Contrast-enhanced ultrasound in focal liver lesions

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The Studies described in this thesis were performed at the Department of Gastroenterology & Hepatology and the Department of Surgery of the Erasmus University Medical Center, Rotterdam, the Netherlands.

ISBN: 978-94-6361-495-5
Cover design: Optima Grafische Communicatie, Rotterdam, the Netherlands
Layout and printing: Optima Grafische Communicatie, Roterdam, the Netherlands

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Contrastversterkte echografie van focale lever laesies

Proefschrift

ter verkrijging van de graad van doctor aan de
Erasmus Universiteit Rotterdam
op gezag van de rector magnificus
Prof. dr. R.C.M.E. Engels
en volgens besluit van het College voor Promoties.
De openbare verdediging zal plaatsvinden op
vrijdag 11 december 2020 om 9:30 uur

door

Pavel Taimr
Geboren te Praag, Tsjechie

Erasmus University Rotterdam

Promotiecommissie:

Promotoren:	Prof.dr. R.A. de Man
	Prof.dr. J.N.M. IJzermans
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Aan Renata, Anna en Martin

CHAPTER 1

General introduction and aims of the thesis

GENERAL INTRODUCTION AND AIMS OF THE THESIS

The incidence of focal liver lesion is increasing. It is probably due to widespread availability of modern radiological imaging. Ultrasound, CT or MRI can detect some liver lesion in up to 1/3 of examinations in general population [1]. The majority of these incidentally detected lesions are benign, but malignancy should be always ruled out. As management and prognosis varies greatly, correct diagnosis is important. The most common benign liver lesions are simple cysts, focal steatosis, haemangiomas, focal nodular hyperplasia (FNH) and hepatocellular adenoma (HCA). Some are completely innocent and their correct and proper diagnosis is the most important step in clinical work-up. Others can have a different course over time. They can be complicated by bleeding or rupture. Some have even potential to undergo malignant transformation over period of years. Thus, in clinical practice, discrimination inside benign tumors group is important, but discrimination between benign and malignant is of utmost importance.

Focal nodular hyperplasia

FNH is a tumor-like malformation consisting of hyperplastic nodules of hepatocytes, separated by fibrous septa, which form a typical central scar [2]. The liver is otherwise morphologically and histologically normal. FNH can be defined as not truly a tumor but a local regenerative mass of normal polyclonal hepatocytes. The prominent central artery lies close to the central scar. The lesion is often found incidentally during an imaging session for unrelated complaints. It may be detected more frequently in women and in 15% to 30% of cases, FNH is multiple [3]. FNH is a benign lesion with no malignant transformation course, the chance of bleeding is very low. If properly diagnosed, specific treatment is very rarely indicated.

Hepatocellular adenoma

Hepatocellular adenoma is a benign neoplasm that originates in a normal liver and is composed of cells that closely resemble normal hepatocytes [2]. Adenomas typically develop in women during the reproductive age, and are almost always associated with oral contraceptive use [4]. They have been also reported in patients with an inherited glycogen storage disease and maturity-onset diabetes of the young, and some are obesity-related. Approximately 33%–80% of patients are asymptomatic with tumors discovered by a chance on imaging [4-5].

Recently a new molecular classification has been established, based on metabolic pathways [6-7]. There are currently four suggested subtypes of liver adenomas: 1) Hepatocycte nuclear factor 1 (HNF1-α)-inactivated adenomas (25%–40%, diffuse intratumoral steatosis), 2) β-catenin mutated (5%–10%), 3) teleangiectatic/inflammatory

(45%–55%, former teleangiectatic focal nodular hyperplasia [FNH]), and 4) unclassified (5%–10%). MRI is the best imaging modality for characterizing HCA, and efforts based on MRI imaging are being made to subclassify them in accordance with the above-mentioned metabolic subtypes [8].

Adenoma may require more invasive treatment. This profoundly differs in relation to FNH and in some cases, a correct differential diagnosis can be challenging against FNH. Adenomas can be complicated by spontaneous rupture, bleeding and even malignant transformation (into hepatocellular carcinoma). Therefore HCA diagnosis is highly important. The most important factor for treatment is size of the tumor. Complications very rarely occurs in HCA smaller than 5 cm. The first step in the treatment of HCA consists of weight reduction (in obese patients) and cessation of oral contraception, with reevaluation after 6 months. Surgical treatment is recommended in all patients with β-catenin mutated variety and all males.

Benign versus malignant focal liver lesions

Contrast-enhanced ultrasound is highly capable of differentiating between benign and malignant liver lesions. The level of accuracy is the same for lesions that are < 2 cm and those that are > 2 cm. CE-US shows sensitivity and specificity above 85%, positive and negative predictive values (PPV and NPV) of 92% and 77%, respectively, and accuracy of 86% [9-10]. The detection of hypoenhancement (wash-out) during the portal or late phase is strongly suggestive of a malignancy in which >90% of malignant lesions show this feature [11-12]. The presence or absence of preexisting diffuse chronic liver disease is an important factor in interpreting CE-US results. In patients with liver cirrhosis, all solid lesions should be regarded as potentially malignant, unless proven otherwise.

A comprehensive review from the National Institute for Health research of the UK strongly stated: “A potential advantage of using CEUS would be the option of completing the assessment at the same time as the initial unenhanced ultrasound…. to provide more rapid diagnosis without repeat hospital visits is likely to be preferred by patients and may also reduce costs.“ [13]

Aims of this thesis

The overall aim of this thesis was to address various aspects of diagnostic contrast-enhanced US and its diagnostic capabilities in relation to benign and metastatic liver tumors, to other radiological methods, its cost-effectivity and various topics related to its use.

The basic questions related to CE-US diagnostics are: “Is the lesion in the liver benign or malignant?” and “What kind of benign (malignant) lesion is it?” [14]. Position of liver CE-US in imaging algorithms is not precisely stated yet. It is positioned

somewhere between quick, generally available, cheap but unspecific and less sensitive B-mode ultrasound and highly specific, costly and academic contrast- enhanced MRI. Questions arise. What is the position of CE-US in relation to other radiological methods (MRI, CT)? Where does it stand in solid benign liver lesions diagnostics and where in ruling out malignancies? Can we make proper diagnosis immediately during first examination or should we use some more specific method later? What CE-US features should we look for and what is their value in relation to others? Can we improve CE-US results by adding another modern imaging technology, such as stiffness measurements? Is CE-US in focal liver lesions diagnostics cost-effective? Currently there is no widespread consensus regarding the optimal management of CEUS and its position in focal liver lesion diagnostics.

In **chapter 2** (*The state of contrast-enhanced ultrasound imaging in benign focal liver lesions: Where are we now?*) we outline current concepts and status of CE-US and its development. The aim of this chapter is to demonstrate the characteristic features, and CE-US diagnostic position in focal liver lesion diagnostics. This chapter is also a brief review of the literature related to the topic. CE-US can substantially help in a differential diagnostic workup.

Continuing along this theme, in **chapter 3** (*Performance of contrast-enhanced sonography versus MRI with liver-specific contrast agent for diagnosis of hepatocellular adenoma and focal nodular hyperplasia*), we evaluate the position of CE-US in relation to the golden diagnostic standard (MRI with liver-specific contrast agent) for diagnosis of the two most important benign liver lesions - focal nodular hyperplasia and hepatocellular adenoma – in a large cohort of patients.

Chapter 4 (*Can point shear wave elastography differentiate focal nodular hyperplasia from hepatocellular adenoma?*). Recently, a new technique for quantifying local stiffness has emerged. Shear wave elastography technology can provide additional information from tumors, and FNH is a natural candidate for exploration. In some reports, it has been shown that FNH has higher stiffness compared to other focal liver lesions [15]. Whether focal elastography can be a complementary tool to CEUS or not still waits further validation [16].

This chapter focuses on the question as to whether point shear wave elastography can differentiate focal nodular hyperplasia from hepatocellular adenoma. When the diagnosis remains uncertain, these patients may undergo a tumor biopsy. In order to avoid a percutaneous biopsy and the associated risk of complications or even the surgical resection of a suspect lesion that turns out to be FNH, improvement of the diagnostic process is needed.

In **Chapter 5 (***A model-based prediction of the probability of hepatocellular adenoma and focal nodular hyperplasia based on characteristics on contrast-enhanced ultrasound*), we describe the development of a model-based prediction for the probability of hepato-

cellular adenoma and focal nodular hyperplasia based on CE-US characteristics. The main objective was to determine how frequently the specific features of HCA and FNH are displayed on CEUS. We also sought to define the predictive value of features for the discrimination between HCA and FNH on CEUS, and a specific formula for adenoma prediction is the result

Chapter 6 (*Liver contrast-enhanced ultrasound improves detection of liver metastases in patients with pancreatic cancer*) goes on to malignant liver metastases of pancreas adenocarcinoma as such lesions type is important in differential diagnosis of liver focal lesions. Thus far no study has evaluated the performance of CE-US compared to CT scan, in assessing liver metastases of patients with suspected pancreatic adenocarcinoma, this study is the first one ever performed. Should we use CE-US in a pre-operative work-up in patients with a known extrahepatic malignant tumor?

In **chapter 7** (*Clinical and economic evaluation*) we evaluates economic aspects of CE-US examination in a patient with an already diagnosed focal liver abnormalities. The main advantage of our study consists of adding a treatment phase to diagnostic one, and we ask whether CE-US has a role in cost-effectiveness?

Chapter *8 (Inflammatory and multiple hepatocellular adenoma are associated with higher BMI)* describes different subtypes of hepatocellular adenomas in relation to other clinical aspect, such as higher body mass index (BMI) and multiple and inflammatory varieties. We investigated whether the presentation of single or multiple adenomas may lead to different management strategies.

Finally, in **chapters 9 and 10** we summarize results and continue to a conclusion for this thesis.

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CHAPTER 2

The state of contrast-enhanced ultrasound imaging in benign focal liver lesions: Where are we now?

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Submitted 2020

ABSTRACT

The importance of non-invasive liver imaging is increasing as the epidemic of obesity and liver cancer is becoming a world-wide medical and sociological question. Also, we encounter an increasing number of focal liver lesions due to the widespread availability of conventional ultrasound. The absolute majority of focal lesions are benign in nature and are discovered incidentally. Contrast-enhanced ultrasound (CE-US) can substantially help in a differential diagnostic workup. It has an advantageous cost/benefit ratio, dynamic real-time description of liver perfusion, no radiation exposure and can be executed on most of the US devices. The aim of this narrative article is to demonstrate the characteristic features of benign liver tumors and to show various clinical applications of CEUS.

GENERAL INTRODUCTION

Contrast-enhanced ultrasound of the liver (CE-US) is a diagnostic imaging diagnostic method using a specific contrast agent during US examination [1]. It has been used in several countries for more than a decade, but is still awaiting for more general acceptance in clinical practice. All clinically approved contrast agents (UCA) are microbubbles.

The acronym CE-US refers to contrast-enhanced techniques in general and DCE-US (dynamic contrast enhanced ultrasound) refers to the quantitative time intensity curve (TIC) analysis [2].

Other liver-related imaging techniques, such as contrast-enhanced magnetic resonance imaging (CE-MRI) and contrast-enhanced computed tomography (CECT) are more expensive and include radiation exposure (CT), higher risk of allergies, and have a limited use in patients with renal insuficiency. An absence of real-time imaging in CT / MRI is also a limiting feature. Some MRI contrast agents can initiate a serious generalized fibrotic reaction in at-risk patients.

Conventional US imaging, including Doppler techniques, has some limitations: for instance, it cannnot visualize the liver parenchyma microcirculation [3]. CE-US tries to overcome those limitations, although the spread of CE-US knowledge is rather slow. After many years it was finally approved by the Food and Drug Administration (FDA) in the United States [4], but it is still not generally used in daily routine, as it should be [5]. CEUS has also spread outside its sole use in echocardiology and liver, and the number of CE-US examinations in kidney, prostate, or other GI areas is increasing.

Nowadays, there is widespread availability and use of liver imaging, mainly abdominal ultrasound B-mode and Doppler. The main application of contrast agents (outside cardiology) is for focal liver lesions. There are only few available studies and data on the frequency of focal liver lesions in the general population [6-7]. Focal liver lesions are usually detected incidentally, and about 30% of the general population has one or more benign focal lesion [8-9]. In fact, the majority of all liver lesions found by ultrasoundare benign [10-11]. Most of the incidentally found lesions on US are small simple cysts, hemangiomas, regenerative nodules, focal fatty sparing, and/or focal steatosis. The specificity for a precise lesion diagnosis by conventional US is not very high [10]. Too often, even color Doppler US has limitations in accuracy, sensitivity, and specificity. Benign and malignant lesions can have a very similar appearence. In a case of a typical oval homogenous hyperechoic hemangioma we can probably make a final diagnosis, but in less typical cases US has a limited applicability. In the presence of an underlying extrahepatic malignant disease or in liver cirrhosis, the capability of conventional US to be used to make a diagnosis is even more restricted. In these cases, CE-US can offer improved characterization of liver

lesions [12]. In addition it can also improve the detection of otherwise undetectable lesion (-s) in the liver as is the case of liver metastasis of extrahepatic tumors [13].

In general, there are three main populations for CEUS [10]:
1) general population, mostly healthy persons
2) patients with known oncologic disease, and
3) patients with a diffuse liver disease, including liver cirrhosis.

