasons. The best way to diagnose HCA is with contrast-enhanced magnetic resonance imaging (MRI) (4). In the past decade, much has changed in terms of diagnosis and treatment of HCA, due to the discovery of various subtypes of this tumor (5, 6). The change in treatment strategy is ongoing, due to the apparent differences in risks of complications for the various subtypes.

As HCAs are well vascularized tumors, hemorrhage—as documented on imaging—is a common complication, occurring in approximately 25% of the patients with HCA (7). OC use, tumor size of >5 cm, exophytic growth of the tumor and inflammatory subtype (I-HCA) are associated with a higher risk of bleeding (7, 8). Most hemorrhages are intratumoral; however, in cases of massive bleeding, rupture of the HCA can occur, resulting in intraparenchymal hemorrhage and subcapsular hematoma. In some cases, the liver capsule can rupture, causing hemoperitoneum.

Hemorrhagic HCAs cause symptoms such as acute-onset right upper abdominal pain and discomfort. In cases of tumor rupture and excessive bleeding, patients may present with hemodynamically unstable conditions and even may show signs of hypovolemic shock. This complication can be life threatening (3).

Another rare complication of HCAs, not related to risk of bleeding, is malignant degeneration to hepatocellular carcinoma, which occurs mostly in β-catenin-positive HCA (9). As both hemorrhage and malignant degeneration arise especially in adenomas >5 cm in size, surgical resection is recommended for HCA which do not regress by 6 mo after cessation of OC and following lifestyle changes, such as weight reduction in overweight patients (10-13).

The management policy for acute bleeding of ruptured HCA has changed over the past years. In the acute phase, a conservative management and hemodynamic stabilization is justified (14). In case of active bleeding with persistent hemodynamic instability or hemoperitoneum, intervention may be considered (7). Liver resection in the acute phase is associated with increased morbidity and mortality and, therefore, not advisable (15).

Laparotomy and gauze packing of the liver had been recommended until the introduction of minimally-invasive techniques. The advent of selective arterial embolization (SAE) 10 years ago has now allowed for patients to be treated even less invasively (16, 17). An unanswered question, however, remains in regards to the chance of rebleeding and, correspondingly, the need for elective tumor resection.

In this study, we evaluated the outcome of acute management in patients with massive hemorrhage due to ruptured HCA and its sequelae, including the risk of rebleeding and need for elective tumor resection.

MATERIALS AND METHODS

Study design

This study was a retrospective cohort study performed in a tertiary referral center for focal liver lesions. With the availability of a large HCA cohort, consisting of 449 patients, we selected all patients who presented with massive hemorrhage as a result of ruptured HCA and who were admitted to a hospital ward for observation and/or intervention between January 1999 (the start of the database) and April 2016, with follow-up time of at least 6 mo.

This study was approved by the accredited local institutional review board (No. MEC-2016-338) and informed consent was waived.

Patient selection

Massive hemorrhage was defined as intrahepatic (grade II) or intraperitoneal (grade III), as reported by Bieze et al(8) in 2014. Patients with intratumoural hemorrhage (grade I) were excluded from this study. The diagnosis of HCA was based on radiological findings from contrast-enhanced magnetic resonance imaging (MRI) or pathological findings from biopsy or resection of the HCA. Hemorrhage was diagnosed based on findings from computed tomography (CT) or MRI. Hepatocellular carcinoma—the main competing differential diagnosis—was excluded by occurrence of tumor regression after hemorrhage, absence of progression in diameter and lack of metastatic disease over time.

On CT, the density of a hematoma is determined by the time elapsed after the initial event. In the acute phase, a hematoma is hyperdense, becoming more isodense in the chronic phase. On MRI, the intensity of a hematoma also changes over time. On T1-weighting, hematoma is hyperintense in the beginning, becoming more and more isointense in the chronic phase. On T2-weighting, a hematoma starts as hyperintense and resolves in the chronic phase with zones devoid of signal (visualized as black space) due to deposition of hemosiderin, and which occurs mostly in the periphery(18) (Figure 1).

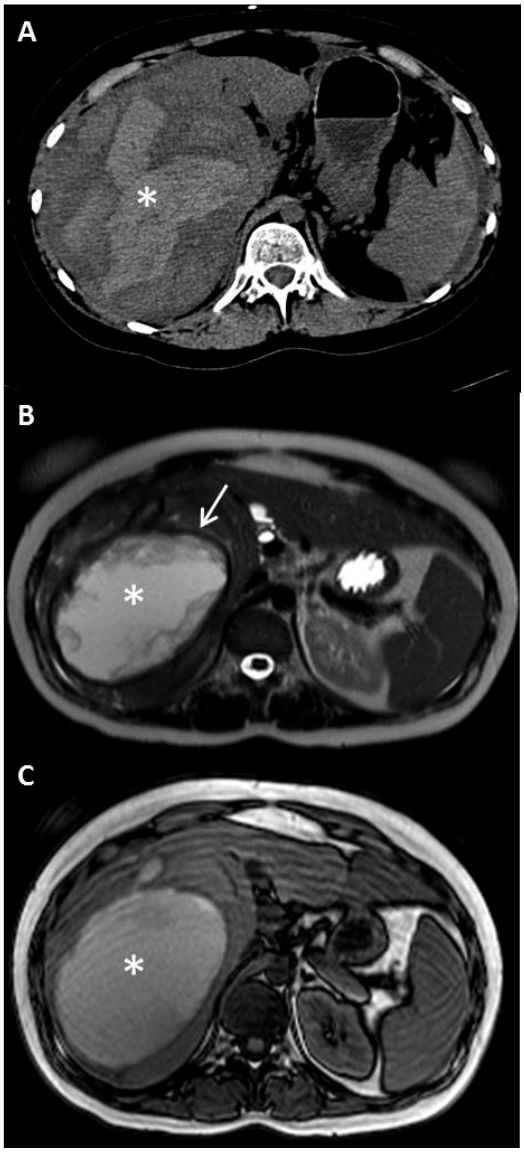

Figure 1. Representative case of grade II bleeding hepatocellular adenoma (HCA) involving a 23-year-old female who presented at the emergency department with an acute hematoma in the right liver lobe.

A: CT without intravenous contrast shows a hyperdense fluid collection in the liver (*); B: Some months later after conservative treatment, the collection appeared hyperintense on T2–weighting, with a hemosiderin ring surrounding the collection (arrow); C: At the same time, the collection appeared hyperintense on T1-weighting.

Data collection for analysis

HCA subtyping—for steatotic, inflammatory, β-catenin-positive and unclassified HCA—was determined based on findings of immunohistochemistry or of the typical MRI features(19-21). In cases that the HCA subtype was not able to be established by MRI or biopsy, previous available MRI scans were reviewed by an experienced radiologist.

Medical records were reviewed to collect each patient's demographic features, clinical presentation, tumor features, initial and subsequent management (including surgery or intervention techniques), short- and long term complications and patient and lesion follow-up. If patients had been referred to our center by another hospital, we requested the data from the referring hospital, with consent of the patient.

Statistical analysis

All statistical analyses were performed using SPSS software, version 21.0 (IBM, Armonk, NY, United States). All non-normal distributed variables were summarized as median and interquartile range (IQR); binary variables were summarized as frequency (n). Differences between groups were investigated using the Mann-Whitney U test for continuous variables. A P-value of <0.05 was considered as the threshold for significance.

RESULTS

Study population and case characteristics

We assessed 23 consecutive patients who were admitted to hospital for massive intrahepatic hemorrhage or hemoperitoneum due to ruptured HCA (Table 1). All patients were female, with a median age of 34-years-old (IQR: 30-44 years). Fifteen of the patients presented with grade II hemorrhage, and 8 with grade III hemorrhage. The median lesion size was 76 mm (IQR: 55-92 mm), with 14/23 (60.8%) located in the right or left lateral liver and the remaining 9/23 (39.1%) located in the right medial or central liver (Table 2). Nineteen out of the 23 patients (82.6%) used OCs at the time of presentation. Two patients had an I-HCA, and a third patient had both an I-HCA as well as a H-HCA (steatotic), of which the I-HCA ruptured. The subtype was not defined in all other patients.

Table 1: Clinical features at presentation

Case no.	Age in years	OC use	Type of bleeding, grade	Initial management	ICU	Blood products	Hospital stay in days	Short-term complications
Initially conservatively treated								
1	49	Yes	II	Cons.	No	No	19	-
2	43	Yes	III	Cons.	Yes	Yes	7	-
3	30	Yes	II	Cons.	No	No	7	-
4	36	No stopped 12 mo prior to bleeding	II	Cons.	No	No	19	-
5	33	Yes	II	Cons.	No	No	8	-
6	23	Yes	II	Cons.	Yes	Yes	28	-
7	30	Yes	II	Cons.	No	No	12	-
8	39	Yes	II	Cons.	No	No	9	-
9	43	Yes	III	Cons.	No	Yes	14	-
10	44	Yes	II	Cons.	No	No	13	-
11	49	Yes	III	Cons.	Yes	Yes	61	Hypovolemic shock, respiratory insufficiency, kidney failure, abdominal compartment syndrome
12	31	Yes	II	Cons.	No	No	10	-
13	33	Yes	III	Cons.	No	No	13	-
Initially treated with intervention								
14	22	Unknown	III	SAE + resection	No	Yes	40	Postoperative abdominal abscess, drainage pleural effusion
15	33	No stopped 18 mo prior to bleeding	III	SAE	Yes	Yes	Unknown	-
16	24	Yes	III	SAE	Yes	Yes	18	-
17	36	Yes	II	SAE	Yes	No	22	Acute liver failure after SAE left and right hepatic artery
18	48	Yes	II	SAE	No	No	10	-
19	48	Yes	III	SAE	Yes	No	6	-

20	34	No stopped 7 mo prior to bleeding	Unknown	SAE	Unknown	Unknown	Unknown	-
21	30	Yes	II	US-guided drainage	No	No	10	-
22	56	Yes	II	SAE	Yes	No	Unknown	-
23	20	Yes	III	SAE	No	Yes	33	Rebleed after 3 mo, drainage hepatic abscess, drainage pleural effusion

ICU: Intensive care unit; OC: Oral contraceptive; SAE: Selective arterial embolization; US: Ultrasound.

Initial presentation and treatment

In all 23 patients, hemorrhage was the first presentation, and none of the patients were diagnosed as having HCA before the bleeding. Fifteen out of the 23 patients (65.2%) were hemodynamically stable at presentation, and one patient presented with hypovolemic shock. A total of 13 patients (56.5%) were treated conservatively. Nine patients (39.1%) underwent SAE in the acute phase, and one of them also underwent an acute resection due to persistent bleeding after the SAE. One patient underwent ultrasound-guided drainage of a presumed liver abscess, through which massive bleeding was found. Additional imaging for this patient showed an undetermined liver tumor, which prompted the patient referral to our hospital, where we confirmed the tumor to be an HCA.

Comparison of the patients initially treated conservatively to those treated with intervention showed no statistically significant differences for age, median HCA diameter, median follow-up time, median hospital stay nor median hemoglobin level at presentation. However, the intervention group showed a statistically greater reduction in median hemoglobin level on day 0-3 compared to the conservative treatment group (0.9 mmol/L vs 2.4 mmol/L respectively, $P = 0.006$) (Table 2).

Table 2: Demographics and features of patients treated conservatively or with intervention

	Initial conservative, n = 13	Initial intervention, n = 10	Total, n = 23	P-value
Median age in years	36 (30.5-43.5)	33.5 (23.5-48.0)	34 (30-44)	0.563
Median HCA diameter at diagnosis in mm	76 (55-101.5)	76.5 (51.3-92.5)	76(55-92)	0.648
Median follow-up time in months	66 (23-87)	22.5 (12.8-60.3)	36 (15-79)	0.257
Type of bleeding				0.349
Grade II	9	5	15	
Grade III	4	5	8	
Median hospital stay in days	13 (8.5-19)	18 (10-33)	13 (9.3-21.3)	0.588
Median Hb level at presentation in mmol/L	8.0 (6.0-8.4)	7.5 (7.0-8.0)	7.6 (7.0-8.1)	0.710
Median decrease Hb day 0-3 in mmol/L	0.9 (0-1.7)	2.4 (1.6-3.5)	1.6 (0.4-2.4)	0.006*

Values are given as median (IQR). *Statistically significant.

Short-term complications

Of the 23 patients, 4 (17.4%) suffered severe short-term complications (Table 1). In 2 patients, abscesses developed after embolization or resection, necessitating additional percutaneous drainage and resulting in hospital stays of 33 and 40 days respectively. One patient suffered acute liver failure after SAE, affecting both the left and right hepatic artery, with laboratory tests showing increases in aspartate transaminase (350-fold), alanine transaminase (170-fold), bilirubin (5.4-fold), lactate (4.5-fold) and the international normalized ratio (INR; 1.8-fold). Spontaneous recovery occurred within 5 days, and the total duration of hospital stay for this patient was 22 days.

The patient who presented with major hypovolemic shock was treated conservatively and suffered major hemoperitoneum, resulting in respiratory insufficiency, kidney failure and abdominal compartment syndrome. This was a patient who had recent history of oral anticoagulants for treatment of deep vein thrombosis. Initially, her case was classified as venous hemorrhage due to an excessively high INR, and therefore she was treated conservatively. After recovery, she was referred to our hospital and the diagnosis of HCA was only made upon liver imaging after the hematoma had become, more or less, absorbed. The total duration of hospital stay for this patient was 61 days.

Elective treatment and follow-up

Median follow-up time was 36 mo (IQR: 15-79 mo). One patient underwent elective resection, 1 underwent elective SAE and 2 underwent elective radiofrequency ablation (RFA) to address residual HCA (Table 3). These patients either had residual HCA of >5 cm in size or a smaller lesion but with an expressed desire for future pregnancy. Out of the total 23 patients in the study, 18 (78.3%) were kept under surveillance for >6 mo, all showing regression of the tumor from a median diameter of 76 mm (IQR: 55-92 mm) to 25 mm (IQR: 17.3-41.5 mm).

Table 3: Tumor features and follow-up

Case no.	Diameter HCA at diagnosis in mm	Location of HCA	Elective treatment of HCA	Last known HCA diameter in mm
Initially conservatively treated				
1	200	Right lateral (sVI/VII)	Surveillance	6
2	76	Right lateral (sVI/VII)	Surveillance	22
3	60	Right lateral (sVI)	Surveillance	26
4	80	Right medial (sVIII)	Surveillance	53
5	80	Right lateral (sVI)	Resection	-
6	75	Right lateral (sVI/VII)	RFA	0
7	75	Right lateral (sVI/VII)	Surveillance	8
8	143	Right medial (sVIII)	Surveillance	35
9	45	Right medial (sV/VIII)	Surveillance	40
10	39	Right lateral (sVII)	Surveillance	18
11	50	Right medial (sV)	Surveillance	21
12	92	Right lateral (sVI/VII)	Surveillance	43
13	111	Left lateral (sII/III)	Surveillance	92
Initially treated with intervention				
14	90	Central (sIV/VIII)	No adenoma tissue after resection	-
15	55	Right medial (sVIII)	Surveillance	20
16	40	Right medial (sV/VIII)	RFA	0
17	73	Central (sIV/VIII)	Surveillance	40
18	80	Right lateral (sVI/VIII)	Surveillance	24
19	100	Left lateral (sII/III)	Surveillance	45
20	100	Central (sIV/V/VIII)	SAE	42
21	55	Right lateral (sVII)	Surveillance	38
22	25	Left lateral (sIII)	Surveillance	17
23	87	Right lateral (sVII)	Surveillance	62

RFA: Radiofrequency ablation; s: Liver segment; SAE: Selective arterial embolization.

Rebleeding was reported in one patient (4.3%), which occurred 3 mo after the initial hemorrhage. This was the only patient in whom OC was continued after the first bleeding because the underlying etiology of the hemorrhage had not yet been established. After the rebleeding, the patient was referred to our tertiary center and the diagnosis of HCA was made. The rebleeding was also managed with a wait-and-watch policy, and cessation of OC lead to regression of the HCA.

None of the patients in this study had long-term complications. Out of the 9 patients who did not have any treatment (initial or elective), 2 became pregnant. In the first patient, the HCA showed growth from 46 mm to 65 mm, but no hemorrhage occurred during pregnancy and after the delivery the HCA regressed. In the second patient, the lesion remained stable in size (30 mm) throughout the pregnancy.

DISCUSSION

In this retrospective cohort study, we evaluated the outcome of acute management of patients with massive hemorrhage due to ruptured HCA and confirmed that a conservative approach is justified in the acute situation if there is a hemodynamically stable condition. In the case of persistent bleeding, SAE can be a solution. Our cohort showed a significant difference in median decrease in hemoglobin level when the conservatively-treated group was compared to the group of patients who underwent initial intervention, with a greater decrease (median: 2.4 mmol/L) occurring in the intervention group. Four patients in our study suffered the following severe short-term complications: 1 patient who had used oral anticoagulants and then presented with hypovolemic shock developed respiratory insufficiency, kidney failure and abdominal compartment syndrome; 2 patients who developed complications after SAE; and 1 patient who developed postoperative complications after both SAE and resection in the acute phase. All complications resulted in longer hospital stay; however, no long-term complications were documented.

After a median follow-up time of 36 mo, all tumors under surveillance showed spontaneous regression, with a median size decrease from 76 mm at presentation to 25 mm at last follow-up. This finding is comparable to the regression reported for non-hemorrhagic HCA after OC discontinuation(10, 11). Only 1 case of rebleeding was reported (4.3%) in a patient who did not discontinue OC, and this occurred at 3 mo after the initial bleed. Secondary treatment, such as elective tumor resection, SAE or RFA, was only performed in patients with HCA >5 cm in size after follow-up and/or in patients with an expressed desire for future pregnancy.

This study, however, did not establish whether hemorrhagic HCA should remain in regular follow-up or when, if ever, it will be safe to end surveillance. Most lesions are >5 cm at the moment of hemorrhage, causing at least a part of the vital adenoma tissue to become necrotic. Therefore, we are unsure if the higher risk of malignant degeneration in HCA >5 cm is still present in hemorrhagic HCA; indeed, it might be advisable to keep these patients in regular follow-up.

It is important to establish an optimal treatment plan for patients with ruptured HCA, as hemorrhage is a frequent complication of lesions >5 cm(7). In 2006, Erdogan et al(14) conducted a study assessing management and outcome in patients treated for ruptured HCA, in which they compared laparotomy and gauze packing with observation. Their results suggested that stable patients could be treated conservatively and that resection of the HCA in the acute situation is accompanied by a higher morbidity. With the advent of SAE a decade ago, Stoot et al (16) conducted a small cohort study including 11 patients, which established the safety and efficacy of SAE for the treatment of ruptured HCA. Recently, the European Association for Study of the Liver issued a European clinical practice guideline for the management of benign liver tumors(13). In this guideline, patients with massive hemorrhage due to ruptured HCA are recommended to be transferred to a center with an interventional radiology department and the possibility to perform SAE.

To the best of our knowledge, this study presented herein is the first to assess outcome and risk of rebleeding in patients with massive hemorrhage due to ruptured HCA. Unfortunately, for most of the included patients, the HCA subtype remained unknown. This is most likely the result of the hemorrhage itself, which makes it very difficult to distinguish HCA subtypes according to imaging characteristics. As we only knew the subtype for 3 of the patients in our study population (all I-HCA), we cannot make a judgement about the distribution of the various subtypes and the relationship to the different variables. Previous studies have shown that the risk for hemorrhage is the greatest in I-HCA (8), which was confirmed in our population.

It was noticed that in our study 60.8% of the ruptured HCA were located in the right or left lateral liver, and the remaining 39.1% were located medial or central. This does not entirely correspond to the study by Bieze et al (8) that was conducted in 2014, in which those authors identified HCA located in the left lateral liver as a risk factor for hemorrhage. It is thought that the medial or central location and maximum surrounding liver tissue prevent rupture of the HCA by tamponade. Our study likewise suggests that medial or centrally located HCA may cause hemorrhage as well.

The biggest limitation of this retrospective study is the design, which has inherent bias. By requesting data from all of the treatment hospitals, the proportion of missing data was

kept to a minimum. In addition, hemorrhage makes the measurement of HCA diameter more difficult and less reliable. Therefore, the reported median diameter of 76 mm for the HCAs at presentation might be an overestimation.

In conclusion, this study confirmed that patients with massive hemorrhage due to ruptured HCA but with cessation of bleeding and in stable condition may be treated conservatively in the acute situation. SAE can be a solution for unstable patients with persistent bleeding and decreasing hemoglobin levels. No long-term complications were documented. As the risk of rebleeding is very low after cessation of OC and most HCAs regress spontaneously, secondary treatments, such as tumor resection, SAE or RFA, may only be considered in patients with HCA >5 cm after follow-up or in patients with an expressed desire for future pregnancy. However, regular follow-up by imaging in these patients should be considered.

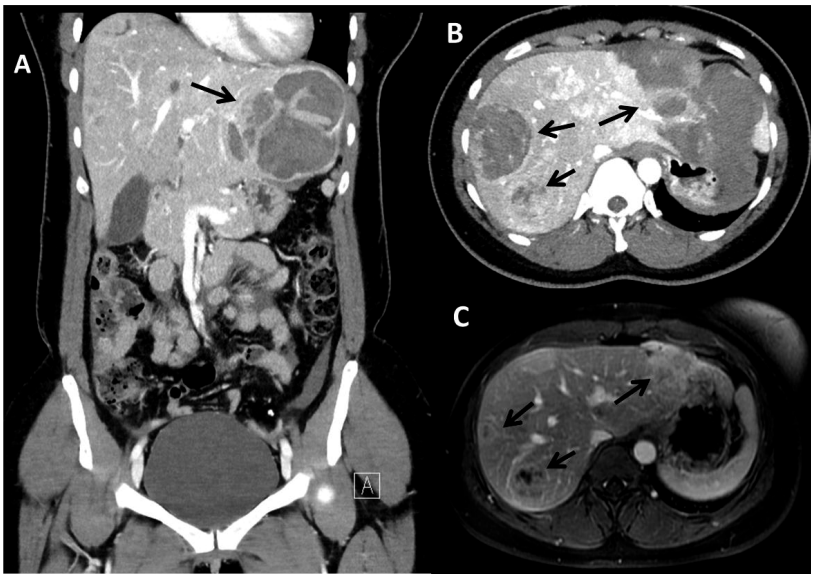

Figure 2. Representative case of grade III bleeding hepatocellular adenoma (HCA) involving a 28-year-old female who presented at the emergency department.

A: Coronal CT image in arterial phase shows a large hematoma in the left liver lobe (arrow); B: On the axial CT slice different bleeding adenomas are present in both lobes (arrows). Extracapsular peritoneal bleeding around the stomach is seen (*); C: One year later on MRI in the venous phase all adenomas have decreased significantly in size.

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CHAPTER 8

Safety and efficacy of transarterial embolization of hepatocellular adenomas in a multicentre cohort study

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ABSTRACT

Introduction: Hepatocellular adenoma (HCA) >5 cm in diameter has an increased risk of haemorrhage and malignant transformation, and is considered an indication for resection. Alternative to resection, transarterial embolization (TAE) may play a role in prevention of complications of HCA, but its safety and efficacy are largely unknown. The aim of this study was to assess outcomes and post-embolization effects of selective TAE in the management of HCA.

Methods: A retrospective, multicentre cohort study included patients aged ≥18 years, diagnosed with HCA and treated with TAE. Patient characteristics, 30-day complications, tumour size before and after TAE, symptoms before and after TAE and need for secondary interventions were analysed.

Results: Overall, 59 patients with a median age of 33.5 years were included among six centres; Fifty-seven out of 59 patients were female. Median tumour size at time of TAE was 76 mm. Six out of 59 (10 per cent) patients had a major complication (i.e. cyst formation or sepsis), which could be resolved with minimal therapy, but prolonged hospital stay. 34 out of 59 (58 per cent)patients were symptomatic at presentation. There were no significant differences in symptoms before TAE and symptoms evaluated on the short term (<3 months) after TAE (P>0.050). First follow-up imaging was performed after a median of 5.5 months after TAE and showed a reduction in size to a median of 48 mm (P<0.001).

Conclusion: TAE is safe and can lead to adequate size reduction of HCA. This minimally invasive intervention offers an alternative to resection.

INTRODUCTION

Hepatocellular adenoma (HCA) is an uncommon, benign tumour of the liver that mostly occurs in middle-aged women.1,2 Over the last few decades, its rising incidence has been associated with the widespread use of oral contraceptives and the obesity pandemic.1-6 Other, less common aetiologies include use of anabolic androgens, a history of Maturity-Onset Diabetes of the Young or glycogen storage disease.7-11

HCA is a hypervascular lesion with exclusively arterial blood supply, resulting in increased intratumoural pressure rendering these tumours susceptible to life threatening bleeding.3,5,12 The reported risk of spontaneous bleeding ranges from 20-40 per cent.13-15 Risk of bleeding is increased in larger diameter tumours, in exophytic growing tumours, and has also been reported in the sonic hedgehog (sh-HCA) and inflammatory (I-HCA) subtypes.16-24 Malignant transformation into hepatocellular carcinoma (HCC) has been reported in 4.3 per cent of HCA patients.25 An established risk factor for malignant transformation is HCA diameter >5 cm.12,18,26 Another important risk factor for malignant transformation is a β-catenin (exon 3) mutation.27-29 MRI, as a non-invasive diagnostic technique, has become a useful diagnostic tool in identifying HCA subtypes.30,31

Patients with HCA may experience complaints such as nausea, pain and tiredness. Although correlation of these symptoms with the presence of HCA remains difficult, they may severely influence patients' quality of life.32 Persistence of these complaints, even when tumour diameter does not exceed 5 cm, may be an indication for resection in selected patients to achieve symptom relief.12,16,19,21,33 To date, elective resection remains the gold standard in the treatment of patients with HCA > 5 cm, to prevent potential bleeding and malignant transformation.18,20,21,23 However, elective liver resection for non-ruptured benign tumours is associated with a reported major morbidity up to 7.2 per cent.34 Alternatively, transarterial embolization (TAE) offers an attractive minimally invasive treatment for non-bleeding HCA35-37. As the majority of patients with HCA are young, otherwise healthy female individuals, cosmetic outcomes can be considered important. TAE could be used in an elective setting to avoid unnecessary resection, in an open or laparoscopic approach.

Conceptually, tumour regression with potentially reduction of the risk of bleeding supports the use of TAE as an elective treatment for HCAs ≥5 cm. The aim of this study was to assess safety and efficacy of TAE in the management of HCAs and to describe its post-embolization effects in a multi-centric, retrospective cohort study.

METHODS

Ethics

Approval of the medical ethical committee of each participating centre was obtained.

Study design and inclusion

An international retrospective multicentre cohort study was performed in patients diagnosed with HCA who underwent TAE between 2001 and 2018 in collaboration with four centres participating in the Dutch Benign Liver Tumour Group (DBLTG). In addition, international data were accrued from the Beaujon Hospital of Paris (France) and the General Hospital of Southampton (United Kingdom). All participating centres collected data from (electronic) patient records and charts. All data were processed and stored anonymously. Patients were excluded if essential data on the TAE procedure or outcomes were lacking. The STROBE guidelines were adhered to in this study.38 Details on data storage and ethical approval are shown in the study protocol in file 1 of the supplementary files.

Definitions and outcomes

The following patient characteristics were collected: age, sex, body mass index (BMI), OC or steroid use, comorbidities associated with HCA, hepatitis B and C viral status, presence of solitary or multiple tumours and the liver segments involved. In addition, data on the type of imaging used for diagnosis was documented (i.e. contrast enhanced magnetic resonance imaging (CE-MRI), MRI without contrast, contrast enhanced computer tomography (CE-CT), CT without contrast, and regular ultrasound). Data on subtype of HCA based on imaging, subtype based on assessment of biopsy or resection specimen and molecular subtype based on molecular diagnostics were collected. Data on subtype were noted as a baseline characteristic, but not used for subgroup analyses due to the limited number of patients available for this study. When no material for pathology was present, HCA subtype was determined based on contrast enhanced MRI. Subtypes were classified as steatotic (H-HCA), inflammatory (I-HCA), β-catenin-activated (β-HCA), combined inflammatory and β-catenin-activated (β-IHCA) HCA 39,40; sh-HCA could not be identified because molecular diagnostics for this subtype were not yet implemented at time of diagnosis of most patients. Data on the presence of bleeding (i.e. clinical bleeding, subclinical bleeding, bleeding after biopsy), indication for TAE (i.e. elective because of size, elective because of symptoms, elective because of (impending) bleeding, acute because of bleeding or poor surgical candidate) and management (i.e. TAE, surgery after TAE, radio frequency ablation (RFA) and TAE combined, TAE and RFA combined followed by surgery) were collected. Lastly, the type of embolization agent used was recorded.

Primary outcome parameters

The primary outcome parameters of this retrospective cohort study were safety, defined as procedure related morbidity consisting of postembolisation syndrome (right upper quadrant pain, fever, nausea/vomiting),41 decrease in renal function,42 sepsis (defined as life-threatening organ dysfunction caused by a dysregulated host response to infection)43, and adverse events (i.e. abscess formation, bleeding, malignant transformation). Complications were graded according to the Society of Interventional Radiology Classification System for Complications by Outcome (SIR).44 Minor complications were classified as: A, no therapy, no consequence; and B, nominal therapy, no consequence, including overnight admission for observation only. Major complications were classified as: C, requiring therapy, minor hospital admission (less than 48 h); D, requiring major therapy, unplanned increase in level of care, prolonged hospital stay (48 h or more); E, permanent adverse sequelae; and F, death. Length of hospital stay was also recorded.

Secondary outcome parameters

The secondary outcome parameters were efficacy: the short-term effects of TAE on symptoms and size of both bleeding and non-bleeding HCAs following TAE. To this end, data on symptoms before and within three months after TAE were obtained. In addition, any decrease in tumour size was measured; tumour size (in mm) was defined as the diameter of the largest tumour present. Tumour size was measured at diagnostic imaging, last imaging before TAE, and first follow-up after TAE and last follow-up. Tumour growth or regression was assessed using the RECIST criteria.45 Large (intra)tumoural hematomas were not included in total tumour size. In case adenomatous tissue in the HCA was not visible due to the hematoma, the patient was not included in this study. The overall success rate was based on how often surgery was prevented by TAE. Of the patients that subsequently underwent surgery, the following data were recorded: indication for surgery, symptoms before and after surgery, type of surgery, perioperative and postoperative complications, length of hospital stay and the occurrence of incisional hernias. All outcomes were divided into two categories for bleeding (defined as a bleeding confirmed on imaging, being the indication for TAE) and non-bleeding HCA.

Statistical analysis

SPSS statistics for Windows version 24.0 (SPSS Inc., Chicago, IL, USA) was used. Outcomes were reported for the complete cohort, and stratified for bleeding and non-bleeding tumours. Categorical data are presented as proportions, continuous data are presented as mean and standard deviation (SD) if normally distributed, and as median and interquartile range (IQR) if not normally distributed. Normality was assessed using both Kolmogorov-Smirnov and Shapiro-Wilk test for normality. The values for asymmetry and kurtosis between -2 and +2 were considered acceptable in order to prove normal univariate distribution.46 The Wilcoxon Signed Ranks Test was used for not normally distributed

data (tumour size). The McNemar's test was used on paired nominal data (symptoms before and after surgery). A two- tailed P<0.050 was considered statistically significant.

RESULTS

Overall, 60 patients were identified, of whom 59 patients met the inclusion criteria (Table 1). Twenty-tree patients underwent TAE for bleeding HCA, and 36 underwent elective TAE for non-bleeding HCA. One patient was excluded because it turned out that the reported embolization was not TAE, but a portal vein embolization. Overall, median age was 33.5 years and 57 out of 59 patients (97 per cent) were female. Median tumour size at time of diagnosis was 84 mm, 85mm in the bleeding patients and 83mm in the non-bleeding patients. Median tumour size measured right before TAE was 76 mm, 81 mm in the bleeding patients and 70.3 in the non-bleeding patients (this was 5.7 months after diagnosis, there was no significant difference in size between the moment of diagnosis and the moment right before TAE). The most frequently used embolization agent in the bleeding group was PVA (*n*=9; 39 per cent) whereas microspheres were mostly used in the non-bleeding group (*n*=14; 39 per cent).

Safety of TAE

Seven out of 59 patients had complications (12 per cent), of whom six had an SIR D complication (10 per cent) (Table 2). Of these, two patients developed an intrahepatic cyst, which both required drainage because of infection. Two other patients developed sepsis, which was successfully treated with supportive care including antibiotic use. One patient died three months after TAE; this patient received TAE of a bleeding HCA in an emergency setting because of haemorrhagic shock. After TAE, the patient was transferred to the intensive care unit of another hospital. The patient subsequently suffered from aspiration pneumonia and died. Any further details on this patient were lacking because of anonymous data collection and no consent to obtain individual data from the other hospital. During follow-up, no patients experienced a clinically overt bleeding nor rebleeding of HCA previously treated with TAE. Post-embolization syndrome (right upper quadrant pain, fever, nausea/vomiting) was observed in one patient, which resolved without intervention. No clinically relevant disturbances of liver function tests were reported. Short-term pain after TAE occurred in 10 (17 per cent) patients.

Table 1: Baseline characteristics, bleeding and non-bleeding HCA

Patient characteristics	Total (*n*=59)		Bleeding (*n*=23)		Non-Bleeding (*n*=36)	
Age (years), median (IQR)	33.5	(26.1-41.2)	34.4	(25.0-42.7)	32.7	(26.7-39.9)
BMI (kg/m²), median (IQR)	29.8	(25.3-37.9)	30.1	(26.3-32.0)	28.7	(24.3-39.5)
Size at time diagnosis (mm), median (IQR)	84	(59-100)	85	(58-100)	83	(57-106.3)
Sex, female, *n (percent)*	57	(96.6)	23	(100.0)	34	(94.4)
Oral contraceptive, *n (percent)*						
Stopped at time of diagnosis	43	(73)	(18)	(78)	(25)	(69)
Stopped prior to diagnosis	10	(17)	(4)	(17)	(6)	(17)
Unknown	4	(7)	(1)	(4)	(3)	(8)
Anabolic steroids	1	(2)	(0)	(0)	(1)	(3)
Never used	1	(2)	(0)	(0)	(1)	(3)
Comorbidities, *n (percent)*						
Diabetes mellitus type I	3	(5)	(2)	(9)	(1)	(3)
Diabetes mellitus type II	2	(3)	(1)	(4)	(1)	(3)
MODY 3	1	(2)	(0)	(0)	(1)	(3)
Glycogen storage disease	1	(2)	(0)	(0)	(1)	(3)
Insulin resistance and PCOS	1	(2)	(0)	(0)	(1)	(3)
None	51	(86)	(20)	(87)	(31)	(84)
Viral status, *n (percent)*						
Hepatitis B positive	2	(3)	(1)	(4)	(1)	(3)
Hepatitis C positive	0	(0)	(0)	(0)	(0)	(0)
Tumour pattern, *n (percent)*						
Solitary	24	(41)	(8)	(35)	(16)	(44)
Multiple	35	(59)	(15)	(65)	(20)	(56)
Location						
Both	30	(51)	(14)	(61)	(16)	(44)
Right	19	(32)	(6)	(26)	(13)	(36)
Left	9	(15)	(2)	(9)	(7)	(19)
Unknown	1	(2)	(1)	(4)	(0)	(0)
Imaging, *n (percent)*						
CE-MRI	50	(85)	(18)	(78)	(32)	(89)
CE-CT	4	(7)	(3)	(13)	(1)	(3)
Ultrasound and CE-CT	4	(7)	(1)	(4)	(3)	(8)
Ultrasound	1	(2	(1)	(4)	(0)	(0)
Molecular subtype, *n (percent)*						
HCA - subtype not specified	33	(56)	(18)	(78)	(15)	(42)
I-HCA	15	(25)	(4)	(17)	(11)	(31)
H-HCA	5	(9)	(0)	(0)	(5)	(14)

	B-IHCA	3	(5)	(0)	(0)	(3)	(8)
	U-HCA	1	(2)	(1)	(4)	(0)	(0)
	Uncertain diagnosis	1	(2)	(0)	(0)	(1)	(3)
	I-HCA + HCC	1	(2)	(0)	(0)	(1)	(3)
Bleeding, *n (percent)*							
	No bleeding	28	(48)	(0)	(0)	(28)	(78)
	Clinical bleeding	20	(34)	(20)	(87)	(0)	(0)
	Subclinical bleeding	6	(10)	(1)	(4)	(5)	(14)
	Bleeding after biopsy	5	(9)	(2)	(9)	(3)	(8)
Indication TAE*, *n (percent)*							
	Size, elective	31	(53)	(0)	(0)	(31)	(86)
	Haemorrhage, acute	19	(32)	(19)	(83)	(0)	(0)
	Haemorrhage, elective	4	(7)	(4)	(17)	(0)	(0)
	Symptoms	4	(7)	(0)	(0)	(4)	(11)
	Poor surgical candidate	1	(2)	(0)	(0)	(1)	(3)
Management							
	TAE	36	(61)	(14)	(61)	(22)	(61)
	TAE followed by surgery	19	(32)	(6)	(26)	(13)	(36)
	TAE and RFA	3	(5)	(2)	(9)	(1)	(3)
TAE and RFA followed by surgery		1	(2)	(1)	(4)	(0)	(0)
Embolic agent							
	PVA	22	(37)	(9)	(39)	(13)	(36)
	Microspheres	16	(27)	(2)	(9)	(14)	(39)
	(Platinum) Coils	6	(10)	(5)	(22)	(1)	(3)
	Gelatine Sponge/foam	3	(5)	(2)	(9)	(1)	(3)
	Foam + PVA	3	(5)	(2)	(9)	(1)	(3)
	Coils + Microspheres	1	(2)	(1)	(4)	(0)	(0)
	Unknown	8	(14)	(2)	(9)	(6)	(17)

*35 out of the 36 non-bleeding patients had tumours larger than 5 cm; Maturity-Onset Diabetes of the Young = MODY; One of the hepatitis B positive patients had suspicion of HCC; Molecular subtype was based on histopathological analysis, and if unavailable, on MRI.

Table 2: Outcomes of patients with complications

	Complication	SIR	Management	Surgical management
Patient 1 – NB	Post-embolization syndrome	A	None	No surgery needed
Patient 2 – NB	Cyst formation	D	Surgical video assisted drainage	Surgery because of tumour growth
Patient 3 – NB	Sepsis	D	Antibiotics. supportive care	No surgery needed
Patient 4 - NB	Analgesia and allodynia of the right cutaneous femoral nerve after abscess the puncture site.	D	Drainage	No surgery needed
Patient 5 – B	Cyst formation	D	Drainage	No surgery needed
Patient 6 – B	Sepsis	D	Antibiotics. supportive care	Surgery because of suspicion of HCC
Patient 7 – B	Thrombosis left iliac vein	D	Anti-coagulant therapy	No surgery needed

NB = patient with non-bleeding HCA, B = patients with bleeding HCA, HCC = hepatocellular carcinoma.

Symptoms before and after TAE

There were no significant differences in symptoms before TAE (58 per cent) when compared to symptoms evaluated at short-term (<3 months) after TAE (38 per cent; *P=0.134*). Upon comparing symptoms before TAE with symptoms post-TAE in the sub-categories bleeding and non-bleeding HCA, no significant differences were noted. Details on symptoms are listed in table 3, and a flow chart on the relief of pre-procedural complaints and development of new complaints is available in figure 2.

Outcomes of TAE and surgery

Details on the outcome of TAE and surgical intervention are listed in table 4. Most patients received only a single session of TAE: 36 out of 59 (61 per cent). Median length of hospital stay after TAE was 3 days, which was significantly longer in patients with bleeding HCA (12 days) compared to patients with non-bleeding HCA (2 days; *P*=0.013).

A third of patients required subsequent resection following initial TAE (20/59). Sixteen patients received one session of TAE, whereas three patients had received multiple sessions of TAE. In one patient, the number of TAE sessions was not reported.

Efficacy of TAE

Details on the change in tumour size during follow-up are reported in figure 1, figure S1 and table S1. Overall, median tumour size at the time of TAE was 76 mm. First follow-up imaging was performed after a median of 5.5 months (table S2) following TAE and showed a reduction of 37 per cent, to a median size to 48 mm, (*P*<0.001). Last follow-up imaging occurred at median 27.4 months after TAE; tumour reduction was 45 per cent, with a median size of 45 mm at end of follow-up, (*P*<0.001).

Table 3: Symptoms before and after TAE

Symptoms, *n* (per cent)	Overall					Bleeding					Non-Bleeding				
	Before TAE		After TAE		*P* [a]	Before TAE		After TAE		*P* [a]	Before TAE		After TAE		*P* [a]
Short-term symptoms	34	(58)	22	(38)	0.134	17	(74)	8	(35)	0.109	17	(47)	14	(39)	0.774
Pain	27	(46)	15	(25)	-	15	(65)	3	(13)	-	12	(33)	12	(33)	-
None	16	(27)	24	(41)	-	5	(22)	9	(39)	-	11	(31)	15	(42)	-
Multiple	5	(9)	1	(2)	-	1	(4)	0	(0)	-	4	(11)	1	(3)	-
Nausea	1	(2)	2	(3)	-	1	(4)	2	(9)	-	0	(0)	0	(0)	-
Tiredness	0	(0)	4	(7)	-	0	(0)	3	(13)	-	0	(0)	1	(3)	-
Missing	10	(17)	13	(22)	-	1	(4)	6	(26)	-	9	(25)	7	(19)	-

a The Mc Nemar's test for paired nominal data was used.

Table 4: Outcomes of TAE and surgery

	Total (n =59)	Bleeding (n =23)	Non-bleeding (n = 36)
TAE			
Number of TAE sessions required, n *(per cent)*			
1 session	36 (61)	13 (57)	23 (64)
2 sessions	6 (10)	2 (9)	4 (11)
3 sessions	5 (9)	2 (9)	3 (8)
4 sessions	1 (2)	0 (0)	1 (3)
Number of sessions not reported	11 (19)	6 (26)	5 (14)
Length of hospital stay IQR [a] (days)	1.0 3.0 10.5	4.8 12.0 18.3	1.0 2.0 5.0
SURGERY			
Surgery needed, *n (per cent)*	20 (34)	7 (35)	13 (65)
Indication surgery, *n (per cent)*			
Persisting size	9 (45)	1 (14)	8 (62)
Bleeding	4 (20)	4 (57)	0 (0)
HCC suspected	3 (15)	1 (14)	2 (15)
Growth	2 (10)	0 (0)	2 (15)
Persisting symptoms	1 (5)	0 (0)	1 (8)
Pregnancy wish	1 (5)	1 (14)	0 (0)
Type of surgery, *n (per cent)*			
Open segment resection	9 (45)	4 (57)	5 (39)
Open hemihepatectomy	6 (30)	2 (29)	4 (31)
Laparoscopic hemihepatectomy	5 (25)	1 (14)	4 (31)
Perioperative complications, *n (per cent)*			
None	18 (90)	6 (86)	12 (92)
Bleeding	2 (10)	1 (14)	1 (8)
Postoperative complications, *n (per cent)*			
None	14 (70)	3 (43)	11 (85)
Bleeding (Clavien-Dindo I)	2 (10)	2 (29)	0 (0)
Abscess (Clavien-Dindo III)	2 (10)	2 (29)	0 (0)
Thrombosis (Clavien-Dindo II)	1 (5)	0 (0)	1 (8)
Thrombosis and sepsis (Clavien-Dindo II)	1 (5)	0 (0)	1 (8)
Length of hospital stay IQR [a] (days)	7.5 10.0 11.0	10.0 10.0 41.0	4.0 8.0 10.0
Incisional hernias [b]	2 (10)	1 (15)	1 (8)

b All symptomatic.

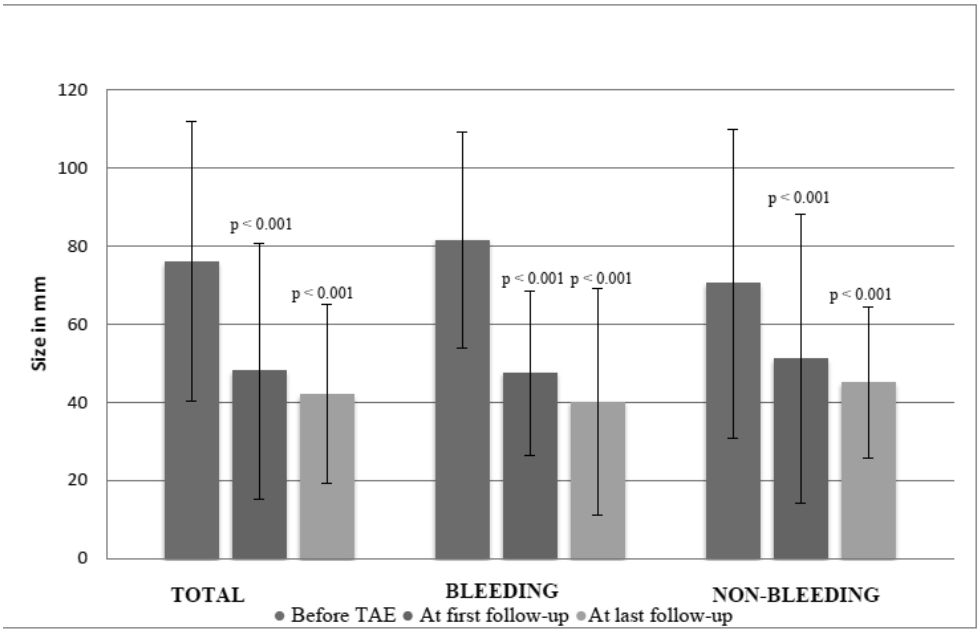

Figure 1. Tumor size before and after TAE.

TAE= Transarterial Embolization. Median tumour size is displayed in mm, with its standard deviation was used to compare size pre- and post TAE. The Wilcoxon Signed Ranks Test was used to compare size pre- and post TAE.

In the subgroup analysis for bleeding HCA, which were 82 mm at the time of TAE, similar regression was found: 47mm (41 per cent reduction) at first follow-up (*n*=18) (*P*<0.001), and 41 mm (50 per cent reduction) at the end of follow-up (*n*=12) (*P*<0.001). A similar pattern was observed in the subgroup of non-bleeding HCA, which were 70 mm at the time of TAE: 51 mm (27 per cent reduction) at first follow-up (*n*=35) (*P*<0.001), and 45 mm (36 per cent reduction) at last follow-up (*n*=21) (*P*<0.001). Of the 36 patients (61 per cent) who had undergone no other procedure than TAE until the end of the study period, 28 had a tumour of 5 cm or more before TAE. After 6 months, 17/36 (47 per cent) had a reduction of the tumour below 5 cm. At last follow-up, 27/36 (75 per cent) patients had reduction of tumour size below 5 cm. No statistical differences were found regarding size reduction and regrowth after TAE for the different embolization agents. Details on median tumour size at the moment of TAE, and subsequently first and last follow-up categorized for the different TAE agents are reported in table S4.

Malignant transformation

Two patients had an uncertain diagnosis at histopathological assessment. The histopathological report stated I-HCA/ highly differentiated HCC in one patient, in the pathology report of a lesion resected preceding TAE. This patient had multiple tumours

in both liver lobes, and underwent embolization of the remaining lesions, which could not be removed during surgery. Of these tumours treated with TAE, no material for pathology was available. Although the pathology report of this patient suggested I-HCA/HCC previously, the clinical course of this patient was uneventful after TAE. The remaining lesions regressed after treatment with TAE, no metastasis occurred, no paraneoplastic signs or elevated tumour markers were present and the patient is still alive after a follow-up of four years with no signs of disease. In the other patient with uncertain diagnosis of HCA or HCC, this was based on indistinctive pathological features after hemihepatectomy. This patient was a male body builder using anabolic steroids who received TAE for treatment of the HCA remaining in the other liver lobe. In addition, two patients with biopsy proven β-HCA underwent TAE and showed no signs of malignant transformation or regrowth during follow-up, after 40 and 39 months, respectively.

DISCUSSION

This study shows that TAE for large hepatocellular adenoma is relatively safe and potentially effective in decreasing tumour size, as tumours decreased to approximately half their size. This decrease was also clinically relevant, as the majority of patients reached a reduction to below 5 cm, therefore not requiring resection. The results warrant a prospective study to compare outcomes of TAE with (minimally invasive) surgery in the management of HCA.

A systematic review comprising 151 (95 non-bleeding) patients who underwent TAE for the treatment of HCA identified no mortality.42 Complete regression on imaging of the tumour was observed in 10 per cent of the patients, whereas a decrease of more than 30 per cent of the tumour diameter was observed in 75 per cent of the patients. The review however could not distinguish size reduction between bleeding and non-bleeding tumours42. A small study proposed that bleeding of HCA leads to size reduction of the tumour, possibly because of disruption of the tumour and subsequent destruction of the adenomatous tissue.47 This may imply that the effect of TAE on regression is merely based on the bleeding event. Therefore, to assess the overall effect of TAE on size reduction, both bleeding and non-bleeding HCAs were included and compared in this study. A similar size reduction was seen in both groups.

Whether the decrease in size also reduces the risk of malignant transformation is subject to speculation. In this study, suspicion of malignancy was the reason for resection in some patients, however in only one of these patients (a male patient) malignancy could not be excluded. No malignant transformation was observed in the two patients with β-HCA after TAE with a follow-up of 40 and 39 months, respectively. However, since these patients are the first and only cases of β-HCA treated with TAE to be reported in literature so far, the implications of a positive β-catenin status after biopsy in regard with treatment with TAE are unknown.

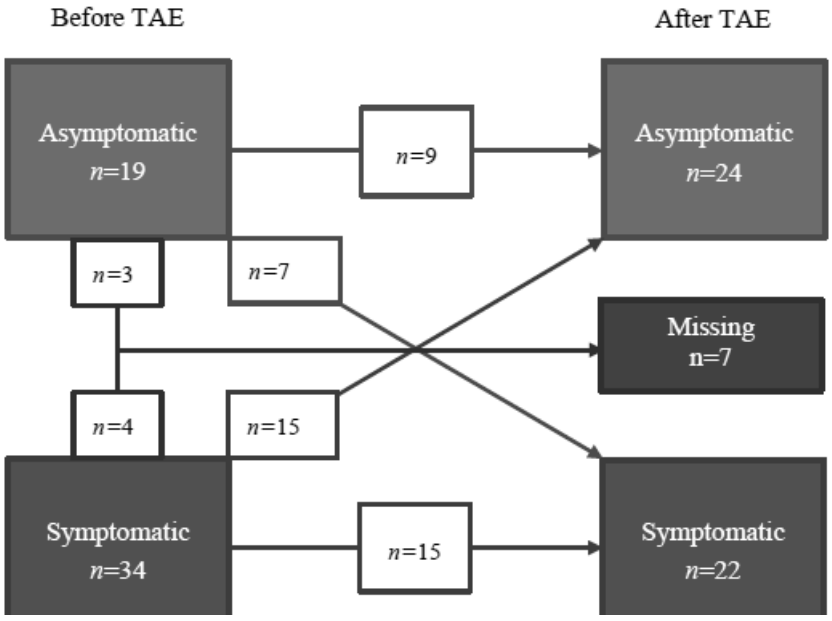

Figure 2. Patients symptomatic before and after TAE.

This figure represents which patients were symptomatic and asymptomatic before TAE, and which patients were symptomatic and asymptomatic after TAE. The newly developed complaints of the patients that were asymptomatic before TAE (n=7) only lasted for a short period. Of the 19 asymptomatic patients, nine remained asymptomatic. Symptoms were resolved in 15 of the 34 symptomatic patients. In 15 of the patients that were symptomatic before TAE, complaints either persisted or were replaced by short-term complaints; five out of theses 15 patients had short-term complaints only.

The question remains whether biopsy should be performed routinely preceding TAE to identify β-HCA, especially since molecular diagnostics have become available. However there are also downsides to routine biopsy such as bleeding, needle-track tumour seeding (the latter two carrying a small risk), and sampling error.

Whether TAE helps to relieve complaints in symptomatic patients remains uncertain. No differences in symptoms before TAE and <3 months after TAE were found in this study. This finding is surprising, especially considering that patients with acute bleeding HCA usually present with severe pain. While one would assume that stopping the bleeding would relief complaints, a counter-argument could be that in intracapsular bleeding, the hematoma remains. We, however, found a trend suggesting relief of symptoms in the bleeding subgroup, although differences were not statistically significant.