A typical healthy incidental patient might be a young woman suffering from a common urinary tract infection. Because of infection recurrence, her general practitioner asks for renal and bladder US to be performed. However, an incidental single hyperechogenic oval lesion was found in the liver and requires further diagnostic work-up. The patient receives another appointment, now for a CT or MRI scan in a local hospital within the following 2-3 weeks. These are anxious weeks for the patient, because of unexcluded malignancy. The patient takes a half or whole day work leave, and the technician will perform a CT scan. Usually within another 2–3 weeks, and during another appointment with general practitioner or specialist, the patient will be finally informed about the diagnosis: a benign hemangioma in the liver. An obvious CE-US advantage should the opportunity to perfom contrast examination during the very first US session. The same diagnosis could have been done immediately by performing CE-US. Using the method produces less stress and less expense for the patient and insurance while also no radiation is used in comparison to a CT.

In patients with known oncological diseases, CEUS can achieve comparable results in liver metastases screening to CE-CT or CE-MRI [13-14].

In patients with diffuse liver diseases, the role of CE-US in hepatocellular carcinoma screening in cirrhotic patients and its cost-effectiveness potential still awaits determination [15]. The role of CEUS in characterization of an indeterminate liver lesion on CT or MRI also awaits for a better determination [16]. The role of CEUS in malignant hepatocellular and cholangiocarcinoma is not discussed in this paper.

Initially, a set of guidelines for liver CE-US examination was organized by the European Federation of Societies for Ultrasound in Medicine and Biology (EFSUMB) and published in Ultraschall in der Medizin in 2004 [17]. A second and updated edition was published in 2008, reflecting the changes in available contrast agents [3]. The latest document was published in 2013, as a joint update with the World Federation for Ultrasound in Medicine and Biology (WFUMB) [18-21].

Dynamics of CE-US

CE-US allows real-time evaluation of contrast agent wash-in and wash-out in a given region of interest (ROI) over a period of several minutes [22]. All approved contrast

agents are microbubbles, which are several μm in diameter large, and they circulate freely in the capillary network. The microbubbles are gas-filled (sulhur hexafluoride) and stabilized by a shell membrane based on phospholipids and albumin. The contrast agent is a pure blood pool agent [17]. It provides a dynamic visualization of different vascular phases in which the liver has a specific advantage thanks to a dual blood supply and arterial (via hepatic artery) and portal (via portal vein) phases. One can thus diferentiate between three different phases: 1) arterial (AP), 2) portal (PVP), and 3) late (LP). The arterial phase starts within 10 to 20 seconds, lasts approximately 35–40 seconds and is characterized by liver arterial filling. The portal phase starts after 30 to 40 seconds. The time delay and shift are caused by filling of other intraabdominal organs and later entrance into the liver via portal vein. The portal phase lasts for approximately two minutes post-injection. Contrast bubbles fill the liver sinusoids (late phase after two minutes) and during the remaining observation time, the contrast agent is progressively cleared. The recommended observational time is around five minutes, after which the signals start to rapidly deteriorate.

Only US machines with so called nonlinear imaging modes with low mechanical index (MI) for CE-US can be used. Microbubbles act as resonant scatterers, for example, they increase US signal and produce dynamic echoes. Microbubbles resist compression better than expansion. During insonation at a low MI, the expansion and compression are not equal anymore and the returning signal results in the generation of fine non-linear signals, which can be visualized. Under higher energy levels, such as levels used in standard B-scan US, a bubble disruption occurs. In the case of disruption, the result is a strong but short transient signal. This technique was used years ago with the first contrast agents (Levovist). Specific contrast software in US devices suppresses linear ultrasound signals from adjacent tissues and visualizes mainly non-linear signals originating from microbubbles. The result is a positive dynamic signal only from areas filled by contrast agents [22].

Two main diagnostic features to characterise focal liver lesions are in use: 1) vascular architecture and 2) changes in contrast enhancement of the lesion compared to surrounding liver tissue. Vascular architecture is visualized during the early wash-in phase, and lesion changes comparedto adjacent tissues can be viewed during the entire course (wash-in, wash-out) [23].

Some new generation contrast agents (perfluorobutane, Sonazoid) are phagocytosed by the Kupffer cells in the liver. Those cells are residential macrophages in the liver sinusoids. The intracellular phase (post-vascular phase) can further improve CE-US diagnostics [24]. The contrast bubble is not a purely intravascular agent anymore. Malignant tumors show contrast defects in the post-vascular phase, while benign tumors display iso-enhancement in the postvascular phase. However, in benign lesions a region of contrast defect is rarely observed due to thrombosis and necrosis [25].

After disruption, contrast agent microbubbles are eliminated mostly by the liver (phosphilipids from shell membrane), and the inert gas is exhaled via the lungs in approximately 10 to 15 minutes [26].

SAFETY

Before performing CE-US, there is no need to plan any laboratory tests (liver or kidney function). Contrast agents are very safe with no hepatotoxic, thyrotoxic, cardiotoxic, or nephrotoxic side effects. In an Italian series of more than 23,000 abdominal patients, anaphylactic reactions, which can be very serious, have been reported with a rate of 0.001% with no deaths [27-29]. The incidence of severe hypersensitivity reaction is lower than with CT contrast agents. CE-US contrast agents can be used in dialysis patients or patients with impaired renal function without risk of contrast agent accumulation. There is no risk of severe fibrotic reaction as is seen with some gadolinium-based MRI agents.

TECHNIQUE, PROTOCOL AND LIMITATION

Firstly, conventional US of the whole liver should be performed [22]. According to the standard protocol, all eight liver segments should be visualized in which focal liver lesions should be searched. Focal liver lesions must be characterized on a B-scan, and their structure, echogenity, relation to surrounding liver structures, and other characteristics should be described according to standard protocol. Color doppler, including tissue harmonic imaging, is part of a liver examination.

Secondly, a sufficiently large needle is placed in the antecubital vein while patient is in the supine position. The best position for the patient should be determined during a pre-contrast examination. A large needle (20 gauge or larger) is important because microbubbles are sensitive to mechanical stress and can be easily destroyed during injection. Also, higher injection pressure during passage should be avoided. A three-way stopcock is preferred because in multiple injections it facilitates saline flushes without syringe manipulation. To avoid bubble destruction by insonation, we can use only a low MI mode on the ultrasound machine, typicaly lower than 0.3.

Generally, 2.4 ml of contrast agent in a bolus injection is administerd in 1 to 2 seconds followed by 5 to 10 ml of saline flush at about 2 ml/sec . The most widely used US contrast agent in Europe is Sonovue (sulfur hexafluoride, produced by Bracco).

Some limitations of CE-US exist. If the general abdominal US is limited because of anatomical conditions (bowel gas, obesity) examination by CE-US will also be insufficient. The scanning conditions and resolution limitations mean that the smallest lesion diameter lies betweeen 3 and 5 mm [30]. A very small focal lesion can therefore be overlooked. There are also areas of the liver, such as SVIII subdiaphragmatical, that are less accessible to conventional ultrasound. Another limitation originates

from attenuated signal penetration and scattering, especially in liver steatosis. Scanning conditions in fibrotic or cirrhotic livers are even less reliable. Both superficial and deeper lesions do not appear well in the picture and indicate that the outcome of CE-US is dependent on both the patient's general condition in addition to the operator's condition. On the other hand, a significant advantage is the opportunity to scan enhancement patterns in real time, with much higher temporal resolution than is possible with CT or MRI

BENIGN versus MALIGNANT LIVER FOCAL LESIONS

CE-US is highly capable of differentiating between benign and malignant liver lesions. The level of accuracy is the same for lesions that are < 2 cm and those that are > 2 cm. CE-US shows sensitivity of 85.75%, specificity of 85.9%, positive and negative predictive values (PPV and NPV, respectively) of 91.6% and 77.1%, respectively, and accuracy of 85.8% [31-32]. The detection of hypoenhancement during the portal or late phase is strongly suggestive of a malignancy in which 91% of malignant lesions show this feature, whereas only 37% of benign lesions show the feature [33].

The presence or absence of preexisting diffuse chronic liver disease is an important factor in while interpreting CE-US results. In patients with liver cirrhosis, all solid lesions should be regarded as potentially malignant, unless proven otherwise. The presence of wash-out in a patient without underlying chronic liver disease is highly specific for malignancy [34].

CYSTIC LIVER LESIONS

Focal cystic liver lesions represent a broad group of benign or malignant lesions. The most common are simple cysts, other benign are infected or hemorrhagic hepatic cysts, abcesses, hematoma, biloma, biliary hamartomas, biliary cystadenomas and many other rare diseases [35]. Malignant cystic liver lesions include biliary cystadenocarcinoma, cystic metastatic disease – typically neuroendocrine, renal, ovary and others [36].

SIMPLE CYSTS

Simple liver cysts are common and usually found incidentally on B-scan US due to US availability and frequent use of hepatic imaging. Generally, they are of no clinical significance [37].

Hepatic cysts can be solitary or multiple. They contain clear fluid and are lined by a single layer of epithelial cells and a thin rim of fibrous stroma. Simple cysts are oval or rounded and completely anechoic with a nearly imperceptible wall. Refraction shadows at the edges indicate typical dorsal US enhancement. They are nonehnancing in all phases of contrast CE-US [38].

Screening liver CT in patients with known extrahepatic malignancy sometimes has difficulties distinguishing simple benign cysts from possible small liver metastasis and too often need confirmation by another imaging method, such as CE-US [13].

COMPLEX CYSTS

Complex cysts are more difficult to characterize, but with a correct diagnosis, malignant versus benign can be diagnosed in 95% of these cases [38], [39]. Malignant tumors have an inclination to show visible washout with hypoechogenic appearence. Benign lesions do not show any enhancement in all three filling phases, and enhancement in portal and/or late phases is sustained.

Biliary hamartoma (von Meyenburg complex) are symptomless and benign cystic malformations of liver parenchyma. They consist of bile ductules in fibrotic stroma. The continuous improvement of liver US technology has made it possible to visualize these structures more often, and the majority are discovered incidentally [40]. On B-scan US, they present small, hyperechoic, sharply delineated lesions without accoustis shadows [41] that are often on the periphery of microcystic or macrocystic liver lesions, sometimes with a hyperechogenic comet tail artifact. On CE-US they behave similarly to other benign lesions in the filling-in parenchymal in addition to the portal phase. Cystic structures show no echo at all.

Infected or hemorrhagic cysts appear as hypoechoic lesions with a lack of internal vascularity [42]. On CE-US, infectious or fluid internal area is not filled-in, and inflammed walls can show some enhancement but no washout as is seen in a malignancy. Other features are variable and include thick fibrotic septations, internal septa, and irregular walls [43].

Cystadenoma and cystadenocarcinoma are solitary complex cysts with thick fibrotic capsules, which are irregular, sometimes even nodular in appearence, with internal septations. Doppler flow might be conspicuous in internal septations [44]. Older literature studies state that CE-US was not helpful in diferentiating between cystadenoma and cystadenocarcinoma [39], [45]. However, in a recent paper, hyperechoic enhancement in the cystic wall, septations or mural nodules in the arterial phase with isoenhancement in portal and late phase was more typical for cystadenoma, and hypoechoic pattern in portal and late was more common in cystadenocarcinoma; thus, the usefulness of CE-US features appears to be the capability to distinguish between these lesions [46].

Echinoccocus cysts (human alveolar echinococcosis), a cestode parasite, are frequently evaluated by US to monitor disease activity. The conditon was only rarely checked by CE-US in the past. In one study, a vascularization pattern was visualized by CE-US in approximately 50% of the cases when compared results from positron

emission tomography (PET)-CT; thus there might be some relationship of with parasitic disease activity and detection on US [47].

ABSCESS AND GRANULOMAS

Proper and correct diagnosis of liver abcsesses is highly important in medical practice. Clinical circumstances often increase vigilance and suspicion with respect to diagnosing abscesses in the liver, including patients with inflammatory conditions, such as appendicitis, cholangitis, and diverticulitis. In the B-mode, abscesses can present a very variable picture because liquefaction and loculation can profoundly change abscess appearances [43]. Sometimes, abscesses contain gas echoes. Margins can be sharp but also irregular or faint. Abscesses can appear heterogenous, homogenous, fluid-filled, or even solid-like. Thick irregular walls with color Doppler flow are sometimes seen. There is rim enhancement in the arterial and portal phases and a tendency toward hypoenhancement in the late phase. This phenomena probably reflects an increase in the blood flow of the surrounding liver tissue. On CE-US, fluid or central debris is always non-enhancing in all three phases, and this phenomenon differs from malignant lesions, which show at least partial filling.

Granulomas can be isoenhancing or hyperenhancing in the first (arterial) phase with washout (later second and third phase) and thus, they blur the booundaries between malignant and benign lesions characteristics [48].

SOLID FOCAL LIVER LESIONS

FOCAL FATTY SPARING AND FOCAL STEATOSIS

Fatty liver refers to various conditions with fat accumulation in hepatocytes. Most fatty liver (steatosis) diffusely involves the whole liver, and occasionally, we encounter local fat deposits [49].

Focal fatty-sparing (focal non-steatosis) is a hypoechoic pseudolesion that is quite common and often located in segment IV close to portal vein bifurcation or gallbladder bed, medial segment of the left lobe adjacent to lig. falciforme, anterior portion of segment I, or posterior portion of segment IV. In comparison with hyperechoic liver parenchyma (picture of liver steatosis), these lesions appear dark [50], [51].

On the other hand, focal steatosis is a hyperechogenic pseudolesion and sometimes presents as a multilobular, mass-like pattern in normal echogenic liver parenchyma. Patients with this pseudolesion often have potential, comorbid clinical conditions, such diabetes mellitus, alcohol abuse, or other metabolic diseases [52]. A focal steatosis does not produce compression of parenchyma (no mass-efect) as it is not a real tumor (fat droplets are intracellular).

On CE-US, the contrast agent dynamic behavior looks the same in all dynamic phases as in other liver parenchyma since there is normal portal and arterial vascularization in the pseudolesion as in the surrounding structures.

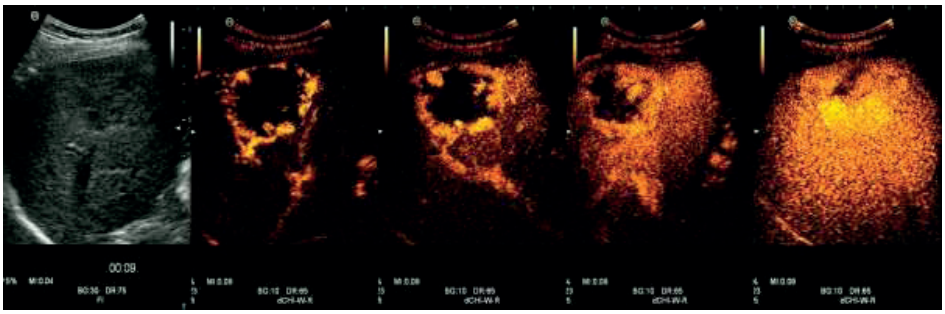
HEMANGIOMAS

Small benign vascular lesions are common in various organs of the body, including the liver. The most common solid benign liver lesions are hemangiomas, and their reported incidence depends on how vigorously they are sought. Their incidence is between < 1% and 20% as incidental hepatic hemangioma when an autopsy is performed [53-54]. The majority of them are small (< 4 cm), asymptomatic and found incidentally. CE-US has improved the accurate diagnosis of hemangiomas, which is now approximately 95% [24], [55].

Typically, hemangioma appearence on B-mode ultrasonography is an oval, round hyperechogenic and homogenous lesion, with well-defined margins, rarely with a small feeding artery and posterior acoustic enhancement. Some are with minimal posterior acoustic enhancement [56]. Doppler flow is too slow to be detected inside the lesion, so Doppler imaging is not useful. In the hyperechogenic surounding parenchyma they can appear either iso- or hypoechogenic.

The typical CE-US pattern is a flame-like or globular enhancement in the arterial phase (PICTURE 1). Contrast agent progressively continues to fill centripetally, and the fill-in is partial or complete, depending on the presence of the thrombus inside the lesion. The filling lasts seconds to minutes, small hemangiomas fill faster.

In a hemangioma during the late phase, there is a typical enhancement, which is sustained for minutes. In some hemangiomas, the filling in the portal or late phase is not complete.



Picture 1. Hemangioma. Baseline ultrasound and CEUS evaluation (a). At B-mode it is not possible to distinct lesion type due to heterogenity of tumor. After contrast administration, slow filling between 15 sec (a) to 1 minute later (e) shows flame-like, centripetal fill-in with complete filling in late phase.

This type of filling occurs mainly in large hemangiomas and probably represents intralesional thrombosis or fibrosis (always avascular). If there is sustained or even progressive enhancement of the nonthrombosed borders during portal or late phases, the lesions are usually of benign in nature. And a hypoechogenic late phase never occurs, and this feature is also a typical trait for benign lesions.

Occasionally, we encounter atypical hemangiomas in which the filling is very rapid and complete, especially when some ateriovenous (AV) shunts are present. Color Doppler is conspicuously strong and usually at the borders of lesion with a strong arterial signal. These types of hemangiomas are the so called high-flow or shunt hemangiomas.

Sclerosed hemangiomas are rare and are characterized by fibrosis and hyalinization resulting from the degeneration of cavernous hemangioma [57]. A filling defect may mimick intrahepatic cholangiocarcinoma in differential diagnosis.

Another atypical feature of this type of hemangioma includes calcifications within the hemangioma.

Sometimes lesions that are isoechogenic in the arterial and portal phases become progressively hyperechogenic compared to the surrounding parenchyma in the late phase [58].

In the differential diagnosis, hypervascular metastasis occurs but usually in this case, the fill-in is very short, and the late phase is completely different (wash-out, hypoenhancement). Another differential is hepatocellular carcinoma (HCC) in which we should also check the portal or late phase (wash-out [59-60]). Sometimes we should wait for 4 to 5 minutes for wash-out to occur in HCC or maybe even longer (so called “late washers“).

HEPATOCELLULAR ADENOMA (HCA)

Hepatocellular adenoma is a benign neoplasm that originates in a normal liver and is composed of cells that closely resemble normal hepatocytes [53]. Older reports of so-called adenomas in cirrhotic liver were very probably examples of macroregenerative nodules.

Adenomas typically develop in women during the reproductive age, and are almost always associated with oral contraceptive use [61]. In our series of 145 adenomas, only 3% were found in men (unpublished data). They have been also reported in patients with an inherited glycogen storage disease and maturity-onset diabetes of the young, and some are obesity-related. Approximately 33%–80% of patients are asymptomatic with tumors discovered by a chance on imaging [61-62]. Recently a new molecular classification has been established, based on metabolic pathways [63-64]. There are currently four suggested subtypes of liver adenomas: 1) Hepatocycte nuclear factor 1 (HNF1-α)- inactivated adenomas (25%–40%, diffuse intratumoral

steatosis), 2) β- catenin mutated (5%–10%, prompted to malignant transformation), 3) teleangiectatic/inflammatory (45%–55%, formery teleangiectatic focal nodular hyperplasia [FNH]), and 4) unclassified (5%–10%). MRI is the best imaging modality for characterizing HCA, and efforts based on MRI imaging are being made to subclassify them in accordance with the above-mentioned metabolic subtypes [65].

On B-mode US, adenomas can be hyperechogenic, isoechogenic or hypoechogenic. They are often homogenous, but previous intratumoral bleeding can change parenchyma into a heterogenous lesion. Sometimes, intralesional calcification can follow an old bleeding episode. On color Doppler US one can find arterial signals with high peak flow and low impedance [66].

HNF1-α inactivated HCAs are often homogenous and hyperechogenic due to fat accumulation. The main feature of inflammatory/teleangiectatic HCAs is heterogenity, which presents an often hyperechoic and prominent Doppler signal similar to FNHs [67]. The other two types (β-catenin-mutated and unclassified) are less characteristically seen on US.

On CE-US, after microbubble injection, adenomas usually show homogenous arterial filling (PICTURE 2), often with a visible feeding artery, and the contrast agent travels very rapidly centripetally from the border to the center part of lesion [68]. In the portal/late phase the lesion becomes isoechogenic, or sometimes faintly hypoechogenic. This event. can even mimic washout and is more prominent in the HNF1-α inactivated HCA subtype [69]. In some patients, this pattern versus a hepatocellular carcinoma can make a diagnosis challenging. An association with hypervascularity, centripetal filling, linear vascularities, peripheral rim of sustained enhancement, and central washout in the late phase teleangiectatic/inflammatory subtype has been shown to exist [69]. These suggestions should, however, be confirmed by other studies [70].

Areas of previous bleeding, thrombi, or necrotic portions remain non-enhancing in all CE-US phases. Higher body mass index (BMI) is associated with the teleangiectatic/ inflammatory subtype and multiple adenomas [71].

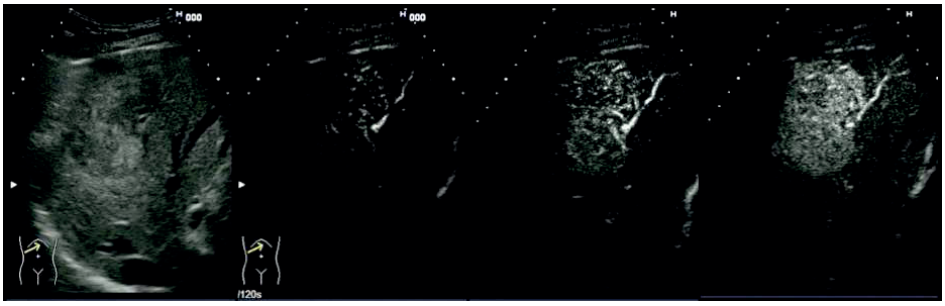
In some cases, a correct differential diagnosis can be challenging against FNH. In adenoma, the centrifugal spoke-wheel pattern is absent, and the central artery with a scar is often more prominent in FNH [72].

FOCAL NODULAR HYPERPLASIA (FNH)

FNH is a tumor-like malformation consisting of hyperplastic nodules of hepatocytes, separated by fibrous septa, which form a typical central scar [53]. The liver is otherwise morphologically and histologically normal. FNH can be defined as not truly a tumor but a local regenerative mass of normal polyclonal hepatocytes (whereas liver

adenoma is a benign monoclonal tumor). The prominent central artery lies close to the central scar.

The lesion is often found incidentally during an imaging session for unrelated complaints. It may be detected more frequently in women (our unpublished data from 184 FNHs suggests 4% incidence in men) and in 15% to 30% of cases, FNH is multiple [73].

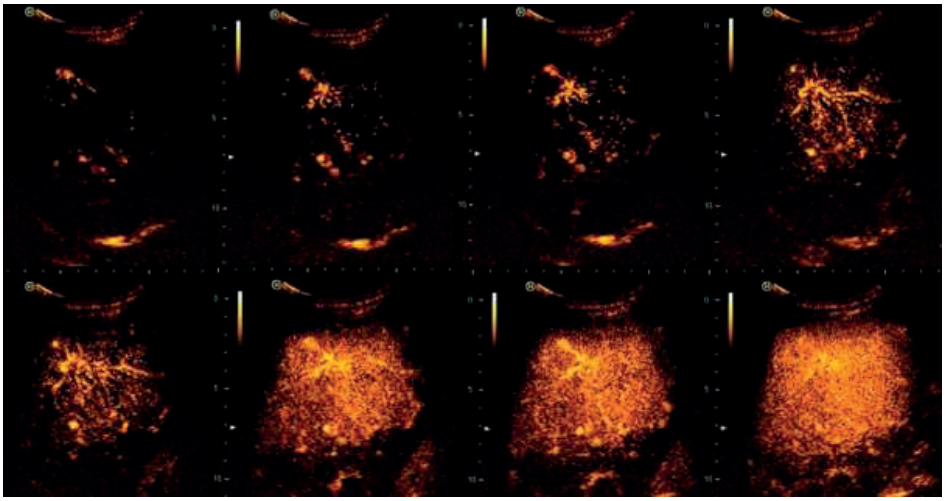


Picture 2: Hepatocellular Adenoma. At conventional B-mode (a) shows heterogenous echopattern. Three CEUS pictures (b-d) show quick filling (sec 10-12). The liver was filled-in portal phase and shows no tumor sings at all (not shown).

In the B-mode, we mostly see an isoechogenic lesion with a central scar and centrally located artery. A fibrotic central scar can be visible as a linear hyperechogenic structure in approximately 70% of the cases. The scar can be very faint, and sometimes multiple scars can be seen. In some patients, a barely visible scar is located more peripherally. The arterial supply can also be multiple or eccentrically located. Central artery imaging can be typical on a color Doppler ultrasound on which it can be seen radiating to the periphery with a high flow and low resistance pattern. FNH parenchyma is usually isoechoic but might be slightly hyper or hypoechogenic.

On CE-US, FNH shows a very short centrifugal spoke-wheel filling pattern in the early arterial phase, which is very rapid, and then is followed by a sustained homogenous faint enhancement during extended observation of the late phase (portal venous) lasting up to 5 minutes [74]. These signs are not present in adenomas. In some patients, FNHs are isoechogenic in the portal or late phase.

The very short nature of rapid spoke-wheel arterial filling is the point at which CE-US shows advantages in time resolution. Recording of the cinematic loop is necessary, and the picture can be checked frame by frame in order to track centrifugal filling of single microbubbles (PICTURE 3).



Picture 3. Focal Nodular Hyperplasia. Typical enhancing filling pattern of a large FNH – centrifugal enhancement within first few seconds in arterial phase.

The central scar appears hypoechoic in the late phase [75-76] and this appearance can be explained by the strict intravascular nature of the ultrasound contrast agent. The central scar is detected in around 40% of the FNHs [77].

The prevalence of typical features of FNH in literature varies considerably from 22% to 70% [64].

Liver adenoma can sometimes display a few fibrotic septa, which in some patients can be a diagnostic chalenge. Lesions originally classified as teleangiectatic FNH are no longer regarded as adenomas. FNH can be also confused with fibrolamellar HCC [1].

Recently, a new technique for quanifying local stiffness has emerged. Shear wave elastography technology can provide additional information from tumors, and FNH is a natural candidate for exploration. In some reports, it has been shown that FNH has higher stiffness compared to other focal liver lesions [78]. Whether focal elastography can be a complementary tool to CEUS or not still waits further validation [79].

REGENERATIVE AND DYSPLASTIC NODULES

Hepatocellular carcinoma develops in a multistep process, consisting of regenerative nodules, low-grade dysplastic nodules, high-grade dysplastic nodules, and early and advanced HCC. HCC is not the topic of this review, but regenerative nodules (RN) are not considered neoplastic lesions, and dysplastic nodules (DN) are considered yet to be premalignant lesions (International Consensus Group 2009). Regenerative nodules can also accompany liver patients with Budd-Chiari syndrome or non-cirrhotic portal hypertension [80].