A limitation of this study is that no validated questionnaire was used and only symptoms <3 months post TAE were evaluated. Newly developed complaints could therefore not be distinguished from pre-existent complaints due to patients crossing-over from the asymptomatic to the symptomatic group after final treatment (figure 2). Moreover, it is unclear whether any newly developed complaints were of a transient nature or not. The pain developing after TAE was most likely caused by intratumoural ischemia induced by TAE. This pain should reside after necrosis of the tumour is complete. A follow-up of at least one year is necessary to assess the long-term impact of TAE on relief of symptoms, using a validated quality of life questionnaire.

An alternative to both surgery and TAE could be local ablation of the HCA. Percutaneous image-guided ablation is less invasive compared to open or laparoscopic surgery. Benefits and harms of percutaneous ablation in patients with HCA have thus far never been assessed in a prospective cohort study, and the largest retrospective study counts only 18 patients.48 The complications that occurred in this study were mainly the formation of cysts because of necrosis. These complications were readily resolved using routine therapeutic interventions. Moreover, percutaneous ablation is typically performed in tumours smaller than 4 cm because of the risk of incomplete ablation.49,50 The majority of HCA with an indication for intervention exceed 5 cm, however, it is uncertain whether incomplete ablation in HCA is an issue. An advantage of TAE is that application is not limited by tumour size or location.

A limitation of our study is the lack of a control group. Ideally, patients should be randomized between TAE and sham TAE to assess the true effect of TAE on relief of complaints. To assess effect on tumour size however, randomization is not strictly necessary, as a placebo effect will not affect tumour size after TAE. The control group in the study by Klompenhouwer et al. could be used as a surrogate group when it comes to tumour size: it showed that HCA that was managed conservatively regressed,51 however in this series HCA treated with TAE showed a larger decrease.

A strength of the current study is the national and international collaboration of the centres taking part in the DBLTG. This collaboration resulted in an increase in volume and in the number of specialized centres involved in the field of benign liver tumours, increasing the quality of the study. Another strength of this study is its clinical relevance; TAE as primary treatment of HCA has potential to be considered in future guidelines.

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SUPPLEMENTARY FILES

Table S1: Change in tumour size during follow-up.

	Total				Bleeding				Non-bleeding			
	N[A]	Mean	SD	P-value[B]	N[A]	Mean	SD	P-value[B]	N[A]	Mean	SD	P-value[B]
Size at time of diagnosis	54	85.7	37.3	-	18	80.2	27.0		36	88.4	41.6	
Size at time of TAE (mm)	54	80.4	35.9	-	18	82.8	27.8	-	36	79.3	39.6	-
Size after TAE at first follow up (mm)	53	52.3	32.7	**< 0.001**	18	45.4	21.0	**< 0.001**	35	55.9	37.1	**< 0.001**
Size after TAE at last follow up (mm)	33	40.8	22.9	**< 0.001**	12	41.8	29.1	**< 0.001**	21	40.2	19.2	**< 0.001**

Table S2: follow-up

	Total		Bleeding		Non-bleeding	
	N	IQR[a]	N	IQR[a]	N	IQR[a]
First follow-up (months)	59	2.6 **5.5** 18.9	23	4.0 **13.5** 27.2	36	2.5 **4.1** 11.2
Last follow-up (months)	59	12.7 **27.4** 65.8	23	20.8 **58.0** 79.5	36	9.9 **18.1** 48.5
Total follow-up (months)	59	21.9 **43.7** 74.8	23	14.4 **45.3** 77.5	36	23.4 **42.6** 47.7

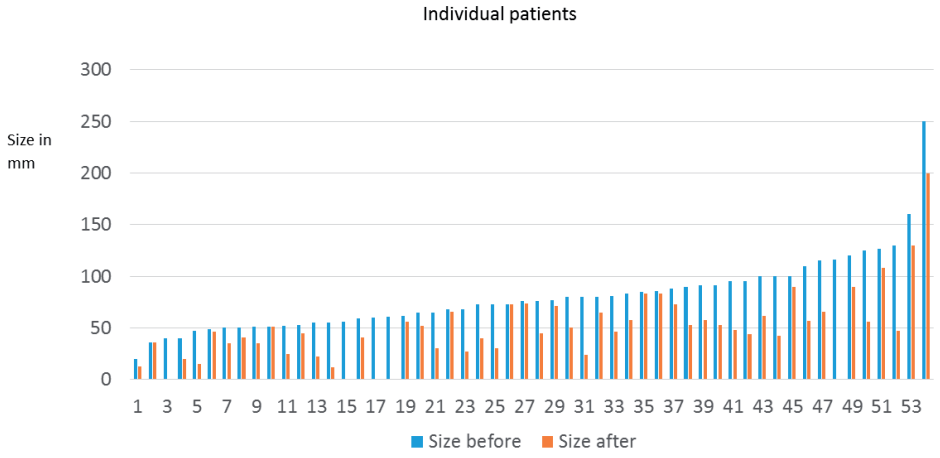

Figure S1. Tumour size before and after TAE, delta per patient.

Four patients had tumours smaller than five cm; of these, two patients had tumours larger than four cm, and two patients smaller four cm. The patients with tumours below 4 cm were treated because of bleeding. The other 2 patients with tumor size above 4cm and below 5 cm were treated (i) because of tumour growth, and (ii) because of severe complaints.

CHAPTER 9

Phenotype or genotype: new decision-making dilemmas in hepatocellular adenoma

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Hepatocellular adenomas (HCA) may undergo malignant transformation to hepatocellular carcinoma (HCC). Several HCA subtypes can be distinguished: H-HCA [characterized by biallelic inactivating mutation of Hepatocyte Nuclear Factor 1A], I-HCA [activating mutations in different oncogenes of the JAK/STAT pathway], B-HCA [mutation in the CTNNB1 gene encoding for β-catenin protein, can be situated on exon 3, 7 or 8], B-IHCA [shares both JAK/STAT pathway activation and CTNNB1 mutation] and sh-HCA [activation of sonic hedgehog signaling pathway](1, 2). In case of multiple lesions, different subtypes of HCA may be observed(1, 3). B-(I)HCA are associated with a higher risk of malignant transformation. It has been described that especially B-(I)HCA with exon 3 mutations (B^{ex3}(I)HCA) are at high risk of HCC development, whereas the risk in those with exon 7/8 mutations ($B^{ex7,8}$(I)HCA) appears to be low(2, 4). In this report we present two patients with HCA and unusual pathological findings that might impact clinical views.

Case 1

A 40-year-old female patient underwent a segmentectomy because of an inhomogeneous hepatic mass. Macroscopic examination showed a solid 90mm lesion, with light and dark brown areas and some small (1-14mm) whitish nodules (fig.1A). Microscopic examination showed a hepatocellular proliferation without atypical features at the background (fig.1B-C). Glutamine synthetase (GS) appreciated faint heterogeneous expression at the center and a reinforcement at the periphery, a surrogate pattern of β-catenin activation. CRP staining was negative (fig.1D). The sections corresponding to the whitish nodules revealed decreased and disorganized reticulin network and positivity for glypican-3 (fig.1E), features pointing towards HCC transformation with good differentiation. Additional molecular analysis of the main lesion confirmed exon 7 mutation in CTNNB1 (B^{ex7}HCA) and telomerase reverse-transcriptase (TERT) promoter mutation in the areas with HCC transformation.

In a benchmark study from 2017, <4% of included patients with HCA had B^{ex7}HCA and malignant transformation was observed in none of the patients with $B^{ex7,8}$HCA(2). The present case shows that although the risk of malignant transformation in B^{ex7}HCA is lower compared to B^{ex3}HCA, it should not be neglected.

Case 2

A 32-year-old woman underwent a segmentectomy because of multiple liver lesions. Macroscopic examination showed two well-demarcated, non-encapsulated lesions of 65 and 13mm (fig.2A-I, B-I). Both had alternating pale areas with dark-red foci. Microscopic examination showed features of I-HCA with sinusoidal dilatation and inflammatory infiltrates in the pseudoportal areas (fig.2A-II, B-II). Immunohistochemistry demonstrated a dense and diffuse staining for CRP (fig.2A-III, B-III). Moreover, in the small nodule a diffuse staining was found for GS, with a more intense staining at the periphery of the

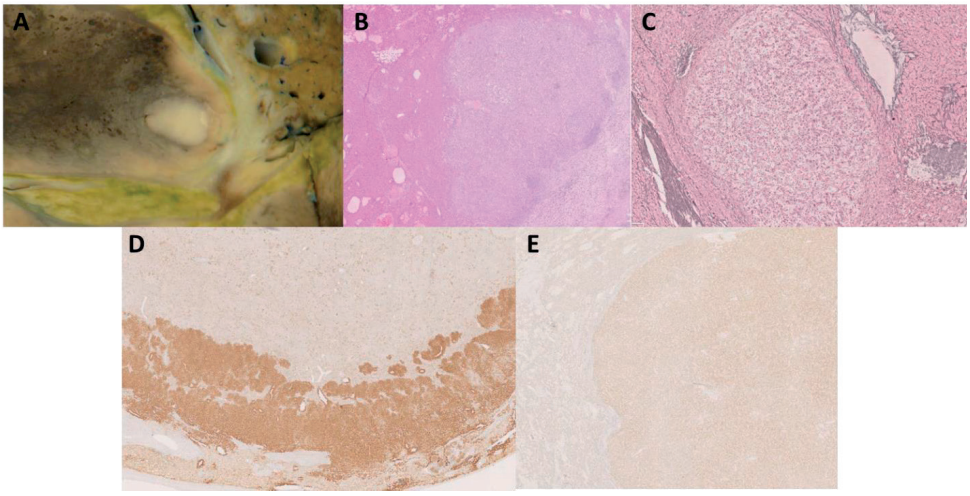

Figure 1. Macroscopic en microscopic images case 1

A. Macroscopy, one of the small whitish nodules in the hemorrhagic background of the HCA
B. Microscopy, H-E staining C. Microscopy, Reticulin staining denoting the disorganized reticulin with forming of broad liver plates D. Immunohistochemistry, CRP with dense reinforcement of expression at the periphery E. Immunohistochemistry, diffuse positive Glypican-3 staining

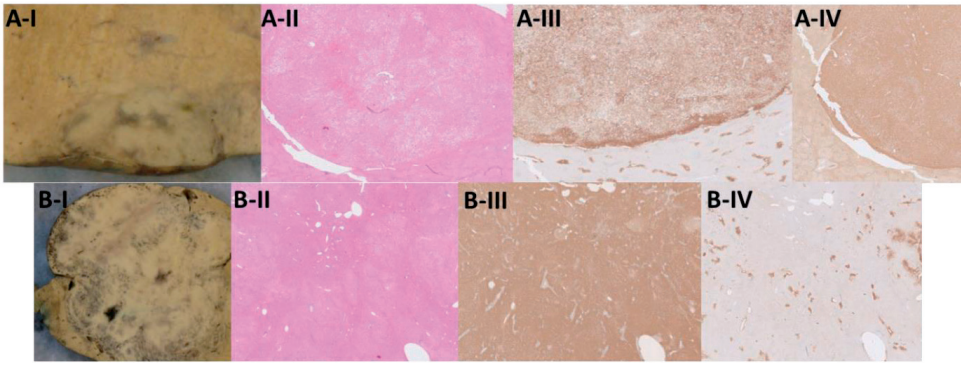

Figure 2. Macroscopic en microscopic images case 2

A. Macroscopic and microscopic images of the small lesion A-I: Macroscopy A-II: Microscopy, H-E staining A-III: Immunohistochemistry, CRP with diffuse positive staining A-IV: Immunohistochemistry, GS with diffuse positive staining B. Macroscopic and microscopic images of the large lesion B-I: Macroscopy B-II: Microscopy, H-E staining B-III: Immunohistochemistry, CRP with diffuse positive staining B-IV: Immunohistochemistry, GS with perivascular staining, no diffuse pattern

lesion, suggesting β-catenin activation (fig.2A-IV). Molecular analysis of the small nodule confirmed CTNNB1 mutation in exon 3. The large nodule was negative for CTNNB1 mutation.

Guidelines on benign liver tumors advise to base management decisions in patients with multiple HCA on the largest lesion(5). The aforementioned benchmark article describes inter-tumor heterogeneity with at least one B^{ex3}(I)HCA in nine patients with multiple HCA(2). Only one patient was described in whom the largest was a H-HCA and a smaller B^{ex3}HCA, in the remaining patients the largest tumor had the CTNNB1 mutation. To our knowledge, the combination of a large IHCA without CTNNB1 mutation and a small B^{ex3}IHCA as described in this case has not been reported up to now.

Conclusively, these cases illustrate the existent risk of malignant transformation in $B^{ex7/8}$HCA. Pending defined indications for molecular analysis, we would propose performing mutational examination in all cases with inconclusive/equivocal immunohistochemistry, at least on biopsy material. Prospectively this will lead to data collection and better understanding of the oncogenic β-catenin exon 7/8 and TERT mutations. Secondly, in the management of multiple HCA, the final decision should be made based on pathomolecular subclassification along with the diameter.

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CHAPTER 10

Can point shear wave elastography differentiate focal nodular hyperplasia from hepatocellular adenoma?

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ABSTRACT

Purpose: Focal nodular hyperplasia (FNH) and hepatocellular adenoma (HCA) are liver tumors needing different management. We assessed the potential of point shear wave elastography (pSWE) to differentiate FNH from HCA and the inter- and intra-observer reliability in these lesions and native liver tissue (NLT).

Methods: We included 88 patients (65 FNH, 23 HCA). pSWE was performed by two experienced liver sonographers (O1 and O2) and acquired within lesion and NLT. Group differences, optimal cut-off for characterization and inter-observer reliability were assessed with Mann-Whitney-U, AUROC and intra-class correlation-coefficient (ICC). Intra-observer reliability in NLT was assessed in 20 healthy subjects using ICC.

Results: Median stiffness was significantly higher in FNH than HCA (7.01 vs 4.98 for O1 ($p=0.017$) and 7.68 vs 6.00 for O2 ($p=0.031$)). A cut-off point for differentiation could not be determined with an AUROC of 0.67 (O1) and 0.69 (O2). Inter-observer reliability was good for lesion-stiffness (ICC=0.86) and poor for NLT-stiffness (ICC=0.09). In healthy subjects, intra-observer reliability for NLT-stiffness was poor for O1 (ICC=0.23) and moderate for O2 (ICC=0.62).

Conclusion: This study suggests that in its present form pSWE cannot reliably differentiate FNH from HCA. Inter- and intra-observer reliability for pSWE in NLT were insufficient. Interpretation of results gained with this method and management decisions based on these results should be done with great caution.

INTRODUCTION

Two clinically important types of benign focal liver lesions are focal nodular hyperplasia (FNH) and hepatocellular adenoma (HCA). Histologically, these two focal lesions differ. FNH is in fact a pseudotumor with a large part consisting of fibrotic stroma making the lesion stiff, while HCA does not have a substantial fibrotic component and resembles the consistency of healthy liver tissue (1).

Differentiating between these two lesions is essential because each require specific management. Follow-up of FNH is not necessary, provided the correct diagnosis was made, but HCA often needs to be resected or at least monitored in view of the risk of bleeding or transformation to hepatocellular carcinoma (2, 3). The current standard diagnostic process makes use of either of two imaging methods, mostly contrast-enhanced ultrasound (CEUS) and contrast-enhanced MRI (CE-MRI) (4-7). The estimated sensitivity and specificity of CEUS for the differentiation of FNH from are 67% and 100% respectively, with a significantly reduced sensitivity in lesions >35mm (8). For CE-MRI with hepatocellular specific contrast the sensitivity is estimated at 91-100% and the specificity at 87-100% (9). When diagnostic uncertainty occurs, these patients may undergo tumor biopsy (10). In order to reduce patient burden and avoid the risk of complications that comes with biopsy (11) or even resection of suspect lesions that turn out to be FNH, improvement of the diagnostic process is warranted.

The first ultrasound elastography method for the liver became available in 2003 in the form of transient elastography with Fibroscan (12). This method uses a mechanic pulse to measure stiffness of the liver tissue. In 2008 a new modality became available which was incorporated in the ultrasound system, named Acoustic Radiation Force Impulse quantification (ARFIÒ, Siemens). This method uses ultrasound point shear wave elastography (pSWE) and measures the speed of the shear wave (perpendicular to the longitudinal axis) in a small region at a freely-chosen depth within 80mm from the skin. Other companies have started producing similar technology, amongst which ElastPQÒ by Philips Healthcare. pSWE can be used as a noninvasive, reproducible, and easy method of assessing liver fibrosis. A few preliminary studies show that pSWE can also be used to determine stiffness of a focal liver lesion such as FNH or HCA and can help in differentiating between these lesions, especially if the lesions are small (13-16).

The primary aim of this study is to assess the diagnostic value of pSWE (ElastPQÒ, Phillips Healthcare) during ultrasound for the differentiation between FNH and HCA. Secondary, we will assess the inter- and intra-observer reliability in these liver lesions and in the native liver tissue (NLT).

PATIENTS AND METHODS

This diagnostic study was performed in a tertiary referral center for focal liver lesions and was approved by the accredited local institutional review board.

Patients with FNH or HCA

Eligible patients were those diagnosed with FNH or HCA between January 1st 2007 and November 30th 2016. Included were all patients who first underwent CEUS and secondly had either contrast enhanced MRI (CE-MRI) or biopsy confirming the diagnosis. If available, histological diagnosis was considered as the reference standard. In all other cases, definitive diagnosis was discussed during a multidisciplinary tumor board (with radiologists, hepatologists and surgeons) and based on the combination of CEUS and CE-MRI characteristics. The following exclusion criteria applied: intervention as treatment for FNH or HCA and severe other liver disease (e.g. cirrhosis, hepatocellular carcinoma, liver metastasis). Furthermore, we excluded women over the age of 50 with an HCA, as these lesions often regress after menopause (17).

We identified potentially eligible patients from the electronic databases of the departments of Gastroenterology and Hepatology and Surgery of the Erasmus MC, Rotterdam. Information on sex, date of birth, date of diagnosis, lesion diameter at diagnosis, CEUS diagnosis, CE-MRI diagnosis and histological diagnosis was retrieved from electronic patient records. CEUS and CE-MRI diagnosis were based on typical imaging characteristics (4-7). HCA subtype (e.g. steatotic, inflammatory, beta-catenin mutated or unclassified) was based on CE-MRI (7, 18, 19) or biopsy (20). An experienced abdominal radiologist (M.T. with 20 years of experience) reviewed the CE-MRI of patients in whom HCA subtype was not yet established and determined the HCA subtype.

Healthy subjects

We asked 20 healthy employees of the department of Gastroenterology & Hepatology and the department of Surgery to volunteer as healthy subjects. Subjects were included if they were male or female, between 20 and 35 years of age and when they were available on the day the measurements took place. Subjects with a known liver disease or a systemic disease requiring medication were excluded.

Ultrasound examination and pSWE

We sent an information letter to all eligible patients and later contacted them by telephone to assess whether they were willing to participate in the study. Patients were scheduled for routine ultrasound examination and pSWE at the outpatient clinic of the Gastroenterology and Hepatology department after we received written informed consent. We performed pSWE using existing transducers (broadband curved array C5-1)

of the Philips Epiq7 ultrasound system. Two experienced liver sonographers (P.T. and R.K., with 25 and 15 years of experience, respectively), hereinafter referred to as O1 and O2, independently performed the measurements in all patients according to the European Federation of Societies for Ultrasound in Medicine and Biology (EFSUMB) guidelines on the use of elastography (21, 22).

In the patients with either FNH or HCA, we measured lesion size in mm and acquired pSWE measurements from the lesion situated best in the field of view, and preferably the largest lesion. In each patient, both sonographers acquired ten measurements in the center of the lesion and ten in the surrounding NLT in the proximity of the focal liver lesion. Patients were asked to hold their breath at the moment of the measurement. Each measurement provides a quantitative value of stiffness in kilopascals (kPa). In the analysis we used the medians of these ten values obtained in the lesion and surrounding NLT. Additionally, we calculated the lesion:liver stiffness-ratio (LLSR).

All healthy subjects were randomly appointed to either O1 or O2. In each of the ten individuals the sonographers performed two rounds of ten measurements in the NLT with a ten-minute interval in between.

Statistical analysis

We used IBM SPSS software version 21.0 (Chicago, Illinois) for statistical analysis and summarized continuous variables as median and interquartile range (IQR) and binary variables as frequency (n) and percentages (%). We used Mann-Whitney U test to assess differences for continuous variables and $\chi 2$ test for categorical variables. Correlation between variables was analyzed using Pearson product-moment correlation coefficient. Performance of the ElastPQ was estimated using receiver operating characteristic (ROC) curves. Interpretation of the ROC was based on the area under the ROC Curve (AUROC): a value between 0 and 1. The accuracy of the diagnostic test was classified using the following point system: < 0.60 fail, 0.60-0.70 poor, 0.70-0.80 fair, 0.80-0.90 good and > 0.90 excellent. Inter-observer and intra-observer reliability were assessed using a two-way mixed effects, consistency, single measures intraclass correlation coefficient (ICC) model. Interpretation of the ICC was based on Cohen's kappa, also a value between 0 and 1. Values < 0.50 were classified as poor inter-rater agreement, 0.50-0.75 as moderate, 0.75-0.90 as good and > 0.90 as excellent. A p-value of < 0.05 was considered as the level of significance.

RESULTS

Patients with FNH or HCA

We found 252 patients (244 females and 8 males) with a focal liver lesion eligible to participate in this study as they underwent both CEUS and CE-MRI or biopsy confirming the diagnosis FNH or HCA. Thirty-three patients were excluded because they either underwent an intervention or because the lesion was not visible at last follow-up and 106 patients were excluded because they were either untraceable or did not consent to participation. One patient was deceased (unrelated to liver disease). Finally, we scheduled pSWE for 113 patients and a total of 88 patients (23 with HCA and 65 with FNH) were included in this study at the end (fig. 1). Eight patients were excluded because the lesion was situated too deep for pSWE and 17 because the lesion could not be found during US examination anymore. O1 performed pSWE in all included patients and O2 repeated the measurements in 62 patients (13 HCA and 49 FNH).

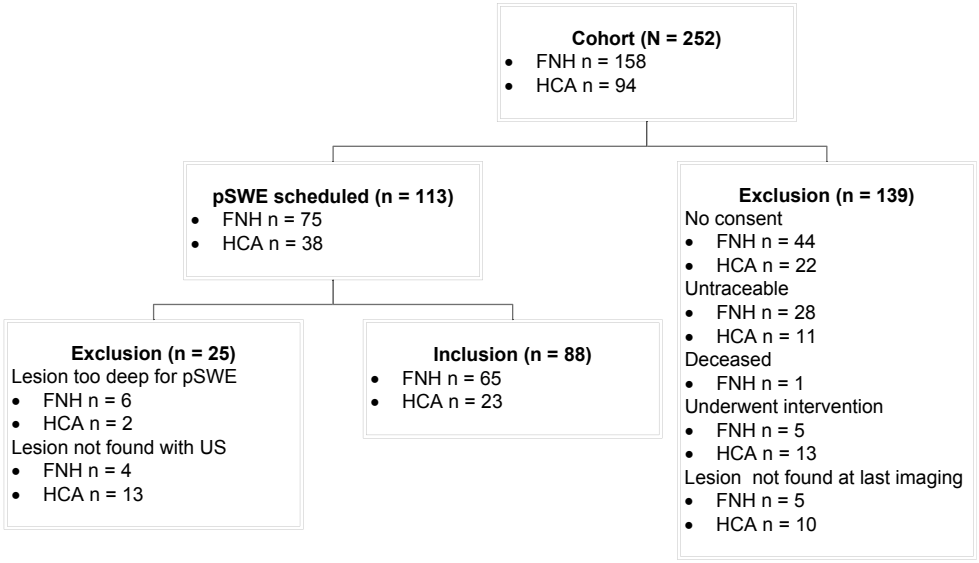

Figure 1. Inclusion flowchart of patients with a focal liver lesion.

FNH = focal nodular hyperplasia. HCA = hepatocellular adenoma. pSWE = point shear wave elastography.

Eighty-seven out of 88 patients included were female (tab. 1). In the FNH group, the diagnosis was confirmed by CEUS in 56 patients (86.2%), seven lesions were characterized as HCA and in two CEUS could not differentiate between FNH and HCA. CE-MRI confirmed FNH diagnosis in 61 patients (93.8%), one lesion was initially characterized as HCA and

Table 1: Baseline characteristics of patients with a focal liver lesion

	FNH (n = 65)	HCA (n = 23)
Sex		
Female	64	23
Male	1	0
Age, years	41 (34 – 52)	43 (33 – 46)
Lesion diameter at diagnosis, mm	50 (35 – 62)	35 (26 – 60)
Lesion diameter at time of study, mm	45 (30 – 60)	20 (12 – 28)
Time since diagnosis, months	71 (62 – 81)	47 (21 – 76)
Diagnosis CEUS		
FNH	56 (86.2)	2 (8.7)
HCA	7 (10.8)	20 (87.0)
FNH or HCA	2 (3.1)	1 (4.3)
Diagnosis CE-MRI		
FNH	61 (93.8)	1 (4.3)
HCA	1 (1.5)	21 (91.3)
FNH or HCA	1 (1.5)	0
Other	0	1 (4.3)
Not performed	2 (3.1)	0
Histopathologic diagnosis		
Yes	11 (16.9)	5 (21.7)
No	54 (83.1)	18 (78.3)

Values are given as n (%) or median (IQR). CEUS = contrast enhanced ultrasound. FNH = focal nodular hyperplasia. HCA = hepatocellular adenoma. CE-MRI = contrast enhanced magnetic resonance imaging.

in one CE-MRI could not differentiate between HCA and FNH. Eleven FNH (16.9%) were biopsy proven. In the HCA group, the diagnosis was confirmed by CEUS in 20 patients (87.0%), two were characterized as FNH and in one CEUS could not differentiate between FNH and HCA. CE-MRI confirmed HCA diagnosis in 21 patients (91.3%), one was initially characterized as FNH and another as a different benign liver tumor (hepatic angiomyolipoma). Five HCA (21.7%) were biopsy proven.