Large regenerative nodules (macroregenerative nodules) are larger than 5 mm in diameter. If larger, regenerative nodules can be called segmental hyperplasia or lobar hyperplasia , [81]. The initial injury can be subacute hepatic necrosis, vascular injury, or chronic biliary disease. They can be found in both cirrhotic and noncirrhotic livers. On B-scans, they form nodules that are mostly isoechogenic on color Doppler US and display peripheral arterial and venous vessels [82].

On CEUS they show a filling similar to surrounding parenchyma in the arterial, portal and late phases, according to the new classificastion system, LI-RADS®-3 [83-84]. LI-RADS can help inexperienced radiologists obtain substantial consistency in differentiating benign from malignant liver lesions [85].

Dysplastic nodules (low- and high-grades) are nodules in cirrhotic liver that are macroscopically distinct from the surrounding cirrhotic nodules [53]. They are usually larger than surrounding nodules and can be detected via imaging studies. They contain dysplastic atypias without frank malignancy. Low-grade dysplastic nodules (LGDN) may not be distinguishable from large regenerative nodules in that they contain portal areas. High-grade dysplastic nodules (HGDN) have histopathologial features suggesting more cellular proliferation. These modules often present the appearence of nodule-in-nodule formation, but they still contain portal tracts and unpaired arteries, which is a typical feature of hepatocellular carcinoma, that are not yet present. On B-scan US, they can show a mildly hypoechogenic and heterogenous pattern. On CE-US they may show diffuse contrast enhancement and mild hypoenhancement in the arterial phase. Accurate depiction of arterial phase hyperenhancement is vital for distinguishing HCC from dysplastic nodules. Contrast arrival time ratio (CAT_R) can be used in multivariable regression models [86]. In a situation without arterial hypervascularity we rely on portal and late phase washout to diagnose malignancy [87].

In several CE-US studies using the Sonazoid contrast agent, regenerative nodules (RNs) showed centrifugal vessels running from the center to the periphery in the arterial phase (central vessel pattern), and HGDNs showed a centripetal pattern (peripheral vessel pattern) [88-89]. These features also await further confirmation [102].

BUDD-CHIARI SYNDROME

Chronic Budd-Chiari syndrome (hepatic vein thrombosis) often causes the formation of nodular liver lesions that are usually small, multiple, and hypervascular. The majority of them are regenerative nodules and are hyperenhanced in the arterial phase as center-to-periphery or homogenous nodules. In the portal/late phase, nodules showed hyperenhancement [90]. These lesions should be monitored, and some of them can change into HCC, which are seen as heterogenous hyperenhanced lesions in the arterial phase and hypoechoic in the portal and late phases. Other types of

nodules closely resemble FNH due to the presence of a central scar. These nodules are larger than 1 cm in diameter [91].

INFLAMMATORY PSEUDOTUMOURS

Inflammatory pseudotumor (IPT) is a rare benign tumour-like lesion of the liver. It often appears to arise from a healing abscess or other inflammatory condition [53]. This lesion contains a mixture of inflammatory cells with a predominance of polyclonal plasma cells that infiltrate the fibrotic stroma. Diagnosis using imaging studies is difficult because findings vary. There are only a few CE-US-related reports of inflammatory pseudotumors, but they seem to show a quick hyperenhancing lesion in the arterial phase, and some lesions showed quick wash-out in the portal and late phase (fast-in, fast-out) [92-93]. Differential diagnosis should thus include malignant lesions, including liver metastasis and HCA [94].

LIVER ANGIOMYOLIPOMA

Hepatic angiomyolipoma (HAML) is a rare, benign mesenchymal tumor that is more common in women. The tumor may become quite large and usually solitary and is found in a normal non-cirrhotic liver. Malignant degeneration in the liver is extremely rare. Microscopically there is typically an admixture of adipose tissue, thick-walled arteries, smooth muscle and haematopoietic cells [53]. On baseline US they appear as a well-defined, sometimes very large, hyperechoic, and often heterogenous nodule without surrounding hypoechoic halo. Their B-scan picture may resemble a hemangioma. On CE-US, they show heterogenous hyperenhancement in the arterial phase that remains hyper- or isoechoic in the portal/late phase [95]. The arterial filling is often quick and visible vascular signals can lead to a misdiagnosis of FNH [96]. A minority may show a hypoenhancing pattern in the portal/late phase, thus mimicking malignant tumours as HCA [95]. Use of the Sonazoid contrast agent may prove no perfusion defect in the post-vascular (intracellular) phase due to the possible presence of macrophages in the tumor, a phenomenon not seen in malignancies [94]. Quantitative CEUS could be explored in these cases [97].

OTHER

Nodular regenerative hyperplasia (NRH) usually mimics diffuse liver cirrhosis and is not likely to be confused with a neoplasm. Occasionally, in B-mode there may be a large regenerative nodule among small cirrhosis-like nodules, causing confusion with liver adenoma [53]. CE-US should differentiate RNs from HCAs becasue arterial hyperenhancement in the adenoma as opposed to the RN.

The application of CE-US was also described in a variety of other liver focal lesions, such as sarcoidosis [98], epithelioid hemangioendothelioma [99], some tropical diseases [100-101] and others.

CONCLUSION

CE-US was introduced more than twenty years ago and is currently a well-established imaging method, mainly in European and Asian countries. It is highly flexible and free of radiation. The method can be used as a second-line imaging method after a basic non-conclusive B-mode or Doppler liver US session. CE-US performance can result in approximately a 15 min delay in a standard US examination. It is inexpensive and can be performed with medium- or high-end US machines. In many incidentally detected and mostly benign lesions, the final diagnosis can be immediately made and if not conclusive, CE-US is a good begining for imaging studies before other methods are used. Its detection rate is comparable to contrast-enhanced MRI or contrast CT. It is a highly effective method for differentiating malignant versus benign liver lesions. CE-US can also be used in a follow-up examination for primary liver tumors or during surveillance in liver cirrhosis.

The psychological aspect of the patient should also be taken into account as a clinically satisfactory result can often be achieved during the first ultrasound examination.

CE-US and MRI are the best complementary imaging methods and usually present correct focal liver lesion (FLL) diagnoses. When results are inconclusive or suggestive of malignancy, a biopsy can promptly be initiated; thus, additional imaging studies are avoided

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CHAPTER 3

Performance of contrast-enhanced sonography versus MRI with a liver-specific contrast agent for diagnosis of hepatocellular adenoma and focal nodular hyperplasia

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AM J Roentgenology 2020

ABSTRACT

OBJECTIVE. The purpose of this article is to compare contrast-enhanced sonography (CEUS) with sulfur hexafluoride with MRI with the liver-specific contrast agent gadobenate dimeglumine in the diagnosis of hepatocellular adenoma (HCA) and focal nodular hyperplasia (FNH) in a cohort of consecutive patients.

MATERIALS AND METHODS. Patients referred to a tertiary center for hepatobiliary disease who had suspected HCA or FNH on MRI performed with an extracellular gadolinium-based contrast agent underwent a prospective workup including CEUS and MRI with a liver-specific contrast agent. Diagnosis was definite when the findings of CEUS and MRI with a liver-specific contrast agent were concordant; histopathologic examination (HPE) was performed for cases with discordant findings. Descriptive statistics and the association between categoric variables were presented as numbers and percentages and were assessed using the Fisher exact test. The primary analysis was patient based. Sensitivity, specificity, and AUC and predictive values for the diagnosis of HCA and FNH were calculated separately for CEUS and MRI with a liver-specific contrast agent.

RESULTS. A total of 181 patients were selected for the first analysis. Findings from CEUS and MRI with a liver-specific contrast agent were concordant for 132 patients (73%) and discordant for 49 (27%). HPE was performed for 26 of the 49 patients with discordant findings (53%), with findings indeterminate for two of these patients, the findings of MRI with a liver-specific contrast agent correct for 21 of the remaining 24 patients (87.5%), and the findings of CEUS correct for three of these 24 patients (12.5%) ($p < 0.05$). For further analysis, 156 patients with concordant findings or HPE-proven cases were included. For CEUS, the sensitivity and specificity for the diagnosis of HCA and FNH were 85% and 87%, respectively; the ROC AUC value was 0.856; and the positive predictive value and negative predictive value were 79% and 90%, respectively. For MRI with a liver-specific contrast agent, the sensitivity and specificity were 95% each, the ROC AUC value was 0.949, and the positive predictive value and negative predictive value were 92% and 97%, respectively, for the diagnosis of HCA and FNH.

CONCLUSION. The findings of CEUS and MRI with a liver-specific contrast agent showed fair agreement for the diagnosis of HCA and FNH. MRI with a liver-specific contrast agent is diagnostically correct significantly more often than CEUS in cases with discordant findings that are HPE proven.

INTRODUCTION

Hepatocellular adenoma (HCA) and focal nodular hyperplasia (FNH) are benign solid liver lesions that are mostly found in young women. Although both entities are benign and often asymptomatic, their pathogenesis and clinical management are different. For some patients with HCA, surgical resection may be considered, whereas patients with typical cases of FNH do not need further follow-up. Therefore, accurate diagnosis is important [1-3]. Contrast-enhanced sonography (CEUS) and MRI with a liver-specific contrast agent are imaging modalities with specific imaging features reported for both HCA and FNH, which may lead to confident diagnosis [3-10]. At present, both CEUS and MRI with a liver-specific contrast agent are regarded as the best complementary techniques for workup-based diagnosis of HCA and FNH, and final diagnosis can be considered definitive when the findings of both CEUS and MRI with a liver-specific contrast agent are concordant [3]. In uncertain or atypical cases, biopsy and histopathologic examination (HPE) are recommended [3] [11]. In addition, a few studies have reported MRI with a liver-specific contrast agent to be highly accurate for the diagnosis of HCA and FNH and can be considered a reference standard in cases with unequivocal lesiontypical findings [1] [6] [12-14]. However, MRI with a liver-specific contrast agent is costly, whereas CEUS has the potential to provide diagnosis of target lesions at a lower cost [15-18].

The purpose of the present study was to compare the diagnostic performance of CEUS with that of MRI with a liver-specific contrast agent in the differentiation of HCA and FNH in a large cohort of consecutive patients.

MATERIALS AND METHODS

Study Population

The ethics committee (institutional review board) at Erasmus University Medical Center approved this retrospective study, and informed consent was waived.

Patients were selected from prospectively collected databases from the departments of radiology and gastroenterology. Patients who met the inclusion criteria were those with a final diagnosis of HCA or FNH who had undergone liver imaging with both CEUS and MRI with a liver-specific contrast agent between May 2008 and December 2016. Exclusion criteria included a final diagnosis other than HCA or FNH, a history of cancer or known chronic liver disease, and the presence of multiple concurrent lesions of both HCA and FNH in the same patient.

Standard Workup Procedure

Patients referred to our institution with suspected HCA or FNH on an initial liver MRI examination performed with an extracellular gadolinium-based contrast agent (gadolinium-enhanced MRI) underwent standard liver workup with CEUS and MRI with a liver-specific contrast agent. CEUS was performed by a hepatogastroenterologist with 21 years of experience in ultrasound of the liver, including 5 years of experience in CEUS. MRI with a liverspecific contrast agent was subsequently performed within 4 weeks of the CEUS examination, and findings were evaluated and reported by one of three abdominal radiologists with expertise in liver imaging (8, 10, and 11 years of experience) who were blinded to the CEUS findings. Those cases for which diagnosis was uncertain at initial evaluation with MRI with a liver-specific contrast agent were discussed by two or three radiologists, and a consensus was reached regarding the final diagnosis.

Contrast-Enhanced Sonography

CEUS was performed using two ultrasound platforms (the Hitachi 900 and Preirus systems, Hitachi Medical Systems) with real-time grayscale, contrast-tuned imaging and a 2.5-5.0–MHz probe. The contrast agent that was used, sulfur hexafluoride (SonoVue, Bracco), was administered at a dose of 2.4 mL and flushed with isotonic saline. Examinations were executed in a standardized fashion. Patients first underwent unenhanced abdominal and hepatic sonography using conventional color Doppler or power Doppler imaging techniques, with the location, number, size, and sonographic features of the focal liver lesions recorded. Contrast agent was subsequently administered, and CEUS was performed. When multiple similar lesions of HCA or FNH were present, only the largest lesion was selected and evaluated with CEUS. Because of the unique network formed by the hepatic artery and the portal vein, three phases can be observed with CEUS [4-5]. Images were acquired in the hepatic arterial phase (10–40 seconds after injection), the portal venous phase (40–120 seconds after injection), and the late parenchymal phase (> 120 seconds after bubble disappearance). The vascularity and enhancement pattern of the lesion were evaluated for up to 5 minutes after injection of the contrast agent. Still images and digital cine loops were saved and were reviewed later for final assessment and report.

Criteria for the diagnosis of HCA and FNH were based on the European Federation of Societies for Ultrasound in Medicine and Biology protocol and guidelines [16] [19-22]. Central arteries were defined by the presence of enhancing central arteries with a spoke-wheel appearance. A central scar was defined as a central stellate hypoechoic area without contrast enhancement in the portal venous phase. Previous intralesional hemorrhage was defined as an irregular heterogeneous area without the presence of contrast filling. Late contrast enhancement (contrast agent reten-

tion) was defined as the presence of hyperechoic contrast filling compared with adjacent liver parenchyma in the portal phase. HCAs usually show homogeneous arterial contrast filling centripetally from the periphery to the center of the lesion. In the portal and late phases, the lesion becomes isoechogenic, sometimes with areas of previous intralesional hemorrhage. Alternatively, FNHs show a centrifugal spoke-wheel filling pattern in the arterial phase, followed by sustained homogeneous enhancement during the portal venous and late phases. A cinematic loop is recommended to check frame by frame for assessment of the filling pattern [23].

MRI

MRI was performed using a 1.5-T system (Signa, General Electrics) with a four-channel, phased-array body coil. The MRI protocol was identical for all patients: a single-shot fast spinecho sequence (slice thickness, 7 mm; TR/TE, 832/80–120; and flip angle, 90°), a fat-suppressed T2-weighted fast spin-echo sequence (slice thickness, 5–8 mm; TR/TE, 6315/90–93; and flip angle, 90°), and T1-weighted in-phase and opposed phase gradient-recalled echo sequences (slice thickness, 7 mm; TE, 4.6 ms [in phase] and 2.3 ms [opposed phase]; flip angle, 80°). Fat-suppressed, dynamic contrast-enhanced T1-weighted gradient-recalled echo imaging (slice thickness, 4–5 mm; TR/TE, 2.7–3.5/1.2; and flip angle, 12°) was performed in at least four phases (unenhanced, arterial, portal, and delayed phases) after administration of an IV bolus (2–2.5 mL/s) of gadobenate dimeglumine (MultiHance, Bracco) at a dose of 0.05 mmol per kilogram of body weight. The optimal arterial phase was based on bolus tracking. Finally, the same scan was repeated during a late hepatobiliary excretory phase 1–1.5 hours after injection.

For HCAs, typical MRI findings include signs of internal bleeding, atoll sign, small cystic areas, and diffuse homogeneous steatosis of the lesion. FNHs typically show the presence of a T2-weighted hyperintense central scar (spoke-wheel appearance). Both lesions show arterial phase hyperenhancement with a tendency toward isointensity in the portal venous phase. In addition, HCA is hypointense and FNH is hyper- or isointense on the hepatobiliary excretory phase image compared with surrounding liver parenchyma.

Reference Standard

Diagnosis was considered definitive in cases for which the findings of CEUS and MRI with a liver-specific contrast agent were concordant. When findings of CEUS and MRI with a liver-specific contrast agent were discordant, HPE of the lesion was performed after percutaneous image-guided biopsy of the target lesion. When CEUS was inconclusive or biopsy was contraindicated or undesirable, MRI with a liver-specific contrast agent was considered the reference standard for final clinical diagnosis in

cases with unequivocal lesion-typical findings. Furthermore, for patients with HCA, repeat MRI examination was performed every 6 months to monitor lesion regression after patient cessation of oral contraceptive use and pursuit of weight loss measures for obesity. For these patients, MRI with a liver-specific contrast agent was evaluated by all three abdominal radiologists, with a unanimous decision reached regarding MRI diagnosis. In addition, these patients were clinically observed for at least 1 year after imaging diagnosis. Final diagnosis and clinical management were discussed and assessed by the multidisciplinary hepatobiliary tumor board, which consisted of dedicated and experienced radiologists, surgeons, hepatogastroenterologists, and oncologists.

Case Evaluation

Patient age and sex, previous imaging reports, CEUS reports, reports from MRI with a liver-specific contrast agent, decisions of the multidisciplinary hepatobiliary tumor board, pathologic reports, final diagnoses, and clinical follow-up information were registered using a standardized and anonymized clinical reporting form in the online clinical software program OpenClinica (version 3.1.3.1, OpenClinica). From the imaging reports, the level of diagnostic confidence with CEUS and MRI with a liver-specific contrast agent was graded on a 5-point scale, with a grade of 5 representing a definite or confident diagnosis; 4, a probable or preferred diagnosis; 3, a relatively uncertain diagnosis; 2, an uncertain diagnosis; and 1, no diagnosis. For final analysis, grades of 5 or 4 were regarded as conclusive outcomes, and grades 3, 2, or 1 were regarded as inconclusive outcomes.

Data Analysis and Statistical Methods

The primary analysis was patient based. Descriptive statistics were used to describe the study population and the outcomes of both imaging modalities. The association between categoric variables was presented as numbers and percentages and was tested using the Fisher exact test. The conclusiveness of CEUS and MRI with a liver-specific contrast agent was presented as numbers and percentages. The sensitivity, specificity, ROC AUC value, positive predictive value (PPV), and negative predictive value (NPV) of CEUS were calculated using SPSS software (version 21, IBM). All tests were regarded as statistically significant when $p < 0.05$.

RESULTS

Study Population and Final Diagnosis

From a database of 196 patients who underwent both CEUS and MRI with a liver-specific contrast agent during the study, we identified 181 patients who fulfilled the inclusion criteria. These 181 patients (154 female patients and 17 male patients) were selected for first analysis. The findings of CEUS and MRI with a liver-specific contrast agent were concordant for 132 patients (73%) and discordant for 49 (27%). HPE was performed for 26 patients with discordant findings (53%); two of these patients had findings indeterminate for FNH or HCA. Patients with concordant findings and HPE proven cases (a total of 156 patients) were included for further analysis (Fig. 1). The mean patient age was 38 years (range, 17– 76 years). Patients had either a single lesion (63 patients; 40%) or multiple lesions (93 patients; 60%). Lesion size ranged from 1.6 to 14 cm. The final diagnosis was FNH for 98 patients (63%) and HCA for 58 patients (37%). Women were significantly overrepresented compared with men ($p < 0.001$), with no significant difference in sex ($p = 0.664$) or age ($p = 0.258$) found between patients with FNH and HCA.

Contrast-Enhanced Sonography and MRI

With a Liver-Specific Contrast Agent for Histopathologically Confirmed Cases

Diagnosis was definite (i.e., findings from CEUS and MRI with a liver-specific contrast agent were concordant) for 132 of 156 patients (85%) (Figs. 2 and 3), and diagnostic findings were discordant for the remaining 24 patients (15%). For both CEUS and MRI with a liver-specific contrast agent, all cases had conclusive findings (confidence level, 4 or 5). Further HPE was performed for 26 of 49 patients with discordant findings (53%) (Figs. 4 and 5). For two of the 26 patients who underwent HPE, findings could not clearly differentiate between HCA and FNH. For the remaining 24 patients (10 with HCA and 14 with FNH), the findings of MRI with a liver-specific contrast agent were correct for 21 of 24 patients (87.5%) and those for CEUS were correct for three of 24 patients (12.5%). This difference was statistically significant ($p < 0.05$). The remaining 23 of 49 patients with discordant findings (47%) included 14 patients with inconclusive CEUS findings (confidence level, 1, 2, or 3). MRI with a liver-specific contrast agent was considered the reference standard for clinical decision making and management for these 23 patients. These 23 patients were classified as having HCA (four patients) or FNH (19 patients) with a follow-up of 1–6 years. FNH lesions were unchanged at 1 year of follow-up and were dismissed from further follow-up. HCA lesions showed a gradual decrease in size with cessation of oral contraceptive use and pursuit of weight loss measures.

Conclusiveness of Contrast-Enhanced Sonography and MRI

With a Liver-Specific Contrast Agent

For CEUS, results were conclusive for 167 of 181 patients (92%) and inconclusive for the remaining 14 patients (8%). The results of MRI with a liver-specific contrast agent were conclusive for 180 of 181 patients (99%), with only one initial inconclusive diagnosis (1%) with an uncertain differentiation between HCA and FNH. At repeat MRI examination, the findings for this patient were considered conclusive.

Diagnostic Performance of Contrast-Enhanced Sonography Versus MRI With a Liver-Specific Contrast Agent

For further analysis, only patients with concordant findings (132 patients) and those with HPE-proven cases (24 patients) were considered, for a total of 156 patients. For CEUS, sensitivity and specificity were 85% (49 of 58 cases [49 cases with concordant findings plus nine that were HPE proven]) and 87% (85 of 98 cases [85 cases with concordant findings plus 13 that were HPE proven]), respectively, for the diagnosis of HCA and FNH, with an ROC AUC value of 0.856. The positive predictive value was 79% (49 of 62 cases [cases with concordant findings plus 13 that were HPE proven]), and the negative predictive value was 90% (85 of 94 cases [85 cases with concordant findings plus nine that were HPE proven]).

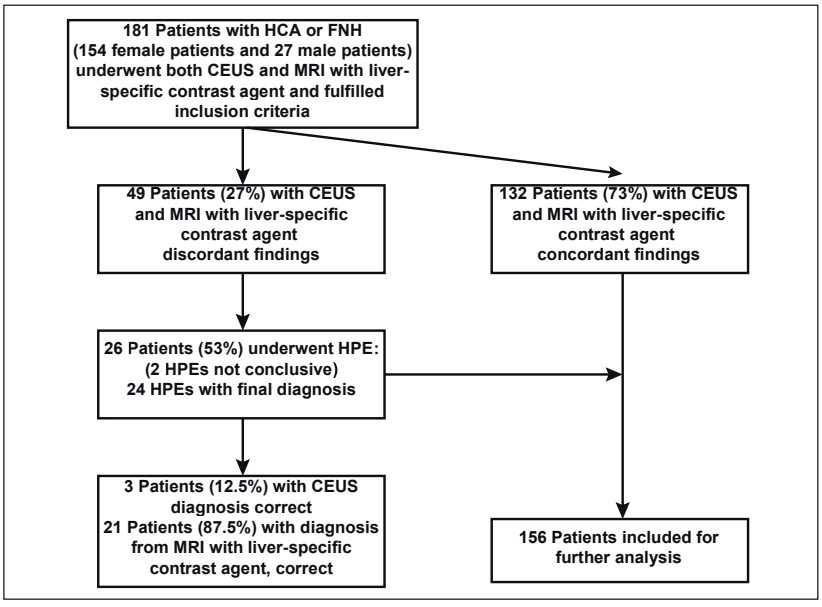

Fig. 1 — Flow diagram summarizing patient sampling and results. HCA = hepatocellular adenoma, FNH = focal nodular hyperplasia, CEUS = contrast-enhanced sonography, HPE = histopathologic examination.

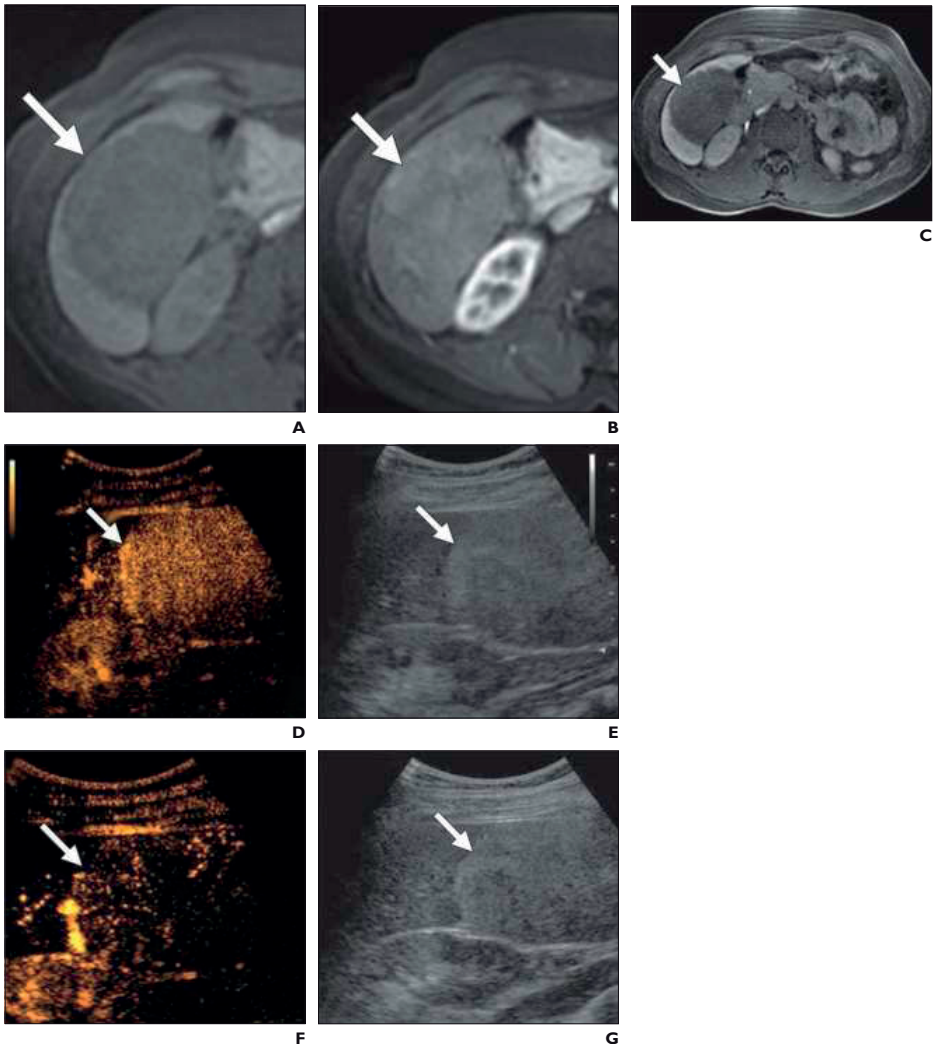

Fig. 2 — 41-year-old-woman with focal liver lesion who underwent contrast-enhanced sonography and MRI with liver-specific contrast agent; findings were concordant for hepatocellular adenoma. **A–C,** Images from MRI with liver-specific contrast agent show solitary lesion in liver segments V and VI (*arrow,* **A–C**). Lesion is hypointense on T1-weighted fat-saturated image (diffuse fatty content of lesion) (**A**), shows enhancement on arterial phase image obtained after IV contrast administration (**B**), and shows marked signal hypointensity on delayed hepatobiliary excretory phase image (**C**). Findings suggest adenoma of hepatocyte nuclear factor 1α– mutated subtype. **D–G,** Contrast-enhanced sonography images show hyperechoic homogeneous lesion with centripetal contrast enhancement (*arrow*, **D** and **F**) and with no washout and no retainment of contrast agent (*arrow*, **E** and **G**). Findings are concordant with MRI findings, resulting in confident diagnosis of adenoma.