We determined the median FNH and HCA values per observer (tab. 2 and fig. 2). For both O1 and O2, the median FNH stiffness value was significantly higher than the HCA stiffness value (7.01 vs 4.98 kPa (p=0.017) and 7.68 vs 6.00 kPa (p=0.031), respectively). We found a median NLT stiffness value of 2.41 kPa (IQR 1.13-3.45) for O1 and 3.50 kPa (2.96-4.45) for O2. For O1, the median FNH-LLSR was 4.00 (IQR 2.05-7.00) and the median HCA-LLSR was 1.35 (0.84-2.71) ($p<0.001$). For O2, these values were 2.44 (1.52-4.44) for FNH-LLSR and 1.34 (0.96-1.97) for HCA-LLSR (p=0.010). No correlation between lesion size and stiffness value was found for both FNH as HCA ($p>0.05$).

Table 2: Stiffness values

	Observer 1	Observer 2
Stiffness – FNH	7.01 (4.02 – 13.37)	7.68 (5.37 – 12.99)
Stiffness – HCA	4.98 (2.89 – 7.25)	6.00 (3.83 – 7.07)
p-value	0.017*	0.031*
Stiffness – NLT	2.41 (1.13 – 3.45)	3.50 (2.96 – 4.45)
Lesion / liver ratio – FNH	4.00 (2.05 – 7.00)	2.44 (1.52 – 4.44)
Lesion / liver ratio – HCA	1.35 (0.84 – 2.71)	1.34 (0.96 – 1.97)
p-value	<0.001*	0.010*

Values are given as median (IQR). FNH = focal nodular hyperplasia. HCA = hepatocellular adenoma. NLT = native liver tissue. *Mann-Whitney U test showed a statistically significant difference in FNH vs HCA stiffness and LLSR for both observer 1 and 2.

ROC analysis for lesion pSWE values showed an AUROC for differentiating FNH from HCA of 0.67 for O1 and 0.69 for O2. Inter-observer reliability analysis showed an ICC of 0.86 for lesion stiffness (95%-CI 0.78-0.92), 0.09 for liver stiffness (95%-CI -0.16-0.33) and 0.78 (95%-CI 0.66-0.86) for LLSR. Subgroup analysis based on lesion diameter was done in 36 lesions <30mm (median 20mm, IQR 14-25) and 52 lesions >30mm (median 50mm, IQR 40-66). It resulted in an ICC for lesions <30mm of 0.18 (95%-CI -0.24-0.55) and for lesions >30mm 0.88 (95%-CI 0.78-0.93).

In 15 patients either the CEUS versus MRI or biopsy diagnosis did not match, or distinction between FNH and HCA could not be made based on that imaging modality. The stiffness values for these FNH ranged from 1.65 to 8.75 kPa and for HCA from 1.29 to 9.36 kPa.

Fourteen HCA were classified as inflammatory, four as steatotic and five were unclassified. The median stiffness values for the inflammatory HCA were 4.99 and 4.46 kPa for O1 and O2 respectively (range 1.44 to 10.08), for the steatotic HCA 4.82 and 7.07 kPa (range 1.29 to 20.03) and for the unclassified HCA 4.98 and 6.10 kPa (range 2.11 to 7.77 kPa).

Healthy subjects

Twenty healthy subjects were included. O1 performed pSWE in the NLT of 4 males and 6 females with a median age of 27.5 years (IQR 25.8-28.3). O2 performed pSWE in 5 males and 5 females with a median age of 27.0 years (IQR 23.5-29.0). Intra-observer reliability analysis showed an ICC of 0.23 (95%-CI -0.13-0.73) and 0.62 (95%-CI 0.02-0.89) for O1 and O2, respectively.

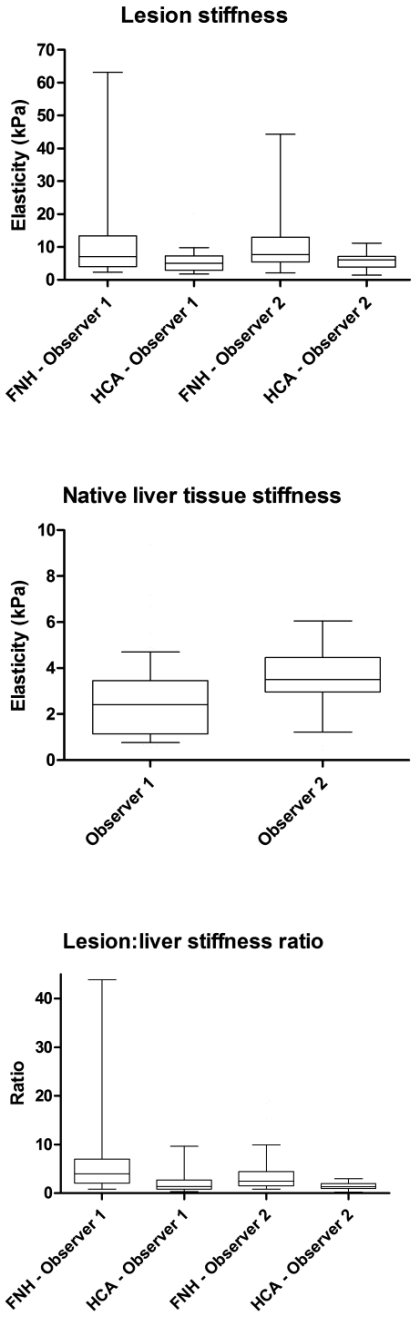

Figure 2. Box and whisker diagrams for lesion stiffness, native liver tissue stiffness and lesion:liver stiffness ratio.

Box: median and IQR, whiskers: 5-95 percentile. FNH = focal nodular hyperplasia. HCA = hepatocellular adenoma.

DISCUSSION

There is a need to improve the non-invasive diagnostic characterization of FNH and HCA in order to avoid overtreatment of FNH and undertreatment of HCA. Due to its benign course, FNH typically does not need treatment or follow-up. In contrast, a proportion of HCA do require regular surveillance or treatment in the form of surgical resection because of a risk of complications. Several preliminary studies have shown a possible benefit from pSWE in differentiating between these two benign liver tumors. However, the present study could not confirm the hypothesis that pSWE (ElastPQÒ, Phillips Healthcare) in its present form can be used to distinguish FNH from HCA. Although median pSWE values were significantly higher in FNH compared to HCA, we were unable to determine an acceptable cut-off point for this characterization due to the great variability in pSWE values.

In a way these results were unexpected, as previous studies suggested pSWE to be a useful supplementary method to distinguish FNH from HCA during regular ultrasound. One of these studies was by Gallotti et al. (14), who also found a significant difference in pSWE values between FNH and HCA. However they did not try to determine a cut-off point for this differentiation.
We can divide elastography methods in pSWE (as used in this study) and multidimensional shear wave elastography. There are some studies available evaluating the diagnostic value of multidimensional SWE, that did determine a cut-off point for the differentiation. One of these studies was performed by Ronot et al. (23), who found that FNH could be differentiated from other lesions (among which HCA, hemangiomas, focal fatty sparing, cholangiocarcinoma and hepatocellular carcinoma) with an AUROC of 0.86. Another study by Brunel et al. (24) focused only on the characterization of FNH and HCA and found a maximal accuracy of 95% for identification with a cut-off of 18.8 kPa (AUROC 0.93). The differences between their results and ours might well be caused by software diversity, as both of these studies used two dimensional SWE (AixplorerÒ, SuperSonic Imaging), whereas in our study pSWE (ElastPQÒ, Phillips Healthcare) was used.

Another possible explanation for the different results might lie in differences between cohorts. We included patients who were diagnosed with FNH or HCA in the past, whereas the previously mentioned studies (23, 24) performed elastography at the moment of diagnosis. This might explain an important difference in patients with HCA. The majority of patients used oral contraceptives at the time of diagnosis and these lesions often spontaneously regress after cessation of these pills (25). We confirmed regression of HCA from 35mm to 20mm in a median follow-up period of 47 months. The influence of this regression process on the lesion stiffness remains an unanswered question. Another remarkable difference between the present study and the study by Brunel et al. (24) is

the stiffness values for the different HCA-subtypes. Brunel et al. found higher values in inflammatory HCA, whereas in our cohort we found high values in both steatotic and inflammatory HCA. We did not perform statistical analysis on these results, as there were only four patients in the steatotic HCA group and five in the unclassified group and therefore statistical analysis would not be reliable.

In this study we also checked whether pSWE could provide a contributory argument in patients in whom there was a discrepancy between CEUS and MRI or biopsy diagnosis. Unfortunately, this was not the case, the pSWE values ranged from low to high for both tumors.

The use of pSWE for focal liver lesions in general might be subject to a few limitations. The first limitation is that it cannot be used in lesions that are situated >80mm from the skin, since current pSWE technology cannot detect them. In this study we had to exclude 8 patients because the lesion was situated to far from the surface. During the execution of this study, we noticed that several factors might result in a higher or lower stiffness value. For example, higher values may be seen in lesions with a fibrotic membrane where the pSWE region of interests exceeds the lesion diameter, in lesions located just underneath the liver capsule or in the proximity of one of the ligaments and in lesions with scar tissue. Lower values may be found in lesions with intralesional arteries or veins or located in the proximity of any liver artery or vein.

In this study we are the first to assess the inter-observer reliability in patients with focal liver lesions using the ICC. We found good inter-observer reliability for lesion stiffness but for the surrounding NLT it was poor. Subgroup analysis showed a better inter-observer reliability in lesions >30mm compared to those <30mm. In 2012, Gallotti et al. (14) also did an inter-operator evaluation while performing ARFI ultrasound imaging in patients with focal liver lesions (hepatocellular carcinomas, hemangiomas, HCA, metastasis and FNH). They compared the mean values between the two operators and did not find a statistically significant difference. However, we believe that the ICC is a more valid method to assess inter-observer reliability and that it would be wise to validate other elastography software with this method as well.

This study also assessed the intra-observer reliability of pSWE in determining the stiffness of NLT in healthy subjects. Remarkably, we found poor to moderate intra-observer reliability while other studies had good to excellent results (26, 27). This is also stated in the most recent update of the EFSUMB guidelines on the use of elastography (28). The differences might be explained by our small sample size of healthy subjects, but could also indicate that the performance of pSWE in determining the stiffness of NLT in healthy subjects is not as good as the first results showed.

As any other study, this one is subject to limitations. The first is the fact that not all lesions were biopsied and therefore only 17% of FNH and 22% of HCA were pathologically proven. Although pathological examination remains to be the reference standard for diagnosing benign liver tumors, clinical practice guidelines advise to rely mainly on imaging findings as CE-MRI has a high sensitivity and specificity. Biopsy should only be performed in case of diagnostic uncertainty after state-of-the-art imaging (29). Secondly, we had a high rate of failed pSWE examinations (25 out of 113 patients) due to either the depth of the lesion or because the lesion could not be found at ultrasound examination. Thirteen HCA could not been found, as has been demonstrated that in time after cessation of oral contraceptives these lesions might regress (17). We must highlight that this was the reason that we purposely excluded all female patients with HCA over 50 years of age. More remarkable was the fact that four FNH also could not be found at ultrasound examination, as these lesions are mostly static and are usually not found to regress over time. The last limitation might lie in the skewed distribution between males and females in this study, as only one male was included. It is known that both lesions have a female predominance, although a clear relationship with female sex steroids has only been demonstrated for HCA. Additionally, guidelines advise to perform a resection in all men with proven HCA as they appear to have a higher risk of malignant transformation (29).

Concluding, this study suggests that pSWE cannot reliably differentiate between FNH and HCA in its present form. Additionally, both inter- as intra-observer reliability for pSWE measurements in the NLT were insufficient. Interpretation of the results gained with this method and management decisions based on these results should be done with caution. Improvement of the software is warranted and the influence of these new updates on the diagnostic accuracy should be prospectively assessed.

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CHAPTER 11

Growth of FNH is not a reason for surgical intervention but patients should be referred to a tertiary referral centre.

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ABSTRACT

Background: 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

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.

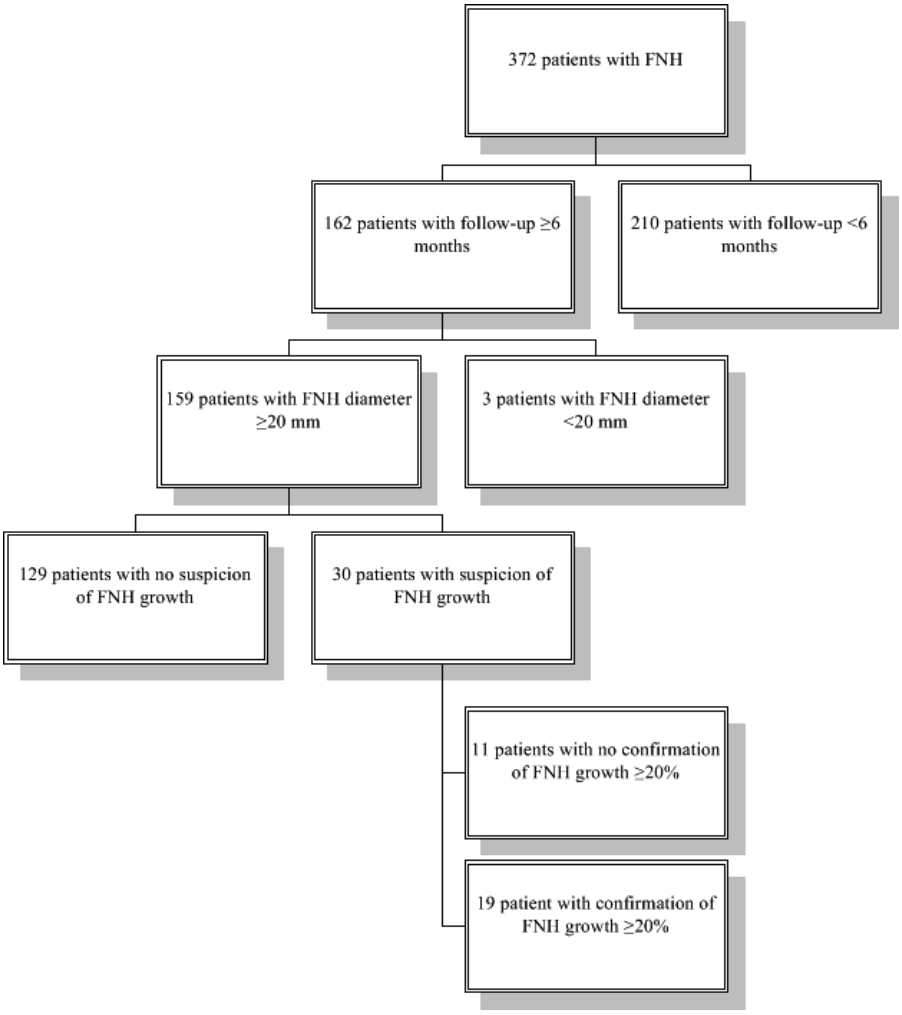

Figure 1. Flowchart inclusion

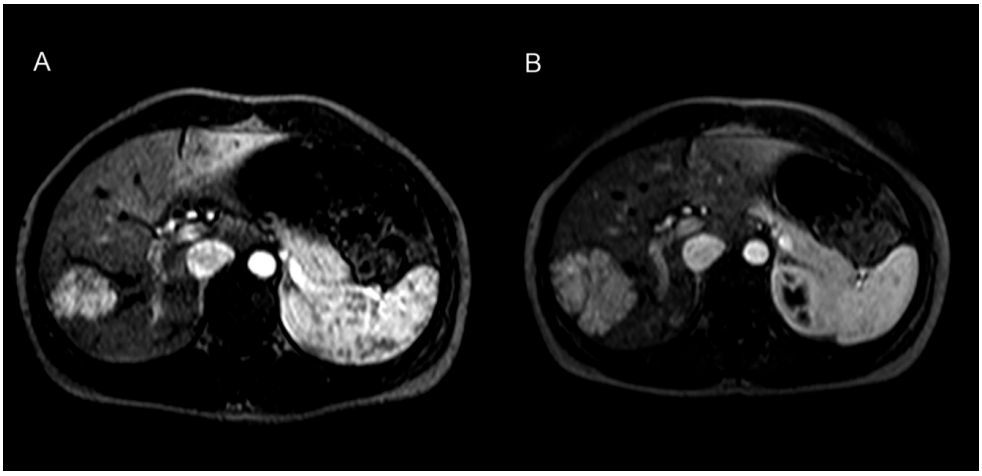

Figure 2. Example of a growing FNH.

A. FNH at T1 (diagnostic scan): diameter 29 mm. B. FNH at T2 (follow-up scan): diameter 55 mm.

Table 1: Patient and lesion characteristics

	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%)	

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.

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 FNH

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.

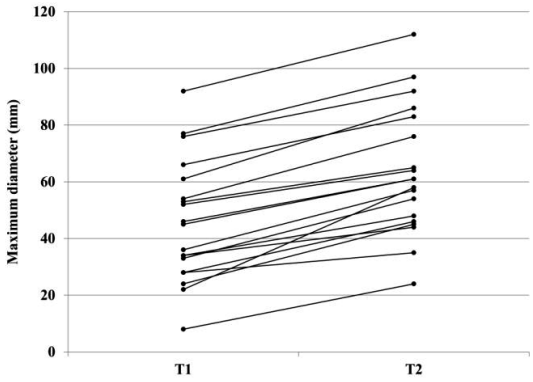

Figure 3. Size of growing FNH.

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

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	108	61*50	32,6%	6,50 (25%)
16	53	1	53	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

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 ($r = -.214$, $p = .340$).

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 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.

It must be noted that the accuracy for diagnosing FNH with MRI has improved significantly in the study period. As of 2008 gadolinium-based contrast agents were used, making distinction from hepatocellular adenoma more accurate (22). 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 (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 12

Management of hepatic angiomyolipoma: a systematic review

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ABSTRACT

Background & Aims: Hepatic Angiomyolipoma (HAML) is a rare mesenchymal liver tumor assumed to be predominantly benign, although incidental cases with malignant behavior such as invasive growth, recurrence after resection and metastases have been reported. The aim of this systematic review was to assess the biological behavior, estimate the risk of HAML related mortality and recommend on a justifiable management strategy.

Methods: We performed a systematic literature search in Embase, Medline, Web-of-Science, Scopus, Pubmed Publisher, Cochrane and Google Scholar. We included all articles published from inception until March 2016 which reported on follow-up of various treatment strategies.

Results: We included 18 articles reporting on 292 patients. Male:female ratio was estimated at 1:3 with gender not reported in 31 cases. Out of 292 patients 247 were treated with surgery, including one liver transplant, 7 with chemotherapy or Sirolimus, 3 with embolization and 35 conservatively. Recurrence after resection was described in 6/247 (2.4%) with pathologically proven HAML resulting in metastases and death in 2/247 (mortality rate 0.8%). Progression was described in 6/35 patients treated conservatively (21.4%). Two out of 12 patients with malignant behavior of HAML had an epithelioid-type HAML, of the remaining 10 histological subtype was undefined.

Conclusions: With a risk estimate of 0.8% in surgically treated patients HAML related mortality is very low. Biopsy is indicated when imaging is inconclusive. In case of certain HAML diagnosis on imaging conservative management with annual imaging is justified. Resection should be considered in case of symptoms, inconclusive biopsy or growth in follow-up.

INTRODUCTION

Hepatic angiomyolipoma (HAML) is a rare mesenchymal liver tumor that belongs to a group of perivascular epithelioid cell (PEC) tumors called PEComa. It is typically composed of blood vessels, smooth muscle and adipose cells (figure 1). The proportions of these aforementioned tissues may vary greatly (1). Previous studies demonstrated that most patients are asymptomatic and HAML may be discovered incidentally during regular health check-ups or follow-up examinations for other diseases (2-4). If patients do present with symptoms of HAML they usually complain of abdominal discomfort (5).The pathogenesis of HAML has not yet been clarified (1, 5). There is an association with tuberous sclerosis complex (TSC) in more than 50% of the angiomyolipomas (AML) in the kidney, but this association has been estimated to be present in only 5-15% of the patients presenting with solitary liver tumors (1). HAMLs usually occur in non-cirrhotic livers and are not accompanied by serological abnormalities (2, 4). Although HAML occurs more frequently in women, sex-hormones do not seem to play a role in the pathogenesis or growth (2, 6).

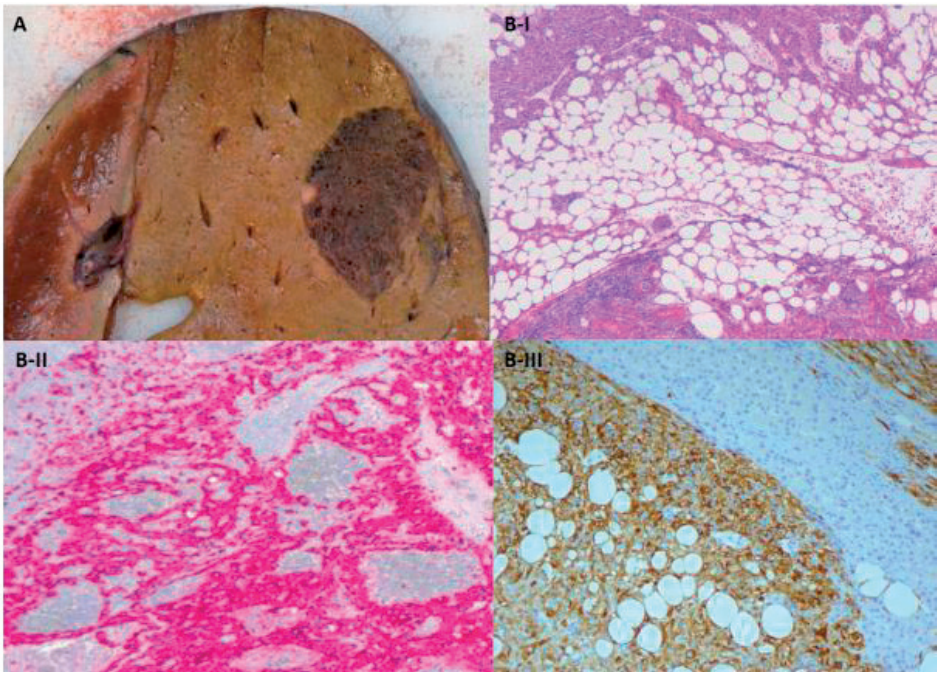

Figure 1. Macroscopic and microscopic image of HAML.

A. Macroscopic image of HAML. Cut section of liver resection: relatively well circumscribed dark brown lesion with irregularly shaped spaces and a whitish nodule at the left. Unremarkable background liver tissue. B-I. Microscopic image of HAML with all components of an HAML present (H&E stain x 50). B-II. Immunohistochemical staining with HMB-45 (x 25). B-III. Immunhistochemical staining with Melan-A (x 100).

The various proportions and distribution of the different tissue components of HAML on imaging impedes to make a diagnosis based on imaging (4). The most important distinctive characteristics on imaging are mature adipose tissue and central thick-wall feeding blood vessels. HAML with mainly fatty tissue are less difficult to diagnose but low- and non-fatty tissue HAML have fewer typical manifestations and may easily be misdiagnosed as other benign or malignant tumors of the liver (2). Therefore, histopathological examination combined with immunohistochemical proven Human Melanoma Black 45 (HMB45) positive (smooth-muscle) cells, are important steps towards the definitive diagnosis and are related to prognosis (4, 5). HAML can be histologically subdivided into the classic type of AML with lipomatous, myomatous or angiomatous predominance (mixed type) on the one hand and an epithelioid variant with the presence of 10-100% epithelioid cells on the other hand.

The majority of HAML are believed to be benign, although a number of cases have been reported with malignant behavior including growth, recurrence after surgical resection, metastasis and invasive growth patterns into the liver parenchyma and alongside the vessels (1). Malignant transformation is thought to occur mostly in the epithelioid type(7). A number of therapeutic strategies have been described but the best treatment option for HAML remains controversial (4). Many researchers advocate surgical resection over a non-surgical approach consisting of regular follow-up. A non-surgical approach however is also being recommended, especially for patients who are asymptomatic and have small tumors or in patients who are deemed unfit surgical candidates. This approach usually involves close follow-up with repeated imaging, preferably with contrast enhanced magnetic resonance imaging (MRI) (5).
There is no evidence-based consensus regarding the best treatment strategy for HAML and a definition of the tumors that should be resected to prevent a dismal outcome. Therefore, the aim of this systematic review was to assess the biological behavior, estimate the risk of recurrence, progression or HAML related mortality and to recommend on a justified management strategy.

METHODS

This review was conducted and reported according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) (8).

Literature search and study selection

A systematic literature search was performed with the help of a clinical librarian (WMB) using descriptors that included synonyms for 'liver' and 'angiomyolipoma' in various combinations (table 1). The search was conducted in Embase.com, Medline (Ovid), Web-

of-Science, Scopus, Pubmed Publisher (the subset as applied by publisher, containing references not yet indexed by Medline), Cochrane Central and Google Scholar and included all articles published from inception until March 2016. Additionally, we examined reference lists from included articles for suitable studies.

Table 1: search terms per database

Database	Search terms
Embase.com	(angiomyolipoma/exp OR (angiomyolipom* OR angiomygolipom* OR (angio NEXT/1 myolipom*) OR (angiomyo NEXT/1 lipom*)):ab,ti) AND (liver/exp OR 'liver tumor'/exp OR 'liver resection'/exp OR 'liver disease'/exp OR 'liver biopsy'/exp OR 'liver histology'/exp OR (liver* OR hepat*):ab,ti)
Medline (OvidSP)	(angiomyolipoma/ OR (angiomyolipom* OR angiomygolipom* OR (angio ADJ myolipom*) OR (angiomyo ADJ lipom*)).ab,ti.) AND (liver/ OR Hepatectomy/ OR exp liver disease/ OR (liver* OR hepat*).ab,ti.)
Cochrane	((angiomyolipom* OR angiomygolipom* OR (angio NEXT/1 myolipom*) OR (angiomyo NEXT/1 lipom*)):ab,ti) AND ((liver* OR hepat*):ab,ti)
Web-of-science	TS=(((angiomyolipom* OR angiomygolipom* OR (angio NEAR/1 myolipom*) OR (angiomyo NEAR/1 lipom*))) AND ((liver* OR hepat*)))
Scopus	TITLE-ABS-KEY(((angiomyolipom* OR angiomygolipom* OR (angio W/1 myolipom*) OR (angiomyo W/1 lipom*))) AND ((liver* OR hepat*)))
PubMed publisher	(angiomyolipoma[mh] OR (angiomyolipom*[tiab] OR angiomygolipom*[tiab] OR (angio myolipom*[tiab]) OR (angiomyo lipom*[tiab]))) AND (liver[mh] OR Hepatectomy[mh] OR liver disease[mh] OR (liver*[tiab] OR hepat*[tiab])) AND publisher[sb]
Google scholar	Angiomyolipoma liver|hepatic

Studies were evaluated for inclusion by two independent reviewers (AJK and DV). We screened title and abstract of all deduplicated references and if the eligibility criteria were met, full manuscripts were procured and reviewed. All disagreement was resolved by discussion. Inclusion criteria were randomized controlled trials, case-control studies, cohort studies and case series ($n \geq 3$) including patients diagnosed with HAML and reporting on outcome of various treatment strategies. We only included manuscripts written in English. Studies describing tumors other than HAML were excluded in addition to reviews, animal studies, cadaver studies, case reports ($n < 3$), surveys, editorials, commentaries, conference abstracts and letters. We also excluded studies lacking details on enrolment in order to prevent reporting on overlapping data. If studies reported on outcomes from the same study population we included the studies providing the most complete data.