For MRI with a liver-specific contrast agent, sensitivity and specificity were 95% (55 of 58 cases [55 cases with concordant findings plus three that were HPE proven]) and 95% (93 of 98 cases [93 cases with concordant findings plus five that were HPE proven]) for the diagnosis of HCA and FNH, respectively, with an ROC AUC value of 0.949. The positive predictive value was 92% (55 of 60 cases [55 cases with concordant findings plus five that were HPE proven]), and the negative predictive value was 97% (93 of 96 cases [93 cases with concordant findings plus three that were HPE proven]).

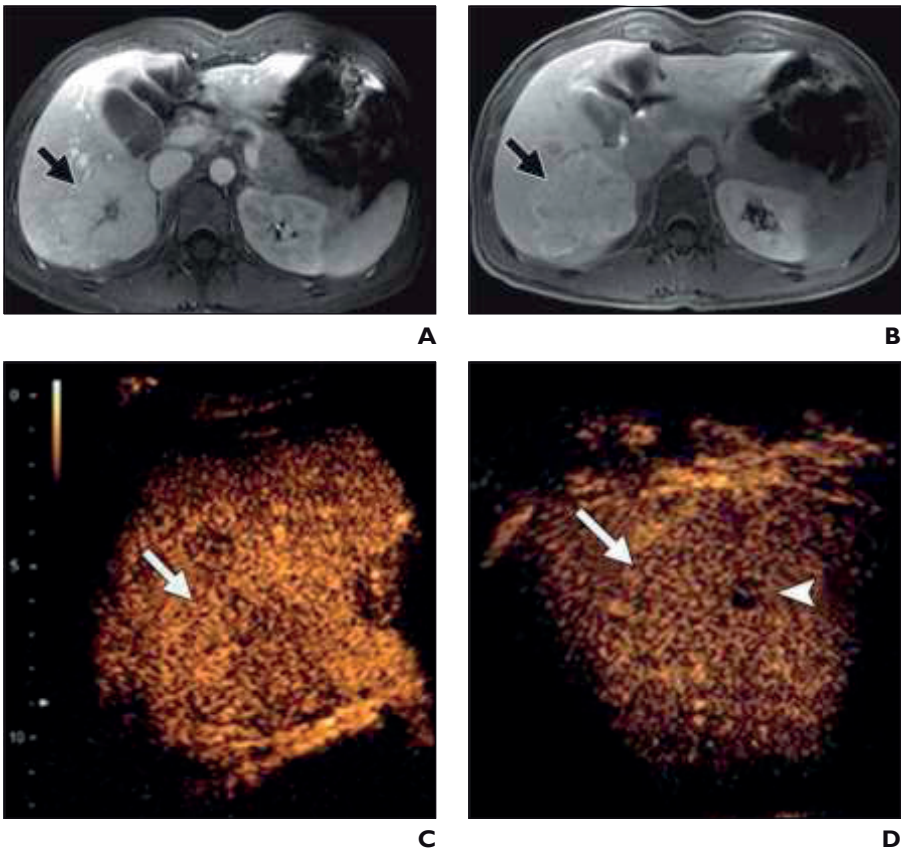

Fig. 3 — 25-year-old woman with focal liver lesion who underwent contrast-enhanced sonography and MRI with liver-specific contrast agent; findings were concordant for focal nodular hyperplasia. **A** and **B,** Images from MRI with liver-specific contrast agent show lesion (*arrow*, **A** and **B**) on right lobe of liver (in liver segments V–VIII) with features typical of focal nodular hyperplasia, including progressive enhancement of central scar (**A**) and hyperintensity on delayed hepatobiliary excretory phase (**B**). **C** and **D,** Contrast-enhanced sonography images show lesion (*arrow*, **C** and **D**) is isodense and heterogeneous compared with surrounding parenchyma. Image obtained after IV contrast administration shows centrifugal filling and retainment of contrast in lesion (**C**), with central scar evident on late phase image (*arrowhead*, **D**).

DISCUSSION

For the diagnosis of FNH and HCA, we found 85% agreement between CEUS and MRI with a liver-specific contrast agent. In addition, for 24 patients with discordant findings that were subsequently pathologically confirmed, MRI with a liver-specific contrast agent provided a correct diagnosis for significantly more patients than did CEUS (21 versus three patients). Another interesting finding is that CEUS resulted in a conclusive diagnosis for 92% of the patients, whereas the diagnosis resulting from MRI with a liver-specific contrast agent was initially conclusive for all but one patient (99%).

To our knowledge, only one other study, performed by Tselikas et al. [24] and involving 54 patients, has compared the added value of MRI performed using a liver-specific contrast agent (i.e., gadobenate dimeglumine) and CEUS with that of inconclusive extracellular gadolinium-based contrast-enhanced MRI (gadolinium-enhanced MRI) for the characterization of FNH and HCA. The authors found that the sensitivity and specificity of these modalities were not statistically different in the diagnosis of HCA and FNH. For lesions larger than 35 mm, although specificities were similar, sensitivity was higher for MRI with a liver-specific contrast agent. Our study was designed to directly compare CEUS with MRI with a liver-specific contrast agent, regardless of the outcome of previous gadolinium-enhanced MRI examination and lesion size. MRI with a liver-specific contrast agent is known to accurately diagnose FNH and HCA, irrespective of lesion size. To achieve a fair comparison, only patients with lesions that were exclusively FNH or HCA were included. Therefore, uncertainty regarding lesion identification in a one-on-one comparison was overcome in cases where patients had multiple different (HCA and FNH) lesions. In addition, in the present study, CEUS and MRI with a liver-specific contrast agent were performed and evaluated by separate readers, so previous personal impressions on lesion interpretation could not influence comparison of the imaging modalities.

The advantage of MRI with a liver-specific contrast agent in comparison with CEUS can be explained by the lack of hepatobiliary excretory properties of the ultrasound contrast medium. Although typical morphologic characteristics, including vascular contrast enhancement patterns, can be assessed by both imaging modalities, the decisive feature in terms of diagnosis with MRI with a liver-specific contrast agent is the ability to differentiate HCA from FNH in the delayed hepatobiliary excretory phase. Previous studies have shown that 20% of FNH lesions lack typical morphologic features, including a central scar, on imaging [13-14]. In addition, a subgroup of HCA (the β-catenin–positive subgroup) may show scarlike features on MRI with gadolinium chelates [25-26]. These cases may pose problems for confident diagnosis with CEUS. On the other hand, few studies have indicated that the inflammatory

subtype of HCA may show isointense or slightly hyperintense signal on delayed phase MRI with a liver-specific contrast agent [9] [27]. One of the three cases missed by MRI with a liver-specific contrast agent in our study was indeed HCA of an inflammatory subtype that was misinterpreted as FNH. Previous reports stated that CEUS is especially valuable in characterizing FNH lesions smaller than 3.5 cm. These small FNH lesions tend to present with classic imaging features more often than do larger lesions [28-29].

By use of MRI with a liver-specific contrast agent, we can combine the functional information (i.e., the hepatobiliary excretion properties) of the lesion with the morphologic information, leading to better diagnosis of FNH and HCA. The use of liver-specific contrast agents in MRI has become standard practice in liver imaging centers around the world. Currently, the two most frequently used agents are gadoxetate disodium (Eovist, Bayer HealthCare [in the United States]]) or gadoxetic acid (Primovist, Bayer Schering Pharma [in Europe]) and gadobenate dimeglumine (MultiHance, Bracco); these agents have extracellular properties but also an affinity for hepatocytes because contrast agent uptake is mediated by OATP1B3 transporters. Furthermore, gadobenate dimeglumine seems to have an advantage over gadoxetate disodium in enhancing visualization of the tumor during vascular phases [12], with no difference observed in the delayed hepatobiliary phase [30]. In a recently published study, gadoxetate disodium proved to have better hepatobiliary excretory properties than did gadobenate dimeglumine, which may result in a better ratio of lesion-to-parenchyma contrast and a more confident reading by the radiologist [31].

The European Medicines Agency approved the use of gadoxetate disodium and gadobenate dimeglumine after a scientific review of gadolinium deposition in the brain and other tissues [32].

Furthermore, the recent approval by the U.S. Food and Drug Administration of the use of microbubbles with the inert gas sulfur hexafluoride and a palmitic acid shell (SonoVue, Bracco) for diagnostic imaging of liver tumors in adults and children represents a potential for CEUS application in liver imaging [33]. We believe that our study may contribute to better and proper application of both imaging modalities in liver imaging performed for the diagnosis of HCA and FNH.

On the basis of our results, we believe that CEUS is less suitable as a stand-alone imaging modality for final diagnosis of FNH and HCA. It may be valuable as an adjunct tool for diagnosis in typical or suggestive cases identified by multiphase CT or gadolinium-enhanced MRI and for follow-up of lesions that have been confirmed as HCA by MRI with a liver-specific contrast agent or HPE. In addition, MRI with a liver-specific contrast agent has the advantage of providing comprehensive evaluation of all lesions, not only for differentiating between HCA and FNH but also for showing features that may be indicative of transformation to HCC in cases of HCA

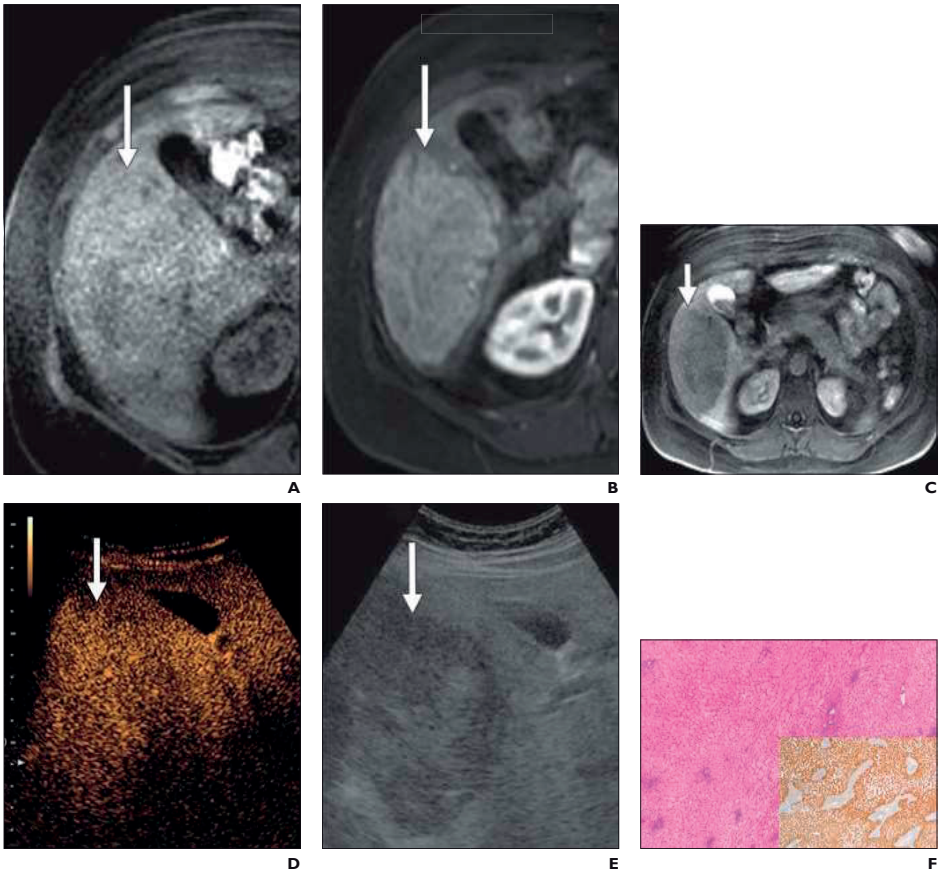

Fig. 4 — 32-year-old woman with large focal liver lesions who underwent contrast-enhanced sonography and MRI performed with liver-specific contrast agent; findings were discordant for hepatocellular adenoma.

A–C, Images from MRI with liver-specific contrast agent show large lesion in liver segments V and VI (*arrow*, **A–C**). Lesion was hypointense on T1-weighted gradient recalled echo sequence with fat saturation (**A**), showed enhancement on arterial phase image obtained after IV contrast administration (**B**), and showed marked hypointensity compatible with hepatocellular adenoma on delayed hepatobiliary excretory phase image (**C**).

D and **E,** Contrast-enhanced sonography images show that lesion (*arrow*, **D** and **E**) is hypoechoic in relation to surrounding parenchyma with suggestion of central scar (**D**) with centripetal contrast filling, homogeneity with retainment, and no washout (**E**). Lesion was interpreted as benign lesion that was probably focal nodular hyperplasia considering suggestive findings of central scar and contrast retainment.

F, Photomicrograph (H and E, ×25) from histopathologic examination of biopsy specimen of lesion proved adenoma of inflammatory subtype, with proliferation of benign hepatocytes, areas of sinusoidal dilatation, and inflammatory infiltrates. Photomicrograph (×100) (*inset*) shows C-reactive protein immunohistochemical staining with characteristic positivity for C-reactive protein.

on follow-up MRI. An increase in size or a change in enhancing properties of HCA on follow-up CEUS evaluation should warrant further evaluation by MRI.

The present study has some limitations, especially its retrospective analysis and the relatively limited number of pathologically confirmed diagnoses. Currently, dedicated MRI with a liver-specific contrast agent that results in a confident diagnosis is considered confirmative of FNH and HCA at centers with radiologists with expertise in liver imaging [6-7] [12-14]. Therefore, it would be impractical and possibly unethical to biopsy all lesions, despite a confident diagnosis provided by MRI with a liver-specific contrast agent. Another potential limitation might be selection bias. Because the analysis was performed retrospectively, patients were selected on the basis of final diagnoses of FNH or HCA. False-positive outcomes in the case of other diagnoses, such as liver hemangioma, were excluded, which may have culminated in an overestimated specificity for FNH or HCA. However, the purpose of the present study was to assess the value of CEUS compared with that of MRI with a liver-specific contrast agent, and we believe that our study design serves this purpose well.

In conclusion, CEUS has fair agreement with MRI with a liver-specific contrast agent for the diagnosis of HCA and FNH. In addition, MRI with a liver-specific contrast agent is superior to CEUS in cases with discordant findings with a pathologically confirmed final diagnosis.

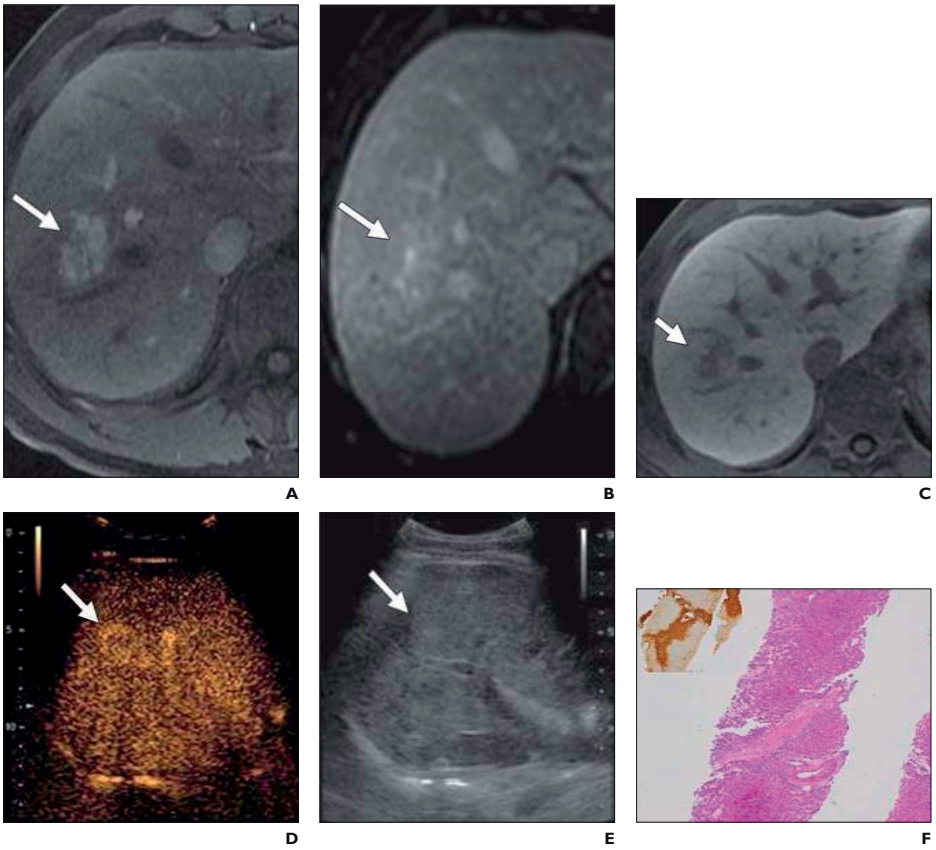

Fig. 5 — 24-year-old man with focal liver lesion who underwent contrast-enhanced sonography and MRI performed with liver-specific contrast agent; findings were discordant for focal nodular hyperplasia (FNH).

A–C, Images from MRI with liver-specific contrast agent show 4-cm large lesion found in right lobe (liver segments VII and VIII) (*arrows*). Lesion shows enhancement on arterial phase image (**A**) and late central scar enhancement on portal venous phase image (**B**). Lesion appears hypointense on hepatobiliary excretion phase image (**C**), and findings were therefore interpreted as hepatocellular adenoma.

D and **E,** Contrast-enhanced sonography images show isoechogenic lesion with central artery and scar (*arrows*), including centrifugal filling of contrast material on arterial phase image (**D**) and mildly hyperechogenic lesion on late phase image (**E**). Diagnostic confidence resulted in grading of lesion as FNH.

F, Photomicrograph (H and E, ×200) from histopathologic examination of biopsy specimen revealed typical features of FNH, such as large dystrophic arteries with thickened and narrowed lumens. Photomicrograph (×100) (*inset*) with glutamine synthetase immunostaining shows characteristic maplike pattern of glutamine synthetase. On retrospective review of MR images, lesion appears partly hypointense centrally on hepatobiliary excretion phase image shown in **C**; contrast excretion

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CHAPTER 4

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

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Journal of Clinical Ultrasound 2018

ABSTRACT

Purpose: Focal nodular hyperplasia (FNH) and hepatocellular adenoma (HCA) are liver tumors that require different management. We assessed the potential of point shear wave elastography (pSWE) to differentiate FNH from HCA and the interobserver and intraobserver reliability of pSWE in the examination of these lesions and of native liver tissue (NLT).

Methods: The study included 88 patients (65 FNH, 23 HCA). pSWE was performed by two experienced liver sonographers (observers 1 [O1] and 2 [O2]) and acquired within the lesion of interest and NLT. Group differences, optimal cutoff for characterization and interobserver reliability was assessed with Mann-Whitney-U, area under the ROC curce (AUROC) and intraclass correlation coefficient (ICC). Intraobserver reliability in NLT was assessed in 20 healthy subjects using ICC.

Results: Median stiffness was significantly higher in FNH than in HCA (7.01 kPa vs 4.98 kPa for O1 (P5 0.017) and 7.68 kPa vs 6.00 kPa for O2 (P 5 0.031)). A cutoff point for differentiation between the two entities could not be determined with an AUROC of 0.67 (O1) and 0.69 (O2). Interobserver reliability was good for lesion- stiffness (ICC5 0.86) and poor for NLT stiffness (ICC 5 0.09). In healthy subjects, intraobserver reliability for NLT-stiffness was poor for O1 (ICC 5 0.23) and moderate for O2 (ICC 5 0.62).

Conclusion: This study shows that pSWE cannot reliably differentiate FNH from HCA. Interobserver and intraobserver reliability for pSWE in NLT were insufficient. Interpretation of results gained with this method should be done with great caution

INTRODUCTION

Focal nodular hyperplasia (FNH) and hepatocellular adenoma (HCA) are two clinically important benign focal liver lesions. Histologically, these two 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 has a consistency similar to that of healthy liver tissue [1]. Differentiating between these two lesions is essential because each requires specific management. Follow-up of FNH is not necessary, provided the correct diagnosis has been made, but HCA often needs to be resected or at least monitored in view of the risk of bleeding or transformation into hepatocellular carcinoma [2-3]. The current standard diagnostic workup includes either contrast-enhanced ultrasound (CEUS) or contrast-enhanced MRI (CE-MRI) [4-7]. The estimated sensitivity and specificity of CEUS in the differentiation of FNH from HCA are 67% and 100%, respectively, with a significantly reduced sensitivity in lesions >35 mm [8]. For CE-MRI with hepatocellular specific contrast, the sensitivity is estimated at 91%–100% and the specificity at 87%– 100% [9]. When the diagnosis remains uncertain, these patients may undergo a tumor biopsy [10]. In order to avoid a percutaneous biopsy and the associated risk of complications [11] or even the surgical resection of a suspect lesion that turns out to be FNH, improvement of the diagnostic process is needed.

The first ultrasound elastography method for the liver became available in 2003 in the form of transient elastography with the Fibroscan device [12]. This method uses a mechanic pulse to measure the stiffness of the liver tissue. In 2008, a new elastography technique named Acoustic Radiation Force Impulse quantification (ARFI®, Siemens) became available and was incorporated in the ultrasound scanner. This method uses ultrasound point shear wave elastography (pSWE) and measures the speed of the shear wave (perpendicular to the axis of the ultrasound beam) in a small region at a selected depth within 80 mm from the skin. Other companies have started to develop similar technologies, including ElastPQ® by Philips Healthcare. pSWE can be used as a noninvasive, reproducible, and easy method of assessing liver fibrosis. A few preliminary studies have shown that pSWE can also be used to measure the stiffness of focal liver lesions 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 was to assess the diagnostic value of pSWE (ElastPQ®, Phillips Healthcare) in the differentiation between FNH and HCA. We also intended to assess the interobserver and intraobserver reliability of pSWE in the evaluation of 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

Patients diagnosed with FNH or HCA between January 1st 2007 and November 30th 2016 were eligible. All patients who first underwent CEUS and subsequently had either contrast enhanced MRI (CE-MRI) or biopsy confirming the diagnosis were included. When available, histological diagnosis was considered as the reference standard. In all other cases, the final diagnosis was discussed during a multidisciplinary tumor board (with radiologists, hepatologists and surgeons) and based on the combination of CEUS and CE-MRI characteristics. Patients who underwent previous intervention for treatment of FNH or HCA or had severe other liver disease (eg, cirrhosis, hepatocellular carcinoma, liver metastasis) were excluded. In addition, women over the age of 50 with an HCA were excluded, 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 diagnoses were based on typical imaging characteristics [4-7]. HCA subtype (eg, 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 examinations of patients in whom HCA subtype was not yet established and determined that 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. Exclusion criteria for healthy subjects were a known liver disease or a systemic disease requiring medication.

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 the Philips Epiq7 ultrasound system equipped with aa C5-1 broadband curved-array (Philips Healthcare Andover, MA). Two experienced liver sonographers (P.T. and R.K., with 25 and 15 years of experience, respectively), hereinafter referred to as observer #1 (O1) and observer #2 (O2), independently performed the measurements in all patients according to the European Federation of Societies for Ultrasound in Medicine and Biology (EFSUMB) guidelines for the performance of elastography [21-22].

In patients with either FNH or HCA, we measured the lesion's size in mm and acquired pSWE measurements from the lesion situated best in the field of view, and preferably the largest lesion. In case there were multiple lesions, we performed the measurements in the lesion situated best in the field of view and preferably the largest 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 of the 10 values obtained in the 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 10 individuals the sonographers performed two rounds of ten measurements in the NLT at 10-min intervals.

Statistical analysis

We used IBM SPSS software version 21.0 (Chicago, Il) for statistical analysis and reported continuous variables as medians and interquartile ranges (IQR) and binary variables as frequencies (n) and percentages (%). We used Mann-Whitney U test to assess differences for continuous variables and v2 test for categorical variables. Correlation between variables was analyzed using Pearson product-moment correlation coefficient. Performance of the ElastPQ was evaluated using receiver operating characteristic (ROC) curves. Interpretation of the ROC curves was based on the area under the ROC Curve (AUROC), which is 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. Interobserver and intraobserver reliabilities were assessed using 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 CEMRI 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 the study at the end (Figure 1).

Eight patients were excluded because the lesion was situated too deep for pSWE and 17 because the lesion could not be found anymore during US examination. O1 performed pSWE in all included patients and O2 repeated the measurements in 62 patients (13 HCA and 49 FNH).

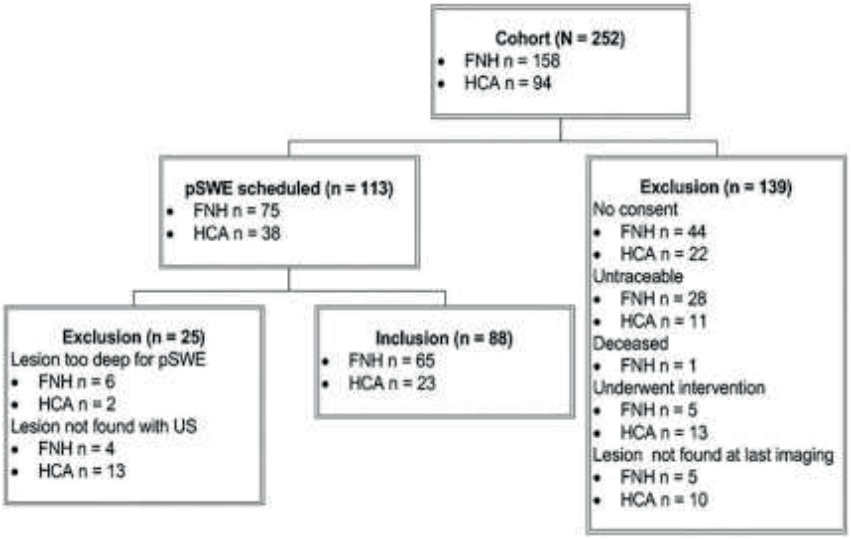

FIGURE 1 Inclusion flowchart of patients with a focal liver lesion. FNH, focal nodular hyperplasia; HCA, hepatocellular adenoma; pSWE, point shear wave elastography

Eighty-seven out of 88 patients included were female (Table 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 patients, 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 in one patient CE-MRI could not differentiate between HCA and FNH. Eleven cases of FNH (16.9%) were biopsy proven. In the HCA

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

TABLE 1 Baseline characteristics of patients with a focal liver lesion

	FNH (n565)	HCA (n523)
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 at 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).
CE-MRI, contrast enhanced magnetic resonance imaging; CEUS, contrast enhanced ultrasound; FNH, focal nodular hyperplasia; HCA, hepatocellular adenoma.