Outcome measures and data extraction

The primary outcome was reported risk estimate or number of events for total mortality. Secondary outcomes included risk estimates or number of events for recurrence or progression of HAML.

Data extraction was performed by AK and DV independently. We collected data including publication details (authors, year, journal), type of study, demographic data (number of subjects, patient characteristics, tumor characteristics), follow-up period, type of treatment applied and the reported outcomes (e.g. death, recurrence, progression, stable disease) and cumulated them to present the clinical features of all patients combined. We also cumulated the reported outcomes per treatment group of all studies and made a distinction between benign behavior (no evidence of disease, stable disease, regression) and malignant behavior (e.g. recurrence, progression and death). The level of evidence was determined using the Oxford Centre for Evidence-based Medicine Levels of Evidence(9).

Statistical analysis

Variables are presented as mean (SD) or median (range), according to the way they were reported in the original article. No meta-analysis or statistical analysis was performed. Cumulative data were tabulated and presented as number with percentages.

RESULTS

The study selection process is depicted in figure 2. The initial search yielded a total of 2926 studies, of which 1037 remained after excluding duplicates. Nine hundred and six articles were excluded after screening of title and abstract, resulting in 131 studies for full text review. In the end, 18 articles were included in this systematic review. Examination of reference lists from included articles did not result in any additional suitable articles.

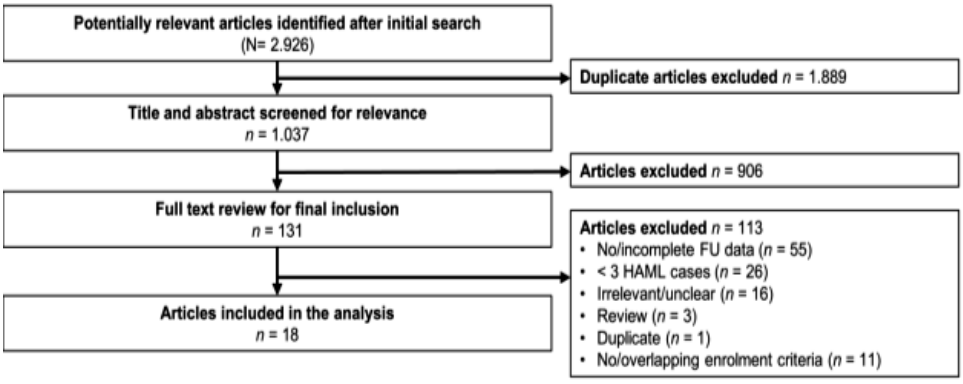

Figure 2. Study selection process

Study and patient characteristics

An overview of the included studies is given in Table 2. All included studies were retrospective case series or cohort studies (level of evidence 4 (9)) describing outcomes after surgical resection, observation, Sirolimus treatment, chemotherapy, embolization and liver transplantation. A total of 292 patients with one or more HAML were included in this systematic review, most of which were female (73.9%). The median age ranged from 24 to 53 years and the median tumor size from 2 to 12.7 cm. Table 3 outlines the cumulated patient characteristics.

Diagnostics

The outcome of imaging as a diagnostic tool was described in 195 patients: 64 tumors (28.2%) were diagnosed as HAML, 89 (39.2%) as a malignancy, 37 (16.3%) as another benign tumor and in 5 (2.2%) patients imaging was inconclusive. The use of biopsy as a diagnostic tool was described in 32 patients; the diagnosis of HAML was confirmed in 25 (78.1%), malignancy in 3 (9.4%), other benign tumor in 1 (3.1%) and inconclusive in 3 (9.4%). Of all studies, only one case was reported to have elevated tumor markers (Alpha-fetoprotein, AFP).

Treatment and follow-up

In all studies combined 246 patients (84.2%) underwent surgical resection for HAML. Among these patients the diagnosis before resection was malignancy of unknown origin in 90, HAML in 71, other benign liver tumor in 37 and inconclusive diagnosis in 8. For the remaining 40 patients there is no information on diagnosis before resection. Seven patients were treated with medication: five with Sirolimus and two with chemotherapy. In three patients embolization of HAML was performed and in one patient a liver transplantation because the patient was thought to have an 18cm hemangiosarcoma involving both liver lobes. A conservative observational approach was applied in 35 patients, 20 of which had a biopsy proven HAML. In 15 cases detailed information on the work-up of diagnosis was missing.

Table 4 shows the reported outcomes per treatment group. Information on follow-up was available in 284 cases (97.3%). Table 5 shows characteristics of patients with HAML showing malignant behavior. The reported overall mortality was 1.4% (4/292 patients) and the HAML related mortality was 0.8% (2/292 patients). Malignant behavior defined as progression, recurrence or mortality was described in 12 patients (4.1%). Recurrence after resection was described in 6/246 patients (2.4%) and progression in 6/29 patients (20.1%) with biopsy proven HAML who were treated conservatively with a cumulative estimated size increase of 0.77 cm per year.

Table 2: Overview of included studies

Reference	Year	Type of study (level of evidence)	No. of patients	Mean/median Age (years)	Female : male	Mean/median Tumor size (cm)	Treatment
Agaimy et al.(16)	2012	Case series (4)	6	49 (21-63)	1 : 5	6 (1.1-18)	Resection Observation
Black et al.(17)	2012	Retrospective cohort (4)	25	24 (8-39)	-	2,3 (0.6-4.3)	Sirolimus Observation
Butte et al.(18)	2011	Retrospective cohort (4)	22	53 (35-75)	18 : 4	7 (1-17)	Resection Chemotherapy Embolization Observation
Ding et al.(10)	2011	Retrospective cohort (4)	79	43 (17-69)	58 : 21	6.1 (1-25)	Resection
Du et al.(19)	2012	Case series (4)	17	40 (27-60)	13 : 4	(1-17)	Resection
Flemming et al.(20)	2000	Case series (4)	12	53 (44-68)	11 : 1	12.7 (4-20)	Resection Observation Transplantation
Huang et al.(21)	2015	Case series (4)	23	49 (27-74)	18 : 5	3.6 (0.9-14.5)	Resection
Li et al.(22)	2008	Retrospective cohort (4)	25	46 (17-57)	19 : 6	5.8 (1.2-17)	Resection
Liu et al.(23)	2015	Case series (4)	5	26-57	4 : 1	-	Resection
Lo et al.(24)	2013	Case series (4)	5	41 (36-70)	5 : 0	11 (1.2-25)	Resection
Ortiz et al.(25)	2016	Case series (4)	4	51 (41-60)	4 : 0	(1-7.5)	Resection Observation
Tan et al.(26)	2012	Case series (4)	6	-	-	-	Resection
Theodosopoulos et al.(27)	2013	Case series (4)	5	49 (33-75)	3 : 2	(2-4)	Resection
Wang et al.(28)	2014	Case series (4)	9	50 (39-62)	4 : 5	(1.4-15.3)	Resection
Yang et al.(11)	2007	Case series (4)	10	44 (34-64)	9 : 1	10.5 (2.5-20)	Resection Observation
Yang et al.(4)	2013	Case series (4)	8	47 (27-56)	6 : 2	3.4 (1.5-8)	Resection Observation
Zeng et al.(29)	2010	Case series (4)	17	41 (23-63)	11 : 6	9.2 (2.5-22)	Resection
Zhong et al.(30)	2000	Case series (4)	14	40 (30-63)	9 : 5	9.4 (2.5-26)	Resection

Table 3: Patient characteristics

	n (%)
Gender	
Male	68 (26.1%)
Female	193 (73.9%)
No information	31
Tuberous sclerosis complex	
No	231 (89.5%)
Yes	27 (10.5%)
No information	34
Presentation	
Incidental	143 (63.8%)
Symptomatic	81 (36.2%)
No information	68
Hepatitis B markers	
Positive	29 (14.2%)
Negative	175 (85.8%)
No information	88
AFP levels	
Normal	189 (99.5%)
Elevated	1 (0.5%)
No information	102
Histology	
Classic mixed	55 (36.4%)
Classic myomatous	64 (42.4%)
Classic lipomatous	16 (10.6%)
Classic angiomatous	4 (2.6%)
Epithelioid	12 (7.9%)
No information	141
Diagnostic outcome	
HAML radiology	64 (28.2%)
HAML pathology	25 (11.0%)
Malignancy radiology	89 (39.2%)
Malignancy pathology	3 (1.3%)
Other benign tumor radiology	37 (16.3%)
Other benign tumor pathology	1 (0.4%)
Unsure diagnosis radiology	5 (2.2%)
Unsure diagnosis pathology	3 (1.3%)
No information	65
HMB45	
Positive	227 (91.5%)
Negative	21 (8.5%)
No information	44

Table 4: Outcome per treatment group

	Surgical intervention			Medication		Conservative	All
	Resection (n = 246)	*Embolization (n = 3)*	*Transplantation (n = 1)*	*Sirolimus (n = 5)*	*Chemotherapy (n = 2)*	*Observation (n = 35)*	*n = 292*
Outcome							
No evidence of disease	234 (95.1%)	-	1 (100%)	-	-	-	235
Stable disease	4* (1.3%)	3 (100%)	-	-	1 (50%)	22 (62.9%)	30
Regression	-	-	-	4 (80%)	-	-	4
Progression	-	-	-	-	1 (50%)	6 (17.1%)	7
Recurrence	6 (1.8%)	-	-	-	-	-	6
Death	3 (0.9%)	-	-	-	-	1 (2.9%)	4
Postoperative complications	7** (2.2%)	-	-	-	-	-	7
No info on follow-up	1 (0.3%)	-	-	1 (20%)	-	6 (17.1%)	8

* 4 patients who had incomplete resections, Butte 2011.

** Postoperative complications specified. Butte 2011: Intraoperative bleeding. Theodosopoulos 2013: Respiratory infection, biloma, urinary tract infection. Zheng 2010: Bile leakage, diffuse intravascular coagulation, acute respiratory distress syndrome.

Table 5: Malignant behavior

RESECTION					
	Cases	**Patient characteristics**	**Tumor size**	**Histology**	**Outcome**
Recurrence (n=4)	Butte 2011	Female, 54yrs	Unknown	Unknown	Recurrence in liver after 53 months.
	Butte 2011	Male, 41yrs	9cm	Unknown	Recurrence and metastases in lungs and retroperitoneum after 41 months, alive with disease.
	Flemming 2000	Female, 51yrs, tumor in left lobe	Primary:10cm and 0.5cm	Epithelioid	Recurrence in right lobe after 36 months of many confluent nodules (together 20cm). Patient underwent another resection and is alive without disease after 18 months.
	Zheng 2010	Gender and age unknown, tumor in left lobe	Unknown	Unknown	Recurrence in right lobe (6cm) after 108 months. Patient underwent another resection and is alive without disease after 36 months.
Death (n=2)	Ding 2011	Female, 31yrs, tumor in right lobe	8cm	Unknown	Recurrence 6 years after resection in residual right liver, death 12 months later.
	Yang 2007	Female, 37yrs, tumor in left lobe	13cm	Unknown	Recurrence in right lobe after 6 months (multiple in right lobe and caudate lobe). After 11 months multiple metastatic nodules in chest. Death 14 months after diagnosis due to hepatic and renal failure.
Death due to unrelated reasons (n=1)	Butte 2011	Female, 53yrs	3cm	Unknown	Death due to metastatic adrenal cancer at a follow-up of 135 months.

OBSERVATION					
Progression (n=6)	Black 2012	Male, 19yrs, TSC	0.1cm	Unknown	Size increase from 0,1cm to 4,1 cm in 25 months (=1,92cm/yr).
	Black 2012	Female, 21yrs, TSC	0.6cm	Unknown	Size increase from 0,6cm to 2,1cm in 42 months (=0,43cm/yr).
	Black 2012	Male 35yrs, TSC	0.1cm	Unknown	Size increase from 0,1cm to 2,1 cm in 30 months (0,8cm/yr).
	Black 2012	Female 8yrs, TSC	2.2cm	Unknown	Size increase from 2,2cm to3,8cm in 40 months (0,48cm/yr).
	Black 2012	Female 14yrs, TSC	2.7cm	Unknown	Size increase from 2,7cm to 3,7cm in 16 months (=0,75cm/yr).
	Flemming 2000	Female 46yrs, seg VI and seg III	3.5cm and 1.0cm	Epithelioid	Progression after 18months: in segment VI growth to 3.6cm (=0.1cm), seg III growth to 2.8cm (=1.8cm) and new tumor segment II 0.8cm = 0.1/yr, 1.2/yr and 0.5/yr.
Death due to unrelated reasons (n=1)	Agaimy 2012	Male, 60yrs	1.1cm	Classic lipomatous	Death due to multi organ failure at a follow-up of 9 months. On autopsy no evidence of malignant progression or further AML's in other organs.

DISCUSSION

To the best of our knowledge, this is the first systematic review that provides an overview of the literature on the management of patients with HAML, including an outline of clinical features, treatment strategies and follow-up outcomes. There is no evidence based consensus on the management of this rare liver tumor, and available literature consists only of retrospective cohort studies or case series. This systematic review therefore provides the highest level of evidence currently available.

We reported on 18 articles describing 292 patients with HAML. Treatment strategies included in this review are resection, observation, Sirolimus, embolization, chemotherapy and liver transplantation. Our findings confirm earlier reported characteristics of patients with HAML; patients are mostly female, aged between 24 to 53 years, usually asymptomatic and without serological abnormalities. Only 10.5% is associated with TSC and HMB45 staining is positive in 91.5% of HAML (5, 6). On imaging, 39.2% of patients were thought

to have a malignancy while only 28.2% were correctly diagnosed with HAML. The use of biopsy as a diagnostic tool was more accurate with HAML as the outcome in 78.1% of the described cases.

Recurrence after resection was described in 6 out of 246 patients (2.4%). One patient had an epitheliod-type HAML, from the others the histological subtype of HAML was not mentioned in the original article. As a result of this recurrence and metastasis two patients died of HAML 12 and 14 months after diagnosis respectively, resulting in a total HAML related mortality rate of 0.8%. The first patient described by Ding et al. was a 31-year-old female who was diagnosed with an 8cm large HAML, recurrence occurred 6 years after initial resection (10). The second patient described by Yang et al. was a 37-year-old female with a 13cm large HAML recurring as multiple tumors in the liver six months after resection and with metastasis in the chest after 11 months (11). In both patients pathologic and histologic examination of the resection specimen showed HAML with positive HMB45 staining, but information on growth patterns, histological characterization of the recurring tumor and the considered treatment strategies after recurrence are missing in the article.

In 6 out of 29 patients (21.4%) treated conservatively, progression was described with a cumulative estimated size increase of 0.77 cm per year. Five of these patients had been diagnosed with TSC. All tumors were initially small (maximum 3.5cm). The one patient that did not have TSC had an epithelioid-type HAML, unfortunately again details on histological characteristics of the other five patients are missing.

Overall, the cohorts of the other four treatment strategies were too small to make a reliable judgement. Treatment with Sirolimus might be effective, but as it may have many side-effects this option is less favourable. Embolization might also be an effective method but only for an undisputed HAML diagnosis. Chemotherapy seems less effective and liver transplantation was performed because the patient was thought to have a hemangiosarcoma.

We report a 4.1% cumulative incidence of malignant behavior of HAML. However, in all probability publication bias leads to an overestimation of the actual risk for malignant behavior. In 2012 Kamimura et al. reported a total of 13 HAML cases in the world literature that showed progressive growth, recurrence or metastasis (1). Three out of these 13 cases are from studies included in this review, the remaining 10 are from case reports and therefore excluded.

The exact prevalence of HAML is unknown, however it is less common than renal AML with an estimated prevalence that ranges from 0.3 to 2.1 percent (12). Most articles state that there have been about 300 cases of HAML reported in the literature until now. We

are certain that this amount is an underestimation, as there are 292 cases reported in this review alone. Our search provided 319 case reports reporting on <3 cases, which makes an estimated total of more than 600 HAML cases described.

Our results show that many of the reported surgically treated patients were initially thought to have a malignancy (90 out of 246), which provides a logical explanation for the skewed distribution in the reported treatment strategies. In the previously mentioned article by Kamimura et al. a recommendation is made regarding indications for resection (1). They recommend to perform a resection of HAML in case of symptoms, aggressive or invasive growth, atypical epithelioid patterns, high proliferation activity and if there is doubt about the diagnosis on imaging or pathology. This is consistent with the recommendations Yang et al. made in 2007 to surgically remove all HAML in symptomatic patients (11). Additionally, this study group states that a conservative approach with close follow-up would be justified in patients with biopsy-proven HAML <5cm with good compliance and who are negative for hepatitis-virus markers in serum. However, as HAML is a very rare tumor, all of these proposals are based on small numbers of patients.

As five out of six patients with progression were diagnosed with TSC and the other one had a HAML of the epithelioid type, these might be risk factors for malignant behavior. Additionally, one patient with recurrence after resection from whom the histopathological subtype of HAML was described also had an epitheliod-type HAML. Unfortunately, we were unable to identify any other risk factors for malignant behavior, due to the many missing data. In 2011 Nese et al. made a risk stratification for the malignant potential of renal epithelioid AMLs(7). They found that risk factors for malignant behavior defined as recurrence, metastasis or death were TSC, necrosis of the tumor, size >7cm, renal vein involvement and/or extrarenal extension and carcinoma-like growth patterns. As renal AMLs and HAMLs are in fact the same entity, it would be logical to assume that the risk factors for disease progression are the same. As mentioned, we found a higher incidence of malignant behavior in the observation group compared to the resection group (2.4% versus 21.4%). However, the high incidence of malignant behavior in the observation group might well be the result of the small sample size and/or publication bias.

HAML is a very heterogeneous tumor which makes distinction from various liver neoplasms on imaging a challenge, despite the advanced techniques available today. Most are misdiagnosed as focal liver lesions or as a malignancy, as was reflected in our results (malignancy in 39.2%, other benign tumor in 16.3% and HAML in 28.2%). The imaging modalities CT scan and MRI have similar diagnostic accuracy rates, but both have a higher diagnostic accuracy when compared to ultrasound (13). A "typical presentation" on CT or MRI is the presence of fatty areas and solid tissue components. However, in practice fat is found to be unreliable since some HCCs also contain fat, mimicking HAML (2). Useful

other imaging features on CT or MRI scan to discriminate between HAML and HCC are the presence of early draining vein, peripheral decreasing enhancement rim and absence of tumor capsules in the hypervascular hepatic tumor (2, 14). The imaging diagnosis of HAML still needs improvement and this should be a focus in future study designs. Furthermore, future studies should also focus on the diagnostic accuracy of biopsy for HAML. Biopsy with HMB45 staining might be superior compared to imaging with a diagnostic accuracy of 78.1%. However, the use of biopsy as a diagnostic tool was only described in 32 cases in this review. Additionally to HMB45, which is considered being the histopathological biomarker for HAML, the detection of other antigens can benefit this diagnostic accuracy as well. Previous studies have shown that next to HMB45 HAMLs commonly express Melan-A and a-SMA (15).

This systematic review was subject to some limitations. It included only retrospective cohort studies or case series (level of evidence 4 (9)), resulting in a fairly high proportion of missing data. Some studies lacked information on patient characteristics while others only reported on patients and follow-up without tumor characteristics. To the best of our knowledge there are no randomised controlled trials or prospective cohort studies available.

Final recommendations

We propose the following algorithm for the management of HAML. In case of a certain HAML diagnosis on imaging, observation and conservative management is justified. As the cumulative estimated size increase in this review was only 0.77cm/year, the first surveillance imaging can take place one year after diagnosis followed by biennial surveillance. When the diagnosis on imaging is inconclusive biopsy can be performed. If biopsy provides an uncertain diagnosis or shows epithelioid features or high proliferation activity, resection is indicated. Other indications for resection are development of symptoms or aggressive/invasive growth. This management algorithm is depicted in figure 3. Additionally, patients with TSC might need longer or more frequent surveillance, as TSC seems to be a risk factor for progression. Prospective multicentre studies are warranted and should focus on assessing the diagnostic accuracy for both imaging and biopsy. Additionally, further evaluation of risk factors for progression, recurrence and metastasis are needed in order to identify those patients requiring aggressive treatment.

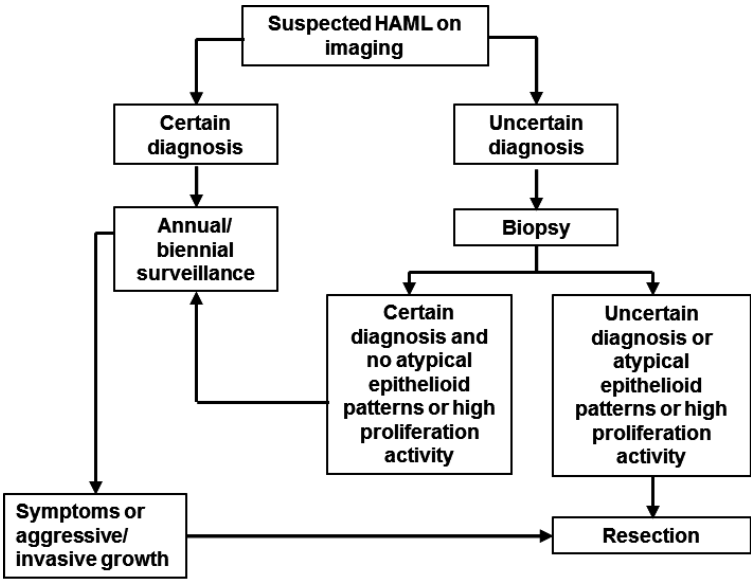

Figure 3. Proposed management algorithm for HAML

Conclusion

In this systematic review we reported on 292 patients with HAML with an overall HAML related mortality risk of 0.8%. The frequency of recurrence after resection was 2.4% and the frequency of progression in the conservatively treated group 21.4%, however these percentages are most likely an overestimation of the true incidence due to publication bias. We recommend to apply conservative management consisting of annual or biennial imaging in case of certain HAML diagnosis on imaging. Biopsy is indicated when imaging is inconclusive and resection has to be considered in case of symptoms, inconclusive biopsy or growth.

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CHAPTER 13

Hepatic angiomyolipoma: an international multicenter analysis on diagnosis, management and outcome

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ABSTRACT

Background: Hepatic angiomyolipoma (HAML) may easily be misdiagnosed as a malignancy. The study aim was to assess diagnostic dilemmas, clinical management and outcome of this rare tumor.

Methods: This retrospective international multicenter study included all patients with pathologically proven HAML diagnosed between 1997-2017. Data on patient characteristics, diagnostic work-up, management and follow-up were analyzed.

Results: Thirty-eight patients were included, 32 female. Median age was 56yrs (i.q.r. 43-64) and median HAML-diameter was 57.5mm (i.q.r. 38.5-95.3). Thirty patients had undergone CT and 27/38 MRI of the liver, diagnostic biopsy was performed in 19/38. Initial diagnosis was incorrect in 15/38 patients, of which 13 were thought to have malignancy. In 84% biopsy resulted in a correct preoperative diagnosis. Twenty-nine patients were managed with surgical resection, 4/38 with surveillance and 3/38 with liver transplantation. Recurrence after resection occurred in two cases. No HAML related deaths or progression to malignancy were documented.

Conclusion: HAML diagnosis proved problematic even in hepatobiliary expertise centers. Biopsy is indicated and may provide valuable additional information when HAML diagnosis is considered on cross-sectional imaging, especially when surgical resection imposes a risk of complications. Conservative management with regular imaging follow-up might be justified when biopsy confirms (classic type) HAML.

INTRODUCTION

Angiomyolipomas (AML) are rare mesenchymal tumors belonging to a group of perivascular epithelioid cell tumors (PEComas) that are frequently described in the kidney (1). Hepatic AML (HAML) are uncommon, with approximately 600 reported cases in the literature (2). HAML typically is composed of blood vessels, smooth muscle cells and fat cells, in varying proportions (3). HAML occur more frequently in women and are usually found in non-cirrhotic livers (4, 5) with an unclear pathogenesis. An association with tuberous sclerosis complex (TSC) has been noted in over 50% of patients with renal angiomyolipoma (AML) versus only 10% in HAML patients (2, 3).

HAML appearance on imaging may vary because of various proportions of the tissue components (5). Detection of intratumoral fatty tissue is essential to suggest the diagnosis of HAML on imaging. As such, lipid- poor HAML may therefore easily be misdiagnosed (4). Histopathological examination along with the distinctive immunohistochemical positivity for Human Melanoma Black 45 (HMB45) and smooth muscle markers such as smooth muscle actin, are confirmative for HAML (6). Epithelioid type HAML is a particular type that is composed of epithelioid cells coexpressing HMB45 and smooth muscle markers (7).

Malignant behaviour of HAML with (invasive) growth, recurrence after surgical resection and even metastasis has been described in approximately 4% of patients (2, 3). This malignant behaviour is thought to occur mostly in epithelioid type HAML (8, 9). Although malignant behaviour is seldom seen in HAML, many researchers still advocate surgical approach. A conservative approach with regular imaging follow-up may be justified in asymptomatic patients with a definitive HAML diagnosis based on imaging and biopsy, without any high risk features (2).

Previously published reports on HAML consist mainly of case reports, small case series or pathology oriented studies, but clear recommendations on the management of patients with HAML are lacking. This study offers a clinical approach to patients with HAML and included patients with histopathological confirmed HAML from six major hepatobiliary centers located in Europe and the United States. The aim is to provide a pragmatic and applicable management strategy for HAML by analyzing cross-sectional imaging, management and outcome of patients HAML.

METHODS

Using an international, multicenter database from 6 major hepatobiliary centers in Europe and North America [Erasmus MC University Medical Center Rotterdam, The Netherlands

(n = 6), Hôpital Beaujon Paris, France (n = 12), University Hospital of Mainz, Germany (n = 6), Charité-Universitätsmedizin Berlin, Germany (n = 2), University of Pittsburgh Medical Center, Pennsylvania, The United States (n = 8) and Ohio State University Columbus, Ohio, The United States (n = 4)], 38 patients who were diagnosed with HAML between 1997 and 2017 were identified [1997-2001: n = 4, 2002-2006: n = 4, 2007-2011: n = 12, 2012-2017: n = 18]. Final diagnosis of HAML was based on pathological examination of either biopsy or resection specimen at the respective institution. The study protocol was reviewed and approved by the accredited institutional review board; informed consent was waived for this retrospective evaluation.

Data collection

Demographic variables including age, sex and baseline comorbidities were collected from institutional electronic medical records. Data on underlying liver disease and presenting symptoms were also recorded. Case images were reviewed in each institution by an experienced liver imaging expert with lesion characteristics on CT and MRI registered on a standard case report form. CT data included lesion type [cystic, solid, mixed cystic/solid], contrast enhancement characteristics [arterial/portal-venous dominant, persistent enhancement, wash-out, homogeneous/heterogeneous enhancement], initial diagnosis [benign, malignant, uncertain] and possibly classifying diagnosis. MRI data included signal intensity on T2W and T1W [high, low, intermediate, homogeneous, heterogeneous], confirmation of fatty content [yes, no], contrast enhancement characteristics [arterial/portal-venous dominant, persistent enhancement, wash-out, homogeneous/heterogeneous enhancement] initial diagnosis [benign, malignant, uncertain] and possibly classifying diagnosis.

In addition, data on clinical management were collected, including surgical treatment, conservative approach and reasons for the selected treatment strategy. Histopathological assessment of biopsy tissue and available resection specimen was performed at each participating center. Among the immunohistochemical markers used were HMB45 and SMA. Epitheliod type HAML were classified as lesions with the presence of 10-100% epitheloid cells. The pre-operative diagnosis was established combining the reports from cross-sectional imaging and, when available, biopsy. When biopsy was performed, the classifying diagnosis emerging from histopathological examination was leading in the pre-operative diagnosis. Long-term outcomes included recurrence after resection and follow-up of non-resected HAML.

Statistical analysis

Continuous variables were summarized as mean ± standard deviation (SD) or as median and interquartile range (i.q.r). Categorical variables were presented as frequency (n) and percentages. Comparative analysis was performed with Mann–Whitney U test for

continuous variables and χ2 test for categorical variables. All statistical analysis was performed with SPSS software version 21.0 (IBM, Armonk, New York, USA).

RESULTS

Clinical features of the 38 patients included in the study are presented in Table 1. The majority of patients was female with a median age at diagnosis of 56 years (i.q.r. 43-64).

Table 1: baseline characteristics

	N = 38
Sex	
Male	6
Female	32
Median age at diagnosis (yr)	56 (43 – 64)
Median BMI (kg/m²)	27.4 (22.0 – 32.5)
Presentation	
Asymptomatic	16
Abdominal pain or distention	19
Acute liver bleeding, ruptured lesion	1
Nausea	1
Other	1
Comorbidities	
None	15
Current or history of malignancy	8
Liver disease	4
Other	11
Median diameter of HAML at diagnosis (mm)	57.5 (38.5 – 95.3)
Number of HAML lesions	
Single	32
2 - 5	4
>5	2
Median follow-up time (months)	12.0 (4.0 – 64.0)

This table shows baseline characteristics of included patients. Values are given in median (IQR) or n.

Eight patients (21.1%) had a history of or a current malignancy (one liver related and seven with non-liver related malignancy) and four were known to have underlying liver disease (three viral hepatitis, one alcoholic cirrhosis). Nineteen patients initially presented

with abdominal pain or distention, 16 were asymptomatic. One female patient, 48 years of age, presented with acute liver bleeding due to a ruptured lesion. The majority had a single HAML lesion with a median lesion diameter of 57.5mm. No diagnosis of TSC was made in this group.

Diagnostic work-up and diagnosis

A summary of the diagnostic work-up is presented in Table 2. All patients underwent cross-sectional imaging with multiphase CT or multiparametric MRI of the liver. Specific findings on imaging are presented in Table 2. Most lesions were characterized as solid with a heterogeneous appearance. Fatty content was confirmed in 21/27 of patients on MRI, the remaining 6 were lipid poor (example of lipid poor HAML in figure 1). In 8/30 and 6/27 wash-out was assessed on CT and MRI, respectively.

Half of the patients (n = 19) underwent a diagnostic biopsy providing a correct diagnosis in 84%. In the patients who underwent surgery, the preoperative diagnosis was correct in 17 patients. Of the 14 patients with an incorrect preoperative diagnosis, 10 were diagnosed with hepatocellular carcinoma (HCC, example in figure 2), 2 with metastasis, 1 with hepatocellular adenoma and 1 with cholangiocarcinoma. One patient did not have a preoperative diagnosis as the lesion was found incidentally during cholecystectomy. Among the 14 patients with an incorrect pre-operative diagnosis, 9 had both CT and MRI as part of the diagnostic work-up and three patients also had an additional biopsy (Table 2). The three patients who had a diagnostic biopsy yet still an incorrect preoperative diagnosis were diagnosed as HCC (n = 1) and metastasis (n = 2, one metastasis of gastrointestinal stromal tumor and one calcified metastasis from colon carcinoma). No relationship was found between year of diagnosis and whether the preoperative diagnosis was correct.

Clinical management and follow-up

The median follow-up time was 12 months (i.q.r. 4-64). The majority of patients (29/38) underwent surgical resection, 4 were managed conservatively with surveillance, 3 underwent a liver transplantation and 2 patients were lost-to-follow-up after diagnosis (Table 3). No statistically significant differences for tumor size were seen between patients who were treated conservatively versus those who underwent surgical treatment (resection or liver transplantation) (p=0.505). The three patients who underwent a liver transplantation all had an incorrect preoperative diagnosis (HCC, 2 based on imaging alone and 1 based on biopsy and imaging), as well as 12 patients who underwent resection.

Table 2: diagnostic work-up and imaging findings

		Total study population N = 38	
Diagnostic work-up			
CT		30	
MRI		27	
Biopsy		19	
	Preop. diagnosis correct n = 17	**Preop. diagnosis incorrect n = 14**	**Total**
Diagnostic work-up			
CT	3	2	5
MRI	3	1	4
CT + MRI	1	8	9
CT + biopsy	3	-	3
MRI + biopsy	2	-	2
CT + MRI + biopsy	5	3	8
Imaging findings			
CT (n = 30)			
Solid		24	
Mixed cystic / solid		1	
Uncertain		5	
Contrast enhancement characteristics			
Arterial dominant		13	
Portal-venous dominant		3	
Persistent enhancement		9	
Wash-out		8	
Homogeneous enhancement		2	
Heterogeneous enhancement		19	
MRI (n = 27)			
Homogeneous signal intensity		5	
Heterogeneous signal intensity		22	
Fatty content confirmed		21	
Contrast enhancement characteristics			
Arterial dominant		19	
Portal-venous dominant		5	
Persistent enhancement		18	
Wash-out		6	
Homogeneous enhancement		0	
Heterogeneous enhancement		20	

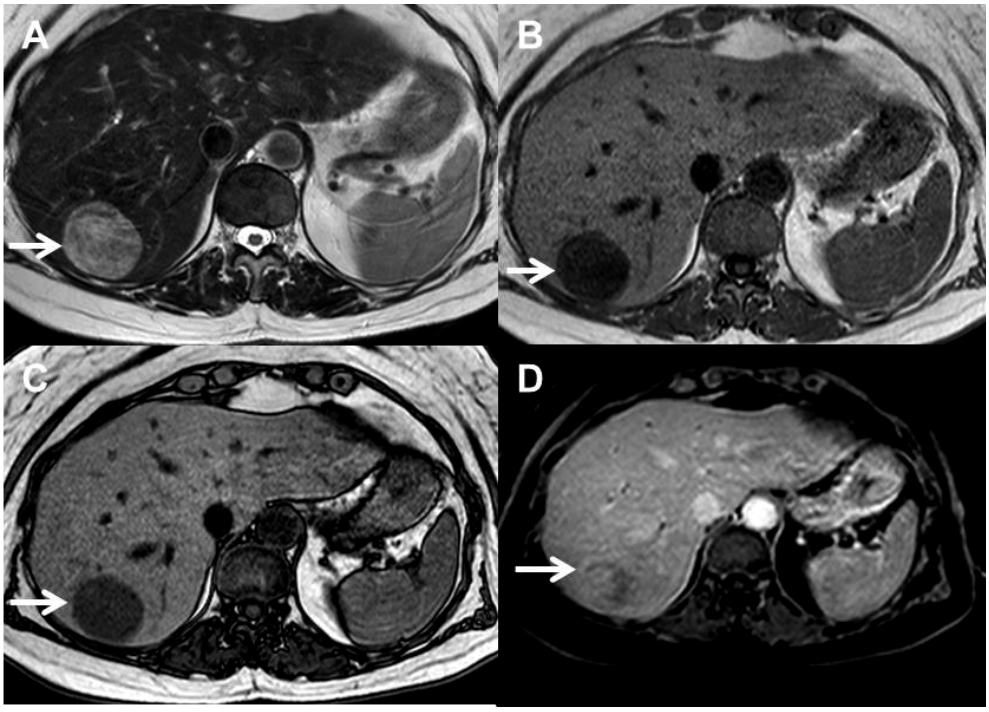

Figure 1. HAML imaging case 1: lipid poor HAML.

27-year-old female patient with history of cervical cancer and a growing focal lesion in liver segment VII (arrows) on the right.
A) T2-weighted image demonstrate a hyperintense lesion with heterogeneous signal intensity;
B,C) on the T1-weighted in-and out-of phase images no obvious fatty content was noted;
D) On the contrast-enhanced series heterogeneous enhancement was seen, including peripheral wash out. The lesion was characterized as a malignant lesion possibly hepatocellular carcinoma or metastasis. Right hemihepatectomy was performed with a lipid-poor HAML proven on histopathology examination.

Table 3: treatment and follow-up

	N = 38
Initial treatment	
Resection	29
Conservative	4
Liver transplantation	3
Unknown	2
Follow-up	
Recurrence after resection	2
Death (due to non-HAML related causes)	1

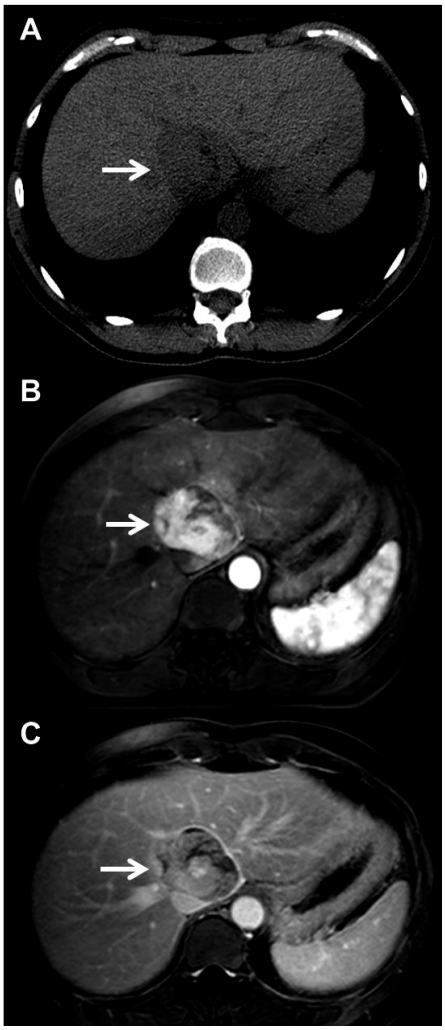

Figure 2. HAML imaging case 2: suspected HCC on imaging.

44-year-old female patient with incidental finding of a focal liver lesion on ultrasound screening for unrelated pain symptoms.
A) Subsequent multiphasic CT scan of the liver demonstrates a centrally located hypodense lesion (arrow) with no macroscopic fat.
B, C) On follow up MRI after 10 weeks, increase in size was noted, including emerging fatty content. Heterogeneous intense arterial enhancement with wash-out and late capsule enhancement was seen which was strongly suggestive for a HCC. Liver transplantation was performed with a HAML proven on the explant.

Recurrence after resection was seen in two patients. The first was a patient who underwent resection of multiple HAML (largest 92mm, right hemiliver) and recurrence was diagnosed one year after resection and was located in the left hemiliver. The second

patient presented with multifocal AML in the liver, pancreatic tail and kidney. Because of rapid growth, left hemihepatectomy and distal pancreatic resection was performed. After seven years, recurrent AML in the liver and progressive and new AML lesions in the abdomen and kidney were noted (Figure 3). TSC was excluded in this patient. Treatment with Everolimus was initiated which resulted in stabilization of the disease. One patient from the entire cohort died as a result of metachronous co-existing disseminated HCC.

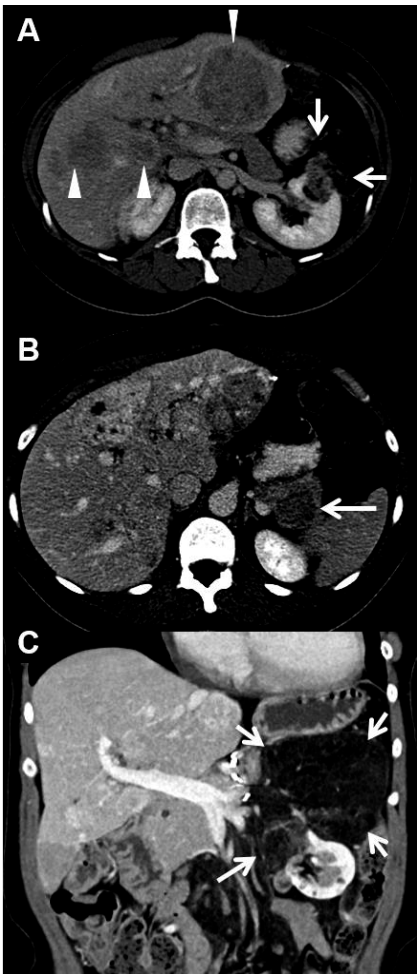

Figure 3. HAML imaging case 3: multifocal AML with recurrence.

35-year-old female patient with multifocal angiomyolipoma:
A) in the liver (arrow heads) and right kidney (arrows);
B) in the pancreatic tail (arrow). Because of rapid growth left hemihepatectomy and distal pancreatic resection was performed. After seven years recurrent AML in the liver and growing and new AML lesions in the abdomen and kidney were found on imaging. Treatment with Everolimus was started, upon which the disease stabilized.
C) Follow up CT after 16 years revealed enlarging AML of the right kidney (arrows) with stable size of others.

Pathology reports of resection specimens revealed 8/38 epithelioid type HAML with the remaining 24 being classic HAML. Four out of these 8 patients underwent a diagnostic biopsy: one was incorrectly diagnosed as metastasis. No patient with the epithelioid type HAML in the cohort had recurrence or metastasis during the follow-up period.

DISCUSSION

In this multi-institutional retrospective analysis, data on diagnosis, management and outcome of 38 patients with pathologically proven HAML from 6 major hepatobiliary centers were analyzed. The majority of patients was female and had a single HAML lesion. All patients underwent cross-sectional imaging and half of the patients also underwent a diagnostic biopsy. In a large subset of patients, the preoperative diagnosis was incorrect.

The results of this study emphasize that misdiagnosis in patients with HAML occurs frequently. In the majority, the incorrect preoperative diagnosis was HCC. Distinction between HAML and fat-containing HCC is particularly challenging when the HAML also demonstrates wash-out on cross-sectional imaging, as was the case in a quarter of our cases. Other misdiagnoses in this cohort were metastasis (of gastrointestinal stromal tumor and colon carcinoma), hepatocellular adenoma and cholangiocarcinoma. The misdiagnosis of these patients may have far-reaching consequences. Three out of fifteen patients who were misdiagnosed (20%) underwent a liver transplantation for suspected HCC. A systematic review noted that a large number of HAML are benign and do not need to be resected (2). As such, future studies need to focus on improving accuracy of (imaging) diagnosis in HAML to prevent avoidable complications in a benign disease.

In this study, more than half of all lesions demonstrate heterogeneous density or signal intensity on CT or MRI. In addition, in almost one out of four lesions contrast wash-out was assessed on both CT and MRI. These features make accurate differentiation from HCC almost impossible based on imaging alone. Biopsy resulted in a correct preoperative diagnosis in 84%. Immunohistochemical markers specific for HAML such as HMB-45 and SMA are the most sensitive and can confirm HAML diagnosis on tissue (10). Thus, if a lesion with intra-lesional fat and washout is found in a non-cirrhotic liver, final confirmation with biopsy and histopathological examination should be recommended to avoid missing HAML, especially when surgical resection imposes the risk of complications. In a systematic review published in 2017, the authors noted that for certain HAML diagnosis on imaging, conservative management with annual imaging was justified (2). However, the current study strongly suggests that imaging alone is not reliable enough and a biopsy should be performed before conservative management can be recommended. Surveillance in case of a clear HAML diagnosis on histopathological examination is indicated because of the uncertainty in biological behavior of HAML. Resection should be considered in case of an inconclusive biopsy, progressive HAML related symptoms and (invasive) growth during follow-up.

The case presented in figure 2 offers a good example in which tissue diagnosis would have provided valuable additional information. In this patients a centrally located lesion thought to be a HCC in non-cirrhotic liver was found, that showed growth during follow-up. When tissue diagnosis would have confirmed HAML, the tumor growth would still have caused suspicion and could have been an indication for resection. Given the central location of the lesion, radical resection was not an option and liver transplantation might still be considered. However, when the biopsy would have confirmed the lesion to be HAML and not HCC, a wait-and-see policy might have been justified given the relative rarity of malignant behavior in HAML. This case illustrates that tissue diagnosis is a pivotal step to make a well-informed decision and to weigh the risk of surgery against the risk of tumor-related complications. In the management of this rare disease, shared decision making and multidisciplinary consultation remains crucial.

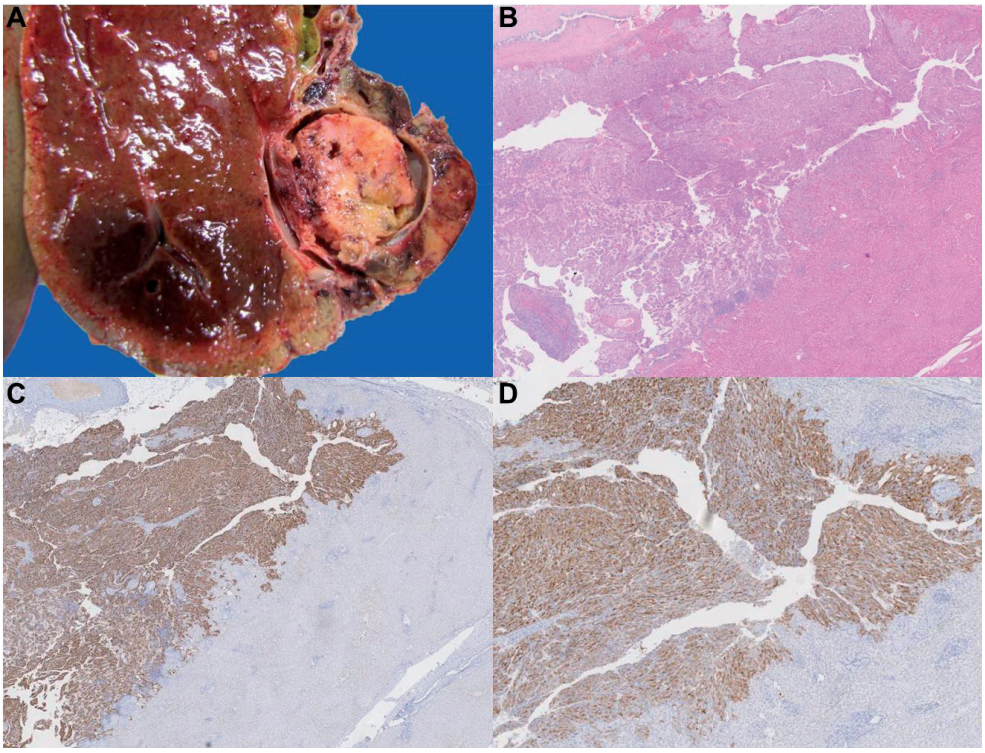

Figure 4. Macroscopic and microscopic image of HAML.

A) Macroscopic image of HAML
B) Microscopic image of HAML, Hematoxylin and Eosin (H&E) staining (x 1.25)
C) Microscopic image of HAML, Human Melanoma Black (HMB) 45 staining (x 1.25)
D) Microscopic image of HAML, Smooth Muscle Actin (SMA) staining (x 2.50)

Some studies suggest that epithelioid type HAML may mimic HCC or hepatocellular adenoma on pathological specimens(11). Epithelioid type HAML are a type of HAML composed almost solely of epithelioid cells, blood vessels and few adipocytes (12). These epithelioid type HAML are thought to have the most malignant course of behavior with recurrence or metastasis. In this cohort, recurrence after resection was observed in 2 patients during a median follow-up period of 12 months. Surprisingly, none of the epithelioid type HAML in this cohort demonstrated recurrence or metastasis during the follow-up period. However, given the small sample size, we would still recommend surgical resection in case of epithelioid type HAML.

In this study, almost half of the patients initially presented with abdominal pain or distention and another large proportion of patients had no specific symptoms, comparable to data reported in the literature (2). One female patient presented with an acute liver bleeding due to a ruptured lesion, at first thought to be a ruptured HCC or hepatocellular adenoma. Pathological examination however noted it to be HAML. Hepatic hemorrhage as a complication of ruptured HAML is very rare and has only been described in a few case reports to date (13-15).

The current study had several limitations. First, selection bias was likely given the retrospective design. Secondly, despite being the largest multicenter series of patients with pathologically proven HAML (aside from the study by Ding et al (16) which was a single center), our study still had a relatively small sample size. Additionally, although the multicenter design offers advantages of an increase of statistical power and generalizability of the results, collaborating with multiple centers limited the standardization of diagnostic and treatment criteria yet reflected daily practice in tertiary referral centers working with multidisciplinary teams. Considering the top level expertise of these centers, central review of the diagnostic scans and pathology specimens was considered unnecessary within the scope of this study as daily practice in these centers already consists of review with more than one radiologist or pathologist (usually fellow and senior) and all diagnostic work-up is discussed in multidisciplinary consultations.

Conclusion

In conclusion, this multicenter study of 38 patients with pathologically proven HAML shows that correctly diagnosing these rare lesions is a great challenge. In the current cohort, 15/38 of lesions initially were misdiagnosed with far-reaching consequences in some cases. As such, when cross-sectional imaging shows a lesion with intra-lesional fat and washout in a non-cirrhotic liver, a diagnostic biopsy should be performed to avoid missing HAML. Conservative management with regular imaging follow-up could be considered when biopsy confirms classic type HAML, given the rarity of malignant behavior. Resection should be considered in case of inconclusive biopsy, progressive

symptoms and growth during follow-up (Figure 5). Most importantly, shared decision making and multidisciplinary consultation in expert centers remains crucial in this rare disease.

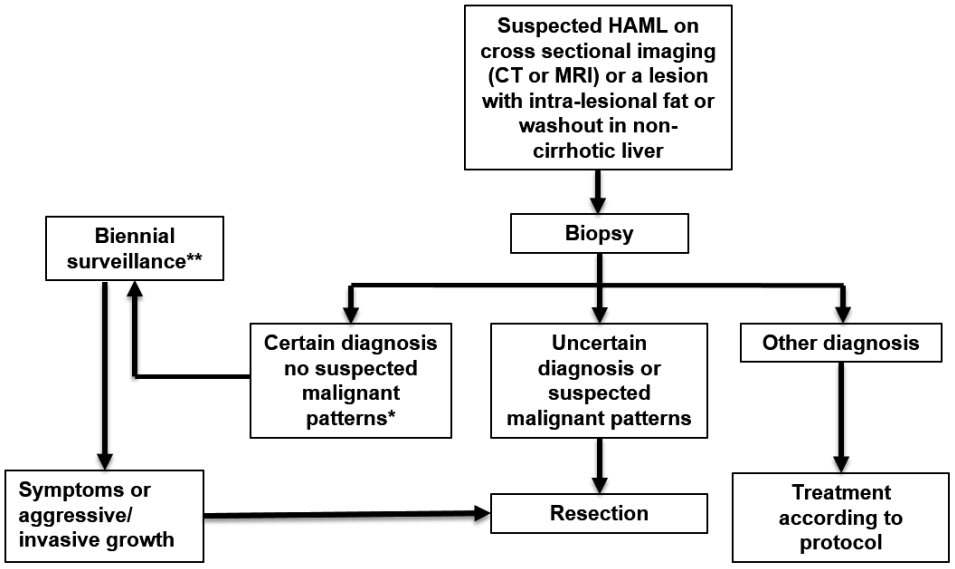

Figure 5. Proposed management algorithm for suspected HAML

*Suspected malignant cellular patterns with dysplasia, for instance well differentiated or dystrophic hepatocytes.
**Surveillance in case of a certain HAML diagnosis on histopathological examination is thought to be indicated because of the uncertainty in biological behavior of HAML.

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CHAPTER 14

The impact of imaging on the surgical management of biliary cystadenomas and cystadenocarcinomas; a systematic review

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ABSTRACT

Background: Biliary Cystadenomas (BCA) are considered to be benign but may transform to Biliary Cystadenocarcinomas (BCAC). The aim of this systematic review was to assess the diagnostic work-up and necessity of complete surgical resection.

Method: A systematic literature search was performed in Embase.com, Medline(Ovid), Cochrane Central, Web-of-Science and Google Scholar. Articles reporting on diagnostic work-up or outcome of various treatment strategies were included.

Results: Fifty-one articles with 1218 patients were included: 971 with BCA and 247 with BCAC. Patients with BCA were more often female (91% vs 63.8%, $p<0.001$). On radiologic imaging BCAC more often had calcifications ($p=0.008$), mural nodules ($p<0.001$) and wall enhancement ($p<0.001$). Reported treatment strategies were resection, enucleation, or fenestration/marsupialization. Recurrence was reported in 5.4% after resection for BCA and 4.8% after resection for BCAC. Recurrence after fenestration/marsupialization varied from 81.6%-100% for both BCA as BCAC. Mortality rate was 0 in patients with BCA and 24% in BCAC.

Conclusion: Due to the difficulty in accurately diagnosing these biliary cystic lesions and the availability of different surgical approaches, patients with suspected BCA or BCAC should be treated in a center specialized in liver surgery with state-of-the-art imaging and all surgical techniques to prevent mismanagement of this rare disease.

INTRODUCTION

Biliary Cystadenomas (BCA) and biliary cystadenocarcinomas (BCAC), are rare complex cystic tumors that may arise within the biliary system of the liver or in the extrahepatic bile ducts including gallbladder (90 vs 10%, respectively)(1). Liver cysts are the most frequent liver lesions with an estimated frequency of 20% of the general population and less than 5% of all liver cysts are considered BCA(2). BCA are considered to be benign, however literature suggests that up to 20% can transform to BCAC(2-4). Due to the rarity of the disease, pathogenesis is still unclear and predictors of malignant behavior are yet to be discovered.

BCA almost exclusively occur in middle aged women(3, 5, 6) while BCAC appear to be more evenly distributed between men and women(7). The average age at which BCA is diagnosed lies around 45 years compared to 55 for BCAC(3, 8). Patients with BCA most often present with abdominal pain or discomfort(9) but jaundice(10-12), nausea and vomiting have also been reported(13).

Computed Tomography (CT) and Magnetic Resonance Imaging (MRI) are most commonly used in diagnosing BCA. Using CT-scanning only, it may be difficult to differentiate simple cysts with septations from BCA. Several studies indicate that with Contrast Enhanced UltraSonography (CEUS) imaging features of cystic and cystic like focal liver lesions can be identified that may be helpful in differentiating simple cysts with septations from BCA(C), whereas conventional UltraSonography is not reliable to make this differentiation(14, 15). Biomarkers such as CA19-9, CA12-5, CEA and AFP may be elevated in both BCA and BCAC(16-20).

For a definite BCA diagnosis histologic examination is required, showing multilocular, cystic lesions with thin walls. BCA are lined by cuboidal to columnar epithelium and have dense cellular ovarian-like stromata (OS)(3). In 2010 the World Health Organization (WHO) established that OS is a requirement for diagnosis and that it should be considered to rename the lesions to Mucinous Cystic Neoplasms (MCN)(21). The cysts are septated and contain mucinous or serous fluid(6). They have been reported to appear more often in the left hemiliver(10, 22) and range in size from 1cm to 40cm(9).

BCA are considered to be slow growing tumors(6, 23). Because of the possible malignant transformation, additional diagnostic tests like biopsy or definitive treatment by resection is recommended. It is still open for discussion which treatment modality is preferable in case of BCA and options may vary from surgical resection to fenestration, marsupialization and drainage(9, 18, 24, 25). Literature suggests resection of every suspected BCA is currently recommended(3, 26, 27), because other treatment options are correlated with a

high recurrence rate(16). However, as not every BCA will transform to BCAC, treatment of these lesions by complex surgical procedures with their associated complications should be carefully considered, against the possibility the resected lesion may be benign(16).

To date, there is no clear evidence-based consensus on the optimal BCA(C) treatment strategy. As the disease is rare and the articles published are limited and potentially biased by expert opinions, this systematic review will assess the diagnostic work-up and necessity of complete surgical resection and aims to recommend on a justified management strategy.

METHODS

This systematic review was conducted and reported according to the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analysis) guideline(28).

Literature search

With the help of a clinical librarian (WMB) a systematic literature search was conducted using various synonyms for 'cystadenoma', 'mucinous cystic neoplasm' or 'cystadenocarcinoma' (appendix A). The search was conducted in Embase.com, Medline (Ovid), Cochrane Central, Web-of-Science and Google Scholar. The date last searched was November 22nd 2017.

Study selection

Two independent reviewers (AJK & DWGtC) evaluated the articles and screened title and abstract of all deduplicated articles. Articles were included if they reported on randomized controlled trials, case-control studies, cohort studies and case series ($n \geq 5$). The articles had to include patients diagnosed with BCA(C) and had to report on diagnostic work-up or outcome of various management strategies. Only articles written in English were included. If the inclusion criteria were met, full articles were reviewed. Disagreement was resolved by discussion. Articles were excluded if they described tumors other than intrahepatic BCA. Reviews, animal studies, cadaver studies, case reports ($n<5$), surveys, editorials, commentaries, conference abstracts and letters were also excluded. Additionally, articles that did not report on enrollment dates or enrollment centers were excluded in order to prevent reporting on overlapping data. When there was an overlap between different articles in study population, the article reporting the most complete data was chosen. Level of evidence was determined using Oxford Centre for Evidence-based Medicine Levels of Evidence(29).

Outcome measures

The primary outcomes were the reported BCA(C) associated mortality rates and recurrence rates after various treatment strategies. Patients who died due to BCA(C) unrelated disease were not included in mortality rate calculations. Secondary outcomes included a comparison of BCA vs BCAC based on patient characteristics and imaging characteristics. Data extraction was performed by AJK and DWG independently. Publication details, type of study, data on diagnostic work-up (biopsy, imaging [modality, diagnosis, characteristics], tumor markers [in serum and cystic fluid]), patient characteristics (sex, age at diagnosis, presentation), tumor characteristics (size, location), follow-up period, type of treatment (fenestration and marsupialization were categorized as one treatment group) and outcome (mortality and recurrence) were collected. Mortality was defined as death of disease or death of other cause.

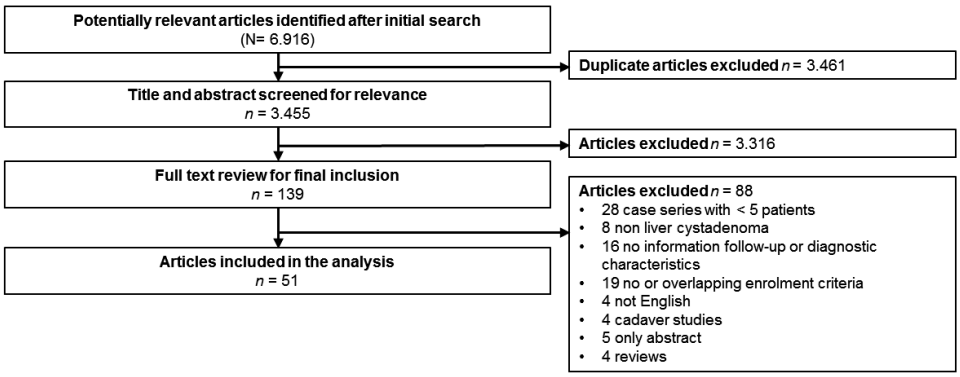
Statistical analysis

Variables are presented as mean (SD) or median (range), according to the way they were reported in the original article. Differences between groups were investigated using Chi-Square test (IBM SPSS software version 21.0 Chicago, Illinois). Cumulative data were tabulated and presented as number with percentages.

RESULTS

The initial search yielded 6919 articles, of which 3455 remained after deduplication. Screening of title and abstract excluded 3316 articles, resulting in 139 articles for full text review. A final 51 articles were included in this systematic review. Article selection is depicted in figure 1.

Figure 1. Flowchart inclusion



Patient characteristics

The included articles are presented in table 1. All studies included were retrospective case series or cohort studies (level of evidence 4). Table 2 describes the included patients in this study. A total of 1218 patients were included, of which 1022 (85.3%) were female. A total of 971 (79.7%) patients were diagnosed with BCA and 247 (20.3%) with BCAC.

A comparison between patients with BCA and BCAC showed a significantly higher proportion of female patients with BCA ($p<0.001$). The age of patients with BCA ranged from 38 to 62 years and for BCAC from 49.4 to 77 years. No significant difference was found for tumor location or symptoms as well as liver function tests, including ALT, AST, Total Bilirubin and Alkaline Phosphatase levels. Analysis of tumor markers in serum and cystic fluid showed that patients with BCAC more often have elevated CA19-9 ($p=0.041$) and CEA in serum ($p=0.024$). No significant differences were found for CA12-5 and AFP in serum nor for any tumor markers in cystic fluid.

Diagnostics

All included patients had histologically confirmed BCA(C). In 678 patients (55.7%) CT was used and in 142 patients (11.7%) diagnosis was based on MRI. Contrast-Enhanced Ultrasound (CEUS) was performed in 41 patients (3.4%). In 79 patients (6.4%) both CT and MRI was used in the diagnostic work-up.

The imaging characteristics are described in table 3. Calcifications were more often reported in patients with BCAC (55 (13.3%) in BCA and 22 (24.4%) in BCAC, $p=0.008$), as were wall enhancement (81 (26.6%) in BCA and 52 (69.9%) in BCAC, $p<0.001$) and mural nodules (81 (17.2%) in BCA and 76 (73.8%) in BCAC, $p<0.001$). No statistically significant differences were found for multiloculation, septation and biliary dilatation. Out of 1218 histologically confirmed BCA(C), the presence of OS was described in 44% of the cases. OS was reported in 389 (88.8%) BCA and 63 (67%) BCAC ($p<.001$).

Table 1: overview of included studies

Reference	Year	Type of study (level of evidence)	No. of patients	Mean/ median age	Female : male	Mean/ median tumor size (cm)	Treatment
1. Ahanatha et al.**(39)**	2012	Case series (4)	13	BCA: 46	11 : 2	-	Resection Enucleation
2. Al-Qahtani et al.**(40)**	2016	Case series (4)	11	BCA: 45.9	11 : 0	-	Resection Enucleation
3. Ammori et al.**(41)**	2002	Case series (4)	8	50*	5 : 3	12*	Resection Percutanous Drainage Conservative

4. Arnaoutakis et al.**(16)**	2015	Retrospective cohort (4)	248	BCA: 51.2 BCAC: 58,9	215 : 33	BCA: 10 BCAC: 10.5	Resection Fenestration Liver Transplantation
5. Buetow et al.**(42)**	1995	Case series (4)	34	BCA: 38 BCAC: 57	31 : 3	BCA: 12	Resection
6. Chen et al.**(43)**	2014	Case series (4)	39	53.7*	30 : 9	-	Resection Enucleation
7. Choi et al.**(19)**	2010	Retrospective cohort (4)	17	BCA: 57	17 : 0	BCAC: 10.1	Resection
8. Devaney et al.	1994	Case series (4)	70	BCA: 45 BCAC: 59	60 : 10	BCA: 15 BCAC: 12.3	Resection
9. Dong et al.**(14)**	2017	Case series (4)	23	BCA: 57 BCAC: 60	11 : 12	BCA: 4.8 BCAC: 6.9	-
10. Doussot et al.**(32)**	2015	Case series (4)	20	62*	17 : 3	9.9*	Resection
11. Emre et al.**(24)**	2011	Case series (4)	9	49*	9 : 0	-	Resection Enucleation
12. Fragulidis et al.**(44)**	2015	Case series (4)	10	BCA: 66 BCAC: 61	9 : 1	BCA: 9.9 BCAC: 13.5	Resection Enucleation
13. Gadzijev et al.**(45)**	1998	Case series (4)	6	BCA: 42.2 BCAC: 58	6 : 0	BCA: 10.8 BCAC: -	-
14. Hai et al.**(46)**	2003	Case series (4)	6	BCA: 56,3 BCAC: 59.6	4 : 2	BCA: 8 BCAC: 8.5	-
15. Hansman et al.**(47)**	2001	Case series (4)	8	-	-	-	Resection
16. Jae et al.**(48)**	2009	Case series (4)	10	BCA: 45.2 BCAC: 62	7 : 3	BCA: 8 BCAC: 9.6	Resection
17. Jwa et al.**(17)**	2017	Case series (4)	30	BCA: 60 BCAC: 68	27 : 3	-	Resection
18. Kim et al.**(49)**	2014	Case series (4)	15	BCA: 44	15 : 0	BCA: 8.7	Resection
19. Kim et al.**(50)**	2010	Case series (4)	12	56.3*	10 : 2	9.5*	-
20. Krige et al.**(51)**	2017	Case series (4)	16	BCA: 46	16 : 0	-	Resection
21. Labib et al.**(20)**	2017	Case series (4)	13	BCA: 46	13 : 0	BCA: 13.7	Resection
22. Lam et al.**(52)**	2008	Case series (4)	8	BCA: 45.7	8 : 0	-	-
23. Lee et al.**(53)**	2015	Case series (4)	21	BCA: 57 BCAC: 67.5	16 : 5	BCA: 8.5	-
24. Lewis et al.**(26)**	1988	Case series (4)	15	BCA: 41	13 : 2	BCA: 12.5	Resection
25. Li et al.**(54)**	2009	cohort study	13	44.4*	11 : 2	11.2*	Resection
26. Li et al.**(55)**	2013	Case series (4)	10	BCA: 45 BCAC: 51.3	8 : 2	-	Resection
27. Lim et al.**(56)**	2007	Retrospective cohort (4)	17	50*	15 : 2	8.9*	Resection
28. Lin et al.**(33)**	2009	Case series (4)	5	-	-	-	-

29. Martel et al.**(57)**	2013	Case series (4)	13	52.1*	12 : 1	12.4*	Resection Enucleation
30. Nakajima et al.**(58)**	1992	Case series (4)	7	BCAC: 55	5 : 2	BCAC: 11.8	Resection Enucleation
31. Pitchaimuthu et al.**(59)**	2015	Case series (4)	29	BCA: 62	28 : 1	-	-
32. Pojchamarn-wiputh et al.**(5)**	2008	Retrospective cohort (4)	12	BCA: 40.6 BCAC: 51.3	10 : 2	BCA: 12 BCAC: 11.9	Resection Enucleation
33. Quigley et al.**(37)**	2017	Case series (4)	36	BCA: 50.4 BCAC: 61	36 : 0	BCA: 11.2 BCAC: 17.5	Resection
34. Ratti et al.**(60)**	2012	Case series (4)	12	BCA: 45	12 : 0	-	Resection
35. Regev et al.**(22)**	2001	Case series (4)	9	BCA: 60.5 BCAC: 77	7 : 2	BCA: 14.3 BCAC: 19	Resection
36. Sanchez et al.**(27)**	1991	Case series (4)	19	BCA: 42	15 : 4	BCA: 11	Resection Aspiration
37. Sang et al.**(61)**	2011	Case series (4)	33	BCA: 44.2 BCAC: 57	22 : 11	BCA: 13 BCAC: 8.3	Resection Enucleation Fenestration
38. Seo et al.**(11)**	2010	Retrospective cohort (4)	20	BCA: 55.2 BCAC: 56.8	17 : 3	BCA: 12.9 BCAC: 11	Resection
39. Song et al.**(62)**	2012	Retrospective cohort (4)	30	BCA: 51.6 BCAC: 49.3	22 : 8	BCA: 7.9 BCAC: 11.7	Resection
40. Teoh et al.**(2)**	2006	Case series (4)	7	BCA: 51.5 BCAC: 74	5 : 2	BCA: 8.6 BCAC: 14.4	Resection Enucleation
41. Thomas et al.**(63)**	2005	Case series (4)	19	BCA: 48.3	18 : 1	-	Resection Enucleation Fenestration
42.Treska et al.**(64)**	2016	Case series (4)	12	BCA: 57.7	12 : 0	-	Resection Enucleation
43. Vogt et al.**(9)**	2005	Case series (4)	22	BCA: 48 BCAC: 60	21 : 1	BCA: 12.5	Resection Enucleation
44. Wang et al.**(10)**	2012	Case series (4)	30	BCA: 44.2 BCAC: 56.9	23 : 7	BCA: 13 BCAC: 7.9	Resection
45. Wang et al.**(65)**	2014	Case series (4)	14	BCA: 48	11 : 3	BCA: 10.4	Resection
46. Wheeler et al.**(6)**	1985	Case series (4)	17	BCA: 41.7 BCAC: 58.8	17 : 0	-	Resection Drainage
47. Wu et al.**(66)**	2008	Case series (4)	7	BCA: 52	5 : 2	BCA: 10	Resection
48. Xu et al.**(67)**	2012	Case series (4)	13	BCA: 44 BCAC: 52	11 : 2	BCA: 8.2 BCAC: 8.6	Resection
49. Xu et al.**(12)**	2015	Retrospective cohort (4)	75	BCA: 45.4 BCAC: 57.5	58 : 17	BCA: 11.7 BCAC: 7.1	Resection Enucleation
50. Zen et al.**(68)**	2011	Retrospective cohort (4)	29	BCA: 45	29 : 0	BCA: 11	Resection
51. Zhang et al.**(13)**	2014	Case series (4)	46	BCA: 53.4 BCAC: 52	36 : 10	-	Resection

*Articles that combined BCA and BCAC in calculations

Table 2. Clinical characteristics and tumor markers

	BCA (N=971) n (%)	BCAC (N=247) n (%)	p-value
Sex			**<0.001**
Male	84 (9.3)	83 (36.2)	
Female	817 (90.7)	146 (63.8)	
No information	70	18	
Age (range)	38-62	49.4-77	
Presentation			0.492
Symptomatic	465 (74.6)	140 (82.4)	
Asymptomatic	158 (25.4)	30 (17.6)	
No information	348	77	
Tumor location			0.467
Left lobe	359 (55)	110 (59.8)	
Right lobe	207 (31.7)	54 (29.3)	
Bilobular	87 (13.3)	20 (10.9)	
No information	318	63	
ALT			0.291
Normal	124 (88.6)	49 (83.1)	
Elevated	16 (11.4)	10 (16.9)	
No information	831	188	
AST			0.888
Normal	124 (95.4)	56 (94.9)	
Elevated	6 (4.6)	3 (5.1)	
No information	841	188	
Total bilirubin			0.191
Normal	138 (89.6)	49 (83.1)	
Elevated	16 (10.4)	10 (16.9)	
No information	817	188	
Alkaline phosphatase			0.382
Normal	128 (94.8)	54 (91.5)	
Elevated	7 (5.2)	5 (8.5)	
No information	836	188	
CA19-9 – serum			**0.041**
Normal	257 (67.5)	62 (56.9)	
Elevated	124 (32.5)	47 (43.1)	
No information	590	138	
CEA – serum			**0.024**
Normal	196 (67.4)	22 (50)	
Elevated	95 (32.6)	22 (50)	
No information	680	203	

CA12-5 – serum			0.786
Normal	24 (82.8)	24 (80)	
Elevated	5 (17.2)	6 (20)	
No information	942	217	
AFP - serum			0.508
Normal	98 (99)	43 (100)	
Elevated	1 (1)	-	
No information	872	204	
CA19-9 – cystic fluid			0.513
Normal	7 (17.9)	1 (33.3)	
Elevated	32 (82.1)	2 (66.7)	
No information	932	244	
CEA – cystic fluid			0.314
Normal	12 (34.3)	-	
Elevated	23 (65.7)	2 (100)	
No information	936	245	
CA12-5 – cystic fluid			-
Normal	-	-	
Elevated	13 (100)	-	
No information	958	247	

Treatment and outcome

Information on treatment was given for 832 patients with BCA and 212 patients with BCAC. Resection was the most reported treatment strategy (BCA 91.7% and BCAC 92.9%). Other reported strategies were: fenestration (BCA 4.4%, BCAC 0.9%), enucleation (BCA 3.5%, BCAC 4.7%), marsupialization (BCA 0.1%, BCAC 0), liver transplantation (BCA 0.2%, BCAC 0.5%), drainage (BCA 0%, BCAC 0.5%), chemotherapy (BCA 0%, BCAC: 0.9%) and conservative treatment (BCA 0, BCAC 0.5%).

BCA recurrence occurred in 5.4% after resection, 3.3% after enucleation and in 81,6% after fenestration or marsupialization (table 4). The reported recurrence rate for BCAC after resection was 4.8%, 9.1% after enucleation and 100% after fenestration or marsupialization.

The BCA associated mortality rate was 0% (7/621 patients died, all causes unrelated to BCA). The BCAC associated mortality rate was 24.2% (42/172), four patients died as a result of BCAC unrelated disease. The difference in mortality between BCA and BCAC was statistically significant ($p<0.001$).

Table 3. Imaging characteristics

	BCA (N=971) n (%)	BCAC (N=247) n (%)	p-value
Multiloculation			0.551
Yes	359 (69.4)	71 (72.4)	
No	158 (30.6)	27 (27.6)	
No information	454	149	
Septation			0.385
Yes	328 (71.3)	69 (67)	
No	132 (28.7)	34 (33)	
No information	511	144	
Calcifications			**0.008**
Yes	55 (13.3)	22 (24.4)	
No	357(86.7)	68(75.6)	
No information	559	157	
Biliary dilatation			0.070
Yes	11 (18)	5 (41.7)	
No	50 (82)	7 (58.3)	
No information	910	235	
Mural nodules			**<0.001**
Yes	81 (17.2)	76 (73.8)	
No	391 (82.8)	27 (26.2)	
No information	499	144	
CEUS wall enhancement			**<0.001**
Yes	81 (26.6)	51 (69.9)	
No	224 (73.4)	22 (30.1)	
No information	666	174	

Table 4. Recurrence after treatment

BCA	
Resection (N=763)	46 (5.4)
Enucleation (N=37)	1 (3.3)
Fenestration or marsupialization (N=38)	31(81.6)
BCAC	
Resection (N=197)	10 (4.8)
Enucleation (N=10)	1 (9.1)
Fenestration or marsupialization (N=2)	2 (100)

DISCUSSION

This systematic review included a total of 1218 patients extracted from 51 articles, 79.7% with BCA and 20.3% with BCAC. Although both BCA and BCAC occur predominantly in females, a significantly higher proportion of men was seen in the BCAC group. Median age of patients with BCAC appears to be higher than BCA (range 49-77 versus 38-62 years, respectively). It has been suggested that occurrence of BCA(C) is related to the embryonic gallbladder development and therefore lesions would be more often located in the left hemiliver(9, 10, 22). However, in this study no statistically significant difference in tumor location between right and left hemiliver was found.

This systematic review shows that liver function enzymes such as ALT, AST, total bilirubin and Alkaline Phosphatase, cannot be used to differentiate BCA from BCAC. Serum tumor markers CA19-9 and CEA are more often elevated in BCAC as compared to BCA. However, tumor markers within the normal range cannot rule out BCAC. No statistically significant differences were found for serum CA12-5 and AFP and for all tumor markers measured in cystic fluid. Even more, as tumor markers in cystic fluid can be elevated even in benign liver cysts(30), these markers should not be used in the diagnostic work-up for BCA(C). Unfortunately, the exact level of the markers (times the upper limit of normal) were not reported.

On imaging, mural nodules, wall enhancement on contrast enhanced CT and calcifications occur significantly more often in BCAC as compared to BCA. Septations, multiloculation and biliary dilatation cannot be used reliably to differentiate BCA from BCAC. A recent study by Kovacs et al. performed in 25 patients with either complex hepatic cysts or BCA showed that the relationship between septations and the wall of the cystic lesion might be more predictive for a diagnosis than previously reported imaging features(31). Although the sample size in this study was small, the results are promising and should be explored further.

The disease related mortality rate for BCA was 0% as compared to 24.2% for patients with BCAC ($p<0.001$). Unfortunately, no data are reported on a wait and see policy for BCA and therefore we cannot comment on the biological behavior and risk of transformation from BCA to BCAC. It would be of interest to study the natural course of these lesions in a well-defined prospective study. The reported recurrence rates of different treatment strategies show a low recurrence after resection (5.4%) or enucleation (3.3%) and high recurrence rates after fenestration or marsupialization (81.6%). Unfortunately, data on the pre-operative diagnosis and the radicality of resection was lacking from the included articles. If the recurrence after resection were all non-radical resections the reason for recurrence may rather be the (inaccurate) treatment and not the disease.

This systematic review investigated BCA and BCAC, and did not look at the differentiation between complex liver cysts and BCA(C). In literature, we found that CT, MRI and ultrasound combined provide a high sensitivity (87.5-100%) for the differentiation of simple liver cysts from BCA(C)(32), but a poor specificity (43.1-53.4%). Another recently introduced imaging technique that might be used is CEUS, providing a sensitivity of 81.3-93.8% and specificity of 47.1-88.2%(33). As all imaging modalities provide a poor specificity, future research should focus on ways to improve the diagnostic work-up, for example by combining CT and MRI with CEUS. A new approach to differentiate simple liver cysts from BCA(C) relies on measurement of intracystic tumor marker TAG-72, thus identifying BCA and BCAC(34). The results of this study are promising and should be explored further.

Complications after surgery were underreported in the included articles. As BCA is essentially a benign disease and liver resections may have a complication rate of up to 20%(35, 36), perhaps resection of BCA should not be performed in all cases. In particular, lesions located nearby larger vessels might be at risk for complications due to surgery and decision-making should include the anatomical characteristics. It has been suggested that frozen section mid surgery could be performed to distinguish simple cysts from BCA and BCAC and avoid unnecessary resection(32). In a study by Doussot et al. frozen section was performed in 36 patients with either simple cysts or BCA. They found a concordance between frozen section and final histopathological examination of resection specimen in all cases. This suggests that frozen section is a reliable way to distinguish simple cysts from BCA. However, a study performed by Quigley et al. found that to prove OS in the lesion, extensive pathological examination is required(37). A selected part of the lesion as is taken for frozen section may not be representative for the lesion as a whole. Additionally, in the process of malignant transformation from BCA to BCAC, a biopsy is unlikely to be representative for the lesion as a whole as a biopsy is usually performed in the part that is easily accessible and not in the most suspect part of a liver lesion. Given the current uncertainty in differentiating BCA from BCAC and the high recurrence rates after fenestration and marsupialization, surgical resection should be the performed in all patients with suspected BCA(C). When BCAC is suspected, fenestration and marsupialization should never be performed.

The main limitation of this study lies in the histopathologic diagnostic criteria for BCA(C). These criteria changed in 2010 when the WHO established that BCA should be called Mucinous Cystic Neoplasms (MCN) and that the presence of OS is a requirement for MCN diagnosis(21). To this day, OS is not widely applied and literature is highly conflicted on the definition of BCA(C) vs MCN(38). One study reports that invasive carcinomas are uncommon in true MCN with OS(37), while another states that OS positive MCN have a worse survival than patients with OS negative lesions(16). In this systematic review, in

over half of the patients (56%) information on stromal characteristics and especially the presence of OS was missing. Additionally, in the majority of included articles the presence of OS was only reported as frequency in the study population and not linked to patient characteristics or outcomes of individual patients, making it impossible for us to make a reliable statement about the association between OS and outcome. The second limitation of this systematic review is the fact that all included articles were retrospective cohort studies or case series (level of evidence 4(29)) resulting in missing data and bias. To the best of our knowledge, no prospective cohorts or randomized controlled trials have been published on this subject.

Table 5. summary of the main implications in the management of BCA(C)

Clinical characteristics	BCA have a female predominance while BCAC occur are more evenly distributed between males and females. Patients with BCAC appear to be older.
Tumor markers	Elevated serum tumor markers (CA19.9 and CEA) are suggestive for BCAC. However, tumor markers within the normal range cannot rule out BCAC.
Imaging	On imaging, calcifications, mural nodules and wall enhancement are more often reported in BCAC. The combination of CT, MRI and CEUS might improve the diagnostic work-up; this should be studied further.
Biopsy	Biopsy cannot be used to differentiate BCA from BCAC, as it is unlikely to be representative for the lesion as a whole.
Treatment	Radical resection should be performed in both BCA as BCAC, as fenestration or marsupialization have high recurrence rates. Fenestration or marsupialization should never be used for BCAC.
General conclusion	Patients with suspected BCA(C) should be treated in a center specialized in liver surgery with the availability of state-of-the-art imaging as well as surgical techniques to prevent mismanagement of this rare disease.

Conclusion

This systematic review shows that present data don't support a role for tumor markers in cystic fluid in differentiating BCA from BCAC. Radiologic imaging such as CT and MRI, but also CEUS, may help in differentiating BCA from BCAC, but their specificity is poor. A non-directed biopsy cannot be used to differentiate BCA from BCAC, as it is unlikely to be representative for the lesion as a whole. In case of suspected BCA(C), radical resection should be performed. Due to the variety in clinical symptoms, the difficulty in rightfully diagnosing BCA(C) and, in BCA, possible complications after resection of a benign neoplasm, patients with suspected BCA or BCAC should be treated in a center specialized in liver surgery with the availability of state-of-the-art imaging as well as surgical techniques to prevent mismanagement of this rare disease.

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CHAPTER 15

General discussion and future perspectives

General discussion and future perspectives

When a patient visits the outpatient clinic with a focal liver lesion the first focus lies on determining whether the tumor is benign or malignant, as their management and prognosis varies greatly. Even if the lesion is thought to be benign on imaging, additional diagnostics may be required to determine whether the lesion carries a risk of complications such as hemorrhage or malignant transformation. Overtreatment of benign liver tumors is up to discussion and the question in which patients surgical treatment should be preferred and in whom a wait and see policy is justified, still has not been answered adequately. In the best case scenario, only patients with a benign liver tumor and a predicted increased risk of complications, including bleeding or malignant degeneration, will undergo surgery. However, to date we are not able to predict an individual patients' disease course reliably and defining proper management for each patient remains a great challenge. Thus, one may question how many patients with liver lesions, suspected to be benign in nature, may be exposed to either a wait and see policy or an intervention to prevent complications of the natural course of the disease? In this thesis I elaborated on this dilemma and aimed to define criteria for a legitimized treatment of the different liver lesions.

In the past two decades, the greatest advances have been made in the field of hepatocellular adenoma (HCA). In 2016, the first EASL guideline on the management of benign liver tumors stated to perform a surgical resection if HCA exceed 5cm six months after implementation of lifestyle changes (cessation of oral contraceptive and weight loss) (1). Before the EASL guideline was issued, patients usually underwent surgery even sooner, meaning that previously overtreatment may have occurred in these patients with HCA. At present, we advocate to await the effect of cessation of oral contraceptives for at least a year in all patients, irrespective of the diameter at diagnosis. Even more, surgery might not even be necessary in HCA >5cm to prevent bleeding as currently there is no data supporting the concept that there is still a risk of bleeding in regressing HCA. Overtreatment has also been an issue in patients with HCA and an active pregnancy wish. As HCA growth may increase due to hormonal stimuli, pregnancy used to be discouraged in patients with unresected HCA. As we demonstrated previously, a large number of women with HCA already had been pregnant without knowing that they had the aberrant liver lesions, and therefore it seemed warranted to study HCA in pregnant women prospectively to get insight in its biological behavior (2, 3). In the PALM study, including pregnant women having HCA <5cm, we demonstrated that monitored pregnancy is safe as only one minimal invasive intervention was performed to prevent rapid increase in growth exceeding 7 cm. The postinterventional course was uneventful for mother as well as child.

In addition, having knowledge of the sensitivity of HCA to hormones in fertile women we questioned whether it would be safe to stop surveillance of HCA after the menopause and

the changes in the hormonal system. In 2012, our study group proposed a standardized decision-making model for the management of HCA in which we hypothesized that it would be safe to stop surveillance of post-menopausal patients. However, the assumptions were based on expert opinions. In this thesis we demonstrated indeed that HCA become smaller after menopause and therefore routine follow-up of HCA <5cm in post-menopausal women after subsequent follow-up is not required.

A great change in the management of HCA lies in the discovery of various HCA subtypes. In the prediction model included in this thesis, we have found that regression occurs sooner in I-HCA as compared to H-HCA. Additionally, other studies have demonstrated that hemorrhage occurs mostly in I-HCA and the recently discovered sh-HCA, whilst malignant transformation occurs mostly in HCA with a β-catenin mutation (4). H-HCA appear to have the most benign course with a low risk of complications. As larger studies validating estimated risks of subtypes are missing we recommended to study these HCA subtypes in larger cohorts. Moreover, the variety of responses in biological behavior of HCA during pregnancy might be related to the subtype and further exploration of this observation in relation to the risk of complications during pregnancy may add important clinical information to legitimize a personalized approach. With the availability of more extensive immunohistochemical staining and molecular characterization, we are moving more towards a subtype based management algorithm as compared to a diameter based algorithm (4). Given the low risk of complications, a conservative approach might be justified in confirmed H-HCA and in I-HCA that regress after cessation of oral contraceptives and do not carry an additional β-catenin mutation. In the past, restraint was required when considering a biopsy due to the perceived risk of biopsy induced hemorrhage. However, biopsy is evolving as a pivotal step in the management of this disease to identify HCA subtypes at risk, as only H-HCA and I-HCA can be identified reliably based on contrast enhanced MRI (5, 6).

One might assume that in the future, the incidence of HCA will increase with the rising incidence of obesity. It has been established that obesity is associated with I-HCA in particular (7). This will mean that weight loss as a potential treatment for obese patients with HCA should be explored further, as the current evidence is based on very limited numbers of patients (8, 9). With all of these changes, the EASL guideline might need an update in the very near future.

Focal nodular hyperplasia (FNH) does not carry a risk of complications and treatment is not indicated in case of a well-established diagnosis. The most important differential diagnosis is HCA and differentiating between the two lesions is of great importance as the management differs. Misdiagnosing FNH as HCA is common and may have a great impact on the lives of these often young female patients, for example in the case of an active pregnancy wish as is described in chapter 5 (PALM study). It has been demonstrated that

MRI with liver specific contrast agent Gd-EOB-DTPA (Primovist® or Eovist®) provides the highest accuracy in differentiating HCA from FNH (10). Growth of FNH is not uncommon and may occur in 12%. This increase in size should not have any implications on clinical management if confident diagnosis by contrast enhanced MRI has been established in a multidisciplinary team.

Hepatic angiomyolipoma (HAML) is the most rare benign liver tumor addressed in this thesis with an approximate 600 cases published in literature. Again, as described the greatest challenge in these rare HAML is to correctly diagnose these lesions. On imaging, HAML may easily be misdiagnosed as a malignancy resulting in overtreatment of this particularly benign tumor. As the risk of malignant behavior in HAML appears to be low (4.1%), resection may only be considered in symptomatic patients or in cases with progressive or invasive growth. In the future, the focus should lie on improving accuracy of (imaging) diagnosis in HAML to prevent avoidable complications in a benign disease. Patients with suspected HAML on imaging should be referred to a tertiary referral center in order to prevent mismanagement of this rare disease.

Patients with suspected biliary cystadenoma (BCA) are the only group addressed in this thesis that should always undergo surgical resection in our opinion. The main future challenge lies in distinguishing patients with complex septated cysts from those with BCA. CT, MRI and ultrasound combined provide a high sensitivity (87.5-100%) but a poor specificity (43.1-53.4%) for this differentiation (11). The same goes for contrast enhanced ultrasound (sensitivity 81.3-93.8% and specificity 47.1-88.2%) (12). By combining CT and MRI with CEUS, the specificity might be increased. Another approach to differentiate complex cysts from BCA relies on adequately describing the relationship of the septa to the cyst wall and measurement of intracystic tumor marker TAG-72 (13, 14). These results are promising and should be explored further in future studies. Just like HAML, patients with suspected BCA on imaging should be referred to a tertiary referral center.

Being rare diseases with a complex diagnostic work-up and possible complications after resection of benign neoplasms, mismanagement of these rare diseases should be prevented by presenting these cases to expert panels. As illustrated by data presented in this thesis on patients with suspected but not confirmed diagnosis of benign liver lesions according to standard criteria, hepatocellular adenoma, focal nodular hyperplasia, hepatic angiomyolipoma and biliary cystadenoma should be treated in centers where the expertise of liver surgeons, hepatologists, radiologists, and pathologists is combined. As all of the aforementioned benign liver tumors are rare, evidence is often limited to single center reports with small sample sizes. To optimize the validity and reliability of future studies, we have to convince all working in this field to collaborate in multicenter consortia and (inter)national collaborations to optimize our insights in diagnosis and treatment of these rare liver lesions.

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CHAPTER 16

English summary
Nederlandse samenvatting

English Summary

In this thesis, we focused on the management of hepatocellular adenoma, focal nodular hyperplasia, hepatic angiomyolipoma and biliary cystadenoma.

In **chapter 2** we performed a retrospective cohort study with the aim of evaluating whether a six month interval is sufficient to expect regression of hepatocellular adenoma (HCA) to less than 5cm. We included 194 patients diagnosed with HCA larger than 5cm and performed a time-to-event analysis. We found that 59% of HCA eventually regressed to less than 5cm after a median of 2 years. No complications were documented during follow-up. We concluded that the cut-off point for assessment of regression of HCA >5cm to ≤5cm could be prolonged to at least twelve months, irrespective of baseline diameter.

Subsequently, in **chapter 3** we sought to develop a model estimating the probability of HCA regression to <5cm at one and two years follow-up. We performed a multicenter retrospective cohort study including 180 patients. Cox proportional-hazards regression was used to develop a multivariable model with time to regression of HCA to <5cm as outcome. The strongest predictors for regression to <5cm were HCA diameter at diagnosis, the regression coefficient and HCA subtype. With this model, regression to <5cm can be predicted at one and two years follow-up.

Chapter 4 is a multicenter retrospective cohort study aiming to determine if liver regeneration after resection is associated with growth of residual HCA in the remnant liver. A total of 134 patients were included. Follow-up of residual HCA showed regression in 24.6%, stable HCA in 61.9%, growth in 11.2%. and recurrence in 2.2%. We suggested that growth of residual HCA is not uncommon but interventions are rarely needed. Surveillance is indicated when residual HCA show growth at the first follow-up after resection.

In **chapter 5** we report on the PALM study, a prospective study investigating the biological behavior and risk of complications in patients with HCA <5cm during pregnancy. Out of 66 patients who completed follow-up, 18 were excluded after because contrast enhanced MRI showed the lesion to be Focal Nodular Hyperplasia (FNH) and not HCA. The remaining 48 patients with confirmed HCA were followed during 51 pregnancies. Growth of HCA was seen in 25% of pregnancies. One patient with a HCA that showed significant growth to >70mm successfully underwent transarterial embolization at week 26 to prevent further growth. No complications were observed during the remaining 50 pregnancies. This study indicated in well-diagnosed patients having a HCA smaller than 5cm pregnancy seems to be safe bearing minimal risk for mother and non for the child.

In **chapter 6** we evaluated whether follow-up of HCA can be safely terminated after the occurrence of menopause. In this cross-sectional cohort study including 48 post-menopausal women with HCA, we found that HCA diameter becomes significantly smaller after the occurrence of menopause and as time progresses this regression increases**.** From these results we concluded that routine follow-up of HCA <5cm in post-menopausal women after subsequent follow-up is not required.

Chapter 7 is a single center retrospective study including all consecutive patients who were admitted for observation and/or intervention at our hospital because of with massive hemorrhage due to ruptured HCA between 1999-2016. Twenty-three patients were included, 4 patients suffered severe short-term complications and none experienced long-term complications. Tumor regression in non-surgically treated patients occurred with a median reduction of 76 mm down to 25 mm. The risk of rebleeding was 4.3%. We concluded that secondary (elective) treatment of HCA after massive hemorrhage may only be considered in patients with persistent HCA >5 cm.

In **chapter 8** we report on a multicenter retrospective cohort study assessing outcomes and post-embolization effects of selective transarterial embolization (TAE) in the management of HCA. A total of 59 patients were included, showing a significant size reduction of the HCA six months after TAE. In the cohort, 10.2% suffered major complication (i.e. cyst formation or sepsis), which could be resolved with minimal therapy. From this study we concluded that TAE is relatively safe and may lead to adequate size reduction of HCA.

Chapter 9 is a case report, presenting two patients who underwent resection of hepatocellular adenoma with unusual pathological findings that may impact clinical management.

In **chapter 10** we assessed the potential of point shear wave elastography (pSWE) to differentiate FNH from HCA. Unfortunately, a cut-off point for differentiation could not be determined and inter- and intra-observer reliability were insufficient. This study suggested that in its present form pSWE cannot reliably differentiate FNH from HCA.

Chapter 11 addresses the implications of growth of FNH for clinical management. We included 162 patients and found that growth occurred in 12%. 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. We concluded that significant growth of FNH should not have any implications on clinical management if confident diagnosis by imaging has been established by a tertiary benign liver multidisciplinary team.

Chapter 12 is a systematic review assessing the biological behavior, estimating the risk of hepatic angiomyolipoma (HAML) related mortality and recommending on a justifiable management strategy. We included 18 articles reporting on 292 patients. Recurrence after resection was described in 2.4% and the HAML related mortality rate was 0.8%. We concluded that in case of certain HAML diagnosis conservative management with annual imaging is justified. Resection should be considered in case of symptoms, inconclusive biopsy or growth in follow-up.

Chapter 13 continues assessing the management of HAML in a retrospective international multicenter study including only patients with pathologically proven HAML. A total of 38 patients was included. We found that the initial diagnosis was incorrect in 14 patients, of which 13 were thought to have malignancy. From this study we concluded that biopsy is indicated when HAML diagnosis is considered on cross-sectional imaging, especially when surgical resection imposes a risk of complications.

In the final chapter, **chapter 14**, we performed a systematic review assessing the diagnostic work-up and necessity of complete surgical resection of biliary cystadenomas (BCA) and cystadenocarcinomas (BCAC). Fifty-one articles with 1218 patients were included: 971 with BCA and 247 with BCAC. Recurrence was reported in 5.4% after resection for BCA and 4.8% after resection for BCAC. Recurrence after fenestration/marsupialization varied from 81.6%-100% for both BCA as BCAC. Mortality rate was 0 in patients with BCA and 24% in BCAC. We concluded that due to the difficulty in accurately diagnosing these biliary cystic lesions and the availability of different surgical approaches, patients with suspected BCA or BCAC should be treated in a center specialized in liver surgery with state-of-the-art imaging and all surgical techniques.

Nederlandse samenvatting

In dit proefschrift hebben we ons gericht op de behandeling van leveradenomen (HCA), focale nodulaire hyperplasie (FNH), angiomyolipomen van de lever (HAML) en cysteadenomen van de lever (BCA).

In **hoofdstuk 2** beschrijven we een retrospectieve cohort studie om te evalueren of een interval van zes maanden voldoende is om regressie van HCA tot minder dan 5cm te zien. Er zijn 194 patiënten geïncludeerd met een HCA >5cm waarop we een time-to-event analyse hebben uitgevoerd. Hieruit bleek dat 59% van de HCA uiteindelijk kleiner werd dan 5cm na gemiddeld 2 jaar. Er traden geen complicaties op tijdens de follow-up. Uit deze resultaten concludeerden we dat het afkappunt voor de beoordeling van regressie tot <5cm verlengd kan worden tot ten minste twaalf maanden, ongeacht de grootte van het HCA bij diagnose.

Hierop verdergaand hebben we in **hoofdstuk 3** geprobeerd een model te ontwikkelen dat de kans op regressie van HCA tot <5cm na één en twee jaar follow-up schat. Het was een multicenter retrospectieve cohort studie met 180 patiënten. Cox proportional hazards regressie werd gebruikt om een multivariabel model te ontwikkelen, met tijd tot regressie van HCA tot <5cm als uitkomst. De sterkste voorspellers waren de diameter van het HCA bij diagnose, de regressie coëfficiënt en het subtype. Met dit model kan regressie tot <5cm worden voorspeld na één en twee jaar follow-up.

Hoofdstuk 4 is tevens een multicenter studie, met als doel bepalen of, in patiënten met multipele HCA, regeneratie na een leverresectie geassocieerd is met groei van de rest HCA. In totaal werden 134 patiënten geïncludeerd. Follow-up van de rest HCA toonde regressie in 24.6%, stabiele HCA in 61.9%, groei in 11.2% en een recidief in 2.2%. Hieruit concludeerden we dat groei niet zeldzaam is maar dat interventies zelden nodig zijn. Geadviseerd werd patiënten onder controle te houden wanneer de rest HCA groei vertonen bij de eerste follow-up na resectie.

In **hoofdstuk 5** rapporteren we over de PALM studie, een prospectieve studie waarin we kijken naar het biologisch gedrag en risico op complicaties bij patiënten met een HCA <5cm tijdens de zwangerschap. Van de 66 initieel geïncludeerde patiënten werden er 18 geëxcludeerd omdat contrast MRI na de zwangerschap bevestigde dat ze een FNH hadden in plaats van HCA. De resterende 48 patiënten met bevestigde diagnose HCA werden gevold tijdens 51 zwangerschappen. Groei van het HCA werd gezien bij 25%. Een patiënt onderging een embolisatie van het HCA in week 26, vanwege groei tot >70mm. Er traden geen complicaties op gedurende de overige 50 zwangerschappen. Deze studie

toont aan dat zwangerschap veilig lijkt te zijn in patiënten met een definitieve diagnose HCA <5cm met een minimaal risico voor de moeder en geen risico voor het kind.

In **hoofdstuk 6** hebben we onderzocht of follow-up van HCA veilig beëindigd kan worden na de menopauze. In deze cross-sectionele studie met 48 postmenopauzale vrouwen zagen we dat de diameter van HCA significant kleiner wordt na de menopauze en dat naarmate de tijd vordert, deze afname toe lijkt te nemen. Uit deze resultaten concludeerden we dat routinematige follow-up van HCA <5cm bij postmenopauzale vrouwen niet nodig is, wanneer zij premenopauzaal in adequate follow-up zijn gehouden. **Hoofdstuk 7** is een retrospectief onderzoek waarin alle patiënten die tussen 1999 en 2016 zijn opgenomen in het Erasmus MC vanwege een massale bloeding als gevolg van een geruptureerd HCA werden geïncludeerd. Dit waren in totaal 23 patiënten, waarvan er 4 kortdurende complicaties ondervonden en er geen langdurige complicaties waren. Regressie bij niet-chirurgisch behandelede patiënten trad op met een gemiddelde afname van 76 tot 25mm. Het risico op een tweede bloeding was 4.3% bij een patiënte die de pil nog gebruikte. Geconcludeerd werd dat electieve behandeling van een HCA na een grote bloeding alleen overwogen hoeft te worden bij patiënten met een persisterend HCA >5cm.

In **hoofdstuk 8** rapporteren we over een multicenter retrospectieve studie waarin de resultaten en post-embolisatie effecten van selectieve transarteriële embolisatie (TAE) als behandeling van HCA wordt beoordeeld. In totaal werden er 59 patiënten geïncludeerd en werd er een aanzienlijke afname in grootte gezien zes maanden na TAE. Complicaties werden gezien in 10.2% en konden allemaal met minimale therapie op worden gelost. Hieruit concludeerden we dat TAE een relatief veilige optie is en dat het kan leiden tot afname van HCA.

Hoofdstuk 9 is een case report waarin we 2 patiënten beschrijven die een resectie van het HCA ondergingen en bij wie pathologisch onderzoek ongebruikelijke bevindingen liet zien.

In **hoofdstuk 10** hebben we gekeken naar de betrouwbaarheid van point shear wave elastography (pSWE) om FNH en HCA van elkaar te onderscheiden. Helaas was de inter- en intraobserver betrouwbaarheid onvoldoende en kon er geen afkappunt voor differentiatie tussen FNH en HCA gevonden worden. Dit suggereert dat pSWE in de huidige vorm onvoldoende onderscheid kan maken tussen FNH en HCA.

Hoofdstuk 11 gaat in op groeiende FNH en wat de implicaties voor het beleid zouden kunnen zijn. Er werden 162 patiënten geïncludeerd en groei trad op bij 12%. Bij 4/19 groeiende FNHs is een resectie uitgevoerd waarbij histologisch onderzoek FNH in

alle gevallen bevestigde. Er zijn geen complicaties ontstaan gedurende follow-up. We concludeerden dat groei van FNH geen implicaties moet hebben voor het beleid wanneer er sprake is van een zekere diagnose op imaging en die is bevestigd door een multidisciplinair team.

Hoofdstuk 12 is een systematic review die ingaat op het biologisch gedrag en kijkt naar de mortaliteit geassocieerd met angiomyolipomen in de lever (HAML). In de review zijn 18 artikelen opgenomen met in totaal 292 patiënten. Recidief na resectie van HAML werd beschreven in 2.4% van de patiënten en de aan HAML geassocieerde mortaliteit bedroeg 0.8%. Hieruit werd geconcludeerd dat in het geval van een zekere HAML diagnose, conservatief beleid met jaarlijkse beeldvorming gerechtvaardigd is. Resectie kan overwogen worden in het geval van symptomen, twijfel over de diagnose of bij groei.

In **hoofdstuk 13** gaan we verder over het beleid van HAML in een retrospectieve, internationale multicenter studie. Er werden 38 patiënten geïncludeerd, allen met een histologisch bewezen HAML. De initiële diagnose was onjuist bij 14 patiënten, waarvan er 13 werden verondersteld een maligniteit te hebben. Uit deze studie concludeerden we dat een diagnostische biopsie uitgevoerd moet worden wanneer de diagnose HAML wordt overwogen op beeldvorming, met name wanneer chirurgische resectie een risico op complicaties met zich meebrengt.

In het laatste hoofdstuk, **hoofdstuk 14**, beschrijven we een systematic review die gaat over de diagnostiek en noodzaak van complete chirurgische resectie van biliaire cysteadenomen (BCA) en cysteadenocarcinomen (BCAC). Er werden 51 artikelen met in totaal 1218 patiënten geïncludeerd, waarvan 971 met BCA en 247 met BCAC. Recidief werd gerapporteerd in 5.4% na resectie voor BCA en 4.8% voor BCAC. Na fenestratie of marsupialisatie varieerde het recidief van 81.6% tot 100%, voor zowel BCA als BCAC. Mortaliteit was 0 bij patiënten met BCA en 24% bij BCAC. Geconcludeerd werd deze cysteuze laesies moeilijk te differentiëren zijn en dat het de voorkeur biedt patiënten met een verdenking op BCA of BCAC te behandelen in een centrum gespecialiseerd in leverchirurgie.

Appendices

List of publications
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List of publications

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PhD portfolio

PhD student:	Anne Julia Klompenhouwer
PhD period:	2016 - 2019
Erasmus MC departments:	Surgery and Gastroenterology & Hepatology
Supervisors:	Prof. dr. J.N.M. IJzermans and prof. dr. R.A. de Man

PhD Training	Year	Workload (ECTS)
Courses		
Biomedical English Writing Course, MolMed	2017	2.0
Workshop Presenting Skills for junior researchers, MolMed	2017	1.0
BROK (Basiscursus Regelgeving Klinisch Onderzoek), NFU	2016	1.0
Research Integrity, Erasmus MC	2016	0.3
Basic Introduction Course on SPSS, MolMed	2016	1.0
Survival Analysis Course, MolMed	2016	0.5
OpenClinica, Erasmus MC	2016	0.1
Graphpad Prism, MolMed	2016	0.3
Scientific presentations		
The Liver meeting, AASLD	2016, 2018	2.0
The International Liver Congress, EASL	2017, 2018	2.0
Annual meeting of AHPBA	2017	2.0
Erasmus Liver Day	2017	2.0
NVGE Digestive Disease Days	2016, 2018	4.0
NVvH Chirurgendagen	2016, 2017	2.0
First Liver MRI Workshop	2018	2.0
Wetenschapsdag Heelkunde Erasmus MC	2017	2.0
NLV Jubileiumcongres	2016	2.0
Attendance at (inter)national conferences and seminars		
Symposium "Current and Future Perspectives in Primary Liver Tumors"	2017	1.0
Erasmus Liver day	2015-2018	3.0
NVGE Najaarsvergadering	2015	1.0
Teaching		
Coaching bachelor students	2016-2018	1.0
Examination Basic Life Support	2016-2019	1.0
Supervision master thesis	2018	2.0
Supervision systematic review elective	2018	1.0
Total		**36.2**

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About the author

Anne Julia Klompenhouwer was born in Horst, The Netherlands on the 5th of July, 1991. After graduating from secondary school in 2009 (Atheneum, Dendron College, Horst), she commenced medical school at Maastricht University. During her clinical rotations she did an elective in gastroenterology at the MUMC (Maastricht) and senior internship gastroenterology and internal medicine at the Catharina Ziekenhuis (Eindhoven). The first steps towards this thesis were made during her research rotation at the department of gastroenterology and hepatology of the Erasmus MC, Rotterdam (supervisors prof. dr. R.A. de Man and dr. D. Sprengers). After obtaining her medical degree in January of 2016, she started her PhD project at the department of Surgery (Erasmus MC, supervisors prof. dr. J.N.M. IJzermans and prof. dr. R.A. de Man), ultimately resulting in this thesis. During her PhD period, she obtained the Master of Health Sciences Degree, specialization Clinical Epidemiology (NIHES, Erasmus University, Rotterdam) and was active as a board member of the Arts Assistenten Vereniging (AAV). From May 2019 onwards she started her residency training in gastroenterology and hepatology at the IJsselland Ziekenhuis (Cappelle a/d IJssel) and Erasmus MC (Rotterdam).