We determined the median FNH and HCA stiffness values per observer (Table 2 and Figure 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 (P5 0.017) and 7.68 vs 6.00 kPa (P 5 0.031), respectively). The median NLT stiffness value for O1 was 2.41 kPa (IQR 1.13–3.45) and 3.50 kPa (2.96–4.45) for O2. For O1, the median LLSR for FNH was 4.00 (IQR 2.05–7.00) and the median LLSR for HCA was 1.35 (IQR 0.84–2.71) (P< 0.001). For O2, these values were 2.44 (IQR 1.52–4.44) for LLSR for FNH and 1.34 (IQR 0.96–1.97) for LLSR for HCA (P 5 0.010). No correlation between lesion size and stiffness value was found for both FNHs and HCAs (P > 0.05).

TABLE 2 Stiffness values and lesion/liver stiffness ratios for focal nodular hyperplasias and hepatocellular adenomas

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

Values are given as median (IQR). FNH, focal nodular hyperplasia; HCA, hepatocellular adenoma; NLT, native liver tissue.
[a] Mann-Whitney U test showed a statistically significant difference in FNH vs HCA stiffness and lesion/liver stiffness ratios for both observers 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. Interobserver reliability analysis showed an ICC of 0.86 for lesion stiffness (95%CI: 0.78–0.92), 0.09 for liver stiffness (95%CI: 20.16–0.33) and 0.78 (95%CI: 0.66–0.86) for LLSR. Subgroup analysis based on lesion longest diameter was done in 36 lesions <30 mm (median, 20 mm; IQR, 14–25) and 52 lesions >30 mm (median. 50 mm; IQR, 40–66). It resulted in an ICC for lesions <30 mm of 0.18 (95% CI: 0.24–0.55) and for lesions >30 mm of 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 CEUS imaging modality. The stiffness values for these lesions ranged from 1.65 to 8.75 kPa for FNHs and from 1.29 to 9.36 kPa for HCAs.

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

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). Intraobserver reliability analysis showed an ICC coefficient of 0.23 (95% CI: 0.13–0.73) and 0.62 (95% CI:0.02–0.89) for O1 and O2, respectively.

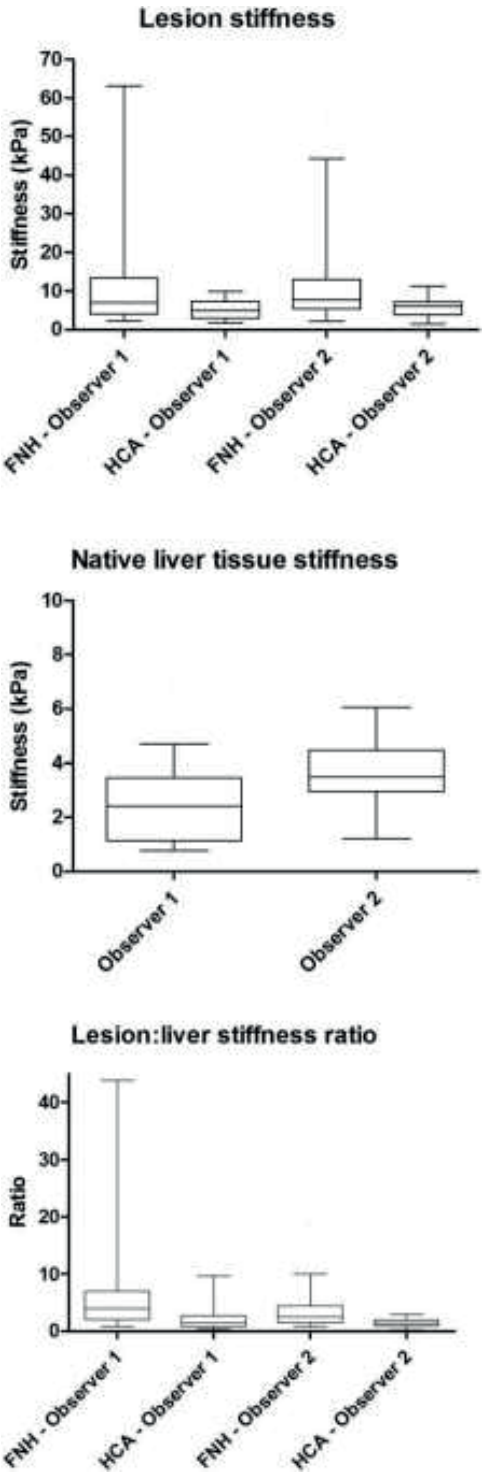

FIGURE 2 Box-and-whisker plots for lesion stiffness, native liver tissue stiffness and lesion:liver stiffness ratio. Box: median and IQR, whiskers: 5–95 percentiles. FNH, focal nodular hyperplasia; HCA, hepatocellular adenoma

DISCUSSION

There is a need to improve the noninvasive diagnosis 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 HCAs 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 performed with ElastPQ® can be used to distinguish FNH from HCA. Although median pSWE values were significantly higher in FNHs compared to HCAs, we were unable to determine an acceptable cutoff point for this characterization due to the great variability in pSWE values.

These results are in disagreement with previous studies, which suggested that pSWE was a useful supplementary method to distinguish FNH from HCA during conventional ultrasound. One of these studies was by Gallotti et al., [14] who found a significant difference in pSWE values between FNHs and HCAs. However, they did not try to determine a cutoff 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 cutoff 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 the highest accuracy (95%) with a cutoff stiffness value of 18.8 kPa (AUROC 0.93). The differences between their results and ours might be caused by the use of different software, as both these studies used two-dimensional SWE (Aixplorer®, SuperSonic Imaging), compared with pSWE (ElastPQ®, Philips Healthcare) in our study.

Another possible explanation for the different results may 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 35 mm to 20 mm in a median follow-up period of 47 months. The influence of this regression process on the lesion stiffness remains unknown. 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 HCAs, whereas in our cohort we found high values in both steatotic and inflammatory HCAs. 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 lesions.

The use of pSWE for the evaluation of focal liver lesions has limitations. The first limitation is that currently it cannot be used in lesions that are situated deeper than 80 mm from the skin. In this study, we had to exclude eight patients because the lesion was too deep. During the execution of this study, we noticed that several factors might affect a lesion's stiffness value. For example, higher values may be seen in lesions with a fibrotic membrane where the pSWE region of interest 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 lesions located in the proximity of any liver artery or vein.

In this study, we also assessed the interobserver reliability in patients with focal liver lesions using the ICC. We found good interobserver reliability for lesion stiffness but a poor one for the surrounding NLT. Subgroup analysis showed a better interobserver reliability in lesions >30 mm compared to those <30 mm. In 2012, Gallotti et al. [14] also did an interobserver evaluation while performing ARFI ultrasound imaging in patients with focal liver lesions, including hepatocellular carcinomas, hemangiomas, HCAs, metastases, and FNHs). 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 interobserver reliability and that it would be wise to validate other elastography software with this method.

This study also assessed the intraobserver reliability of pSWE in determining the stiffness of NLT in healthy subjects. Remarkably, we found poor to moderate intraobserver 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.

This study has limitations. The first is the fact that not all lesions were biopsied and therefore only 17% of FNHs and 22% of HCAs were pathologically proven. Although pathological examination remains the gold standard for diagnosis of benign liver tumors, clinical practice guidelines advice 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]. Second, 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 HCAs could not been found, as these lesions may regress after cessation of oral contraceptives . 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 FNHs also could not be found at ultrasound examination, as these lesions usually do not regress over time. The last limitation is the possible skewed distribution between males and females in this study, as only one male was included. It is known that both types of 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].

In conclusion, this study suggests that pSWE cannot reliably differentiate between FNH and HCA. Additionally, both interobserver and intraobserver reliability for pSWE measurements of the NLT were insufficient. Interpretation of the results gained with this method should be done with caution.

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CHAPTER 5

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

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Ultrasound in Medicine and Biology 2017

ABSTRACT

Contrast-enhanced ultrasound (CEUS) is an emerging imaging technique that is increasingly used to diagnose liver lesions. It is of the utmost importance to differentiate between the two most common solid focal liver lesions (i.e., hepatocellular adenoma [HCA] and focal nodular hyperplasia [FNH]), because their management and follow-up differ greatly. The main objective of this study was to determine how frequently the specific CEUS features of HCA and FNH are visible on CEUS and to define their predictive value for discrimination between HCA and FNH. We included 324 CEUS examinations performed on patients with FNH (n = 181) or HCA (n = 143). Patients with HCA and FNH significantly differed with respect to age and CEUS features of steatosis, echogenicity, homogeneity, the presence of a central scar, central artery, arterial enhancement pattern, necrosis or thrombus and enhancement in the late venous phase.

INTRODUCTION

Abdominal ultrasound examination is readily available and frequently used in virtually every hospital. Consequently, during examination of complaints that are not directly related to the liver, many patients are misdiagnosed with a focal lesion in the liver on ultrasound. Most of these lesions are of benign origin, such as hemangiomas, simple cysts, focal nodular hyperplasia (FNH) or hepatocellular adenoma (HCA). Some lesions, such as simple cysts, can be diagnosed on ultrasound. However, solid liver lesions, such as FNH and HCA, need further characterization. Accurate diagnosis is of the utmost importance because treatments for the conditions differ greatly. FNH is a benign lesion with no malignant transformation, symptoms that may resolve during follow-up and a very low incidence of bleeding [1-2]. Therefore, if the contrast agent Lumason (Sonovue, Bracco Diagnostics, Monroe Township, NJ, USA) will increase interest in and use of contrast-enhanced ultrasound (CEUS) in clinical medicine.

CEUS is an emerging imaging technique that is increasingly used to diagnose solid focal liver lesions. The use of microbubble ultrasound contrast agents allows detailed assessment of vasculature patterns. The detection and characterization of solid liver tumors has improved considerably using CEUS [3].

The extensively described washout phase, defined as negative enhancement in the tumor 75 s after injection of the microbubble contrast agent, is being used to differentiate between benign and malignant liver lesions [4]. Furthermore, a centrifugal hypervascular enhancement pattern (FNH), diffuse arterial enhancement in the arterial phase (HCA), a central scar (FNH), contrast-enhancement in the late phase (FNH) and the presence of thrombus or necrosis (adenoma) are CEUS characteristics that help to differentiate between FNH and HCA. Moreover, a centrifugal hypervascular enhancement pattern in the arterial phase may be an essential feature for the diagnosis of non-typical FNH [5]. However, the frequency of the presence of features for HCA and FNH on CEUS and its capacity to differentiate between HCA and FNH have the diagnosis is firmly established, treatment is rarely indicated [2] [6]. HCA, on the other hand, has a risk of hemorrhage, rupture and malignant transformation, and treatment might be indicated [7].

Macroscopically, FNH tends to be lobulated and in most cases it has a central stellate scar (central element) that radiates into nodules of normal hepatocytes [6]. The central scar contains a fibrous stroma and malformed vascular structure, the central artery. From this anomalous central artery, the arterial blood often flows centrifugally (stellate-type contrast agent distribution), which is in contrast to HCA [8]. HCA tends to have peripheral subscapular vessels that cause diffuse homogenous arterial filling. These characteristics can be used to discriminate the two conditions.

Until recently, magnetic resonance imaging (MRI) or needle biopsy were needed for characterization [9-10]. However, recent US Food and Drug Administration approval of capacity to differentiate between HCA and FNH have only been described in a few small series [11-13]. A metaanalysis concluded that a detailed evaluation of HCA by CEUS was not possible because of the low numbers of patients with HCA [11]. Thereafter, 28 patients with FNH and 10 patients with HCA have been described and showed 66% of the lesions using CEUS were correctly diagnosed compared with 40% of the lesions using color Doppler ultrasound [12-13] described 40 patients (31 patients with only FNH, 7 patients with only HCA and 2 patients with both FNH and HCA) and suggests that CEUS is a useful adjunct tool, especially in assessing smaller lesions, with an almost perfect interobserver agreement [13].

Guidelines outline steps for diagnosing benign solid liver tumors with CEUS and indicate that the specific feature in FNH is a centrifugal hypervascular enhancement pattern in the arterial phase. This specific feature can be used to differentiate FNH from HCA and could even be an essential step for the diagnosis of nontypical FNH [5].

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

According to the literature that describes the characteristics of FNH and HCA in MRI some findings are more typical than others [9]. For example, the central scar, which is more commonly described in FNH, was also found in 21% of confirmed HCA cases [8] [10].

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

MATERIALS AND METHODS

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

Patients

We included 324 CEUS examinations performed between 2007 and 2014 in patients with confirmed FNH or HCA for review in this study. CEUS findings were only included if the diagnosis of the lesion had been confirmed using at least 2 radiologic modalities, including at least 1 MRI examination with the use of a liver-specific contrast agent. Consensus on the diagnosis was reached after discussion within our multidisciplinary tumor board committee or if the lesion was histologically confirmed (by biopsy or surgical resection). Patient characteristics were collected from the electronic hospital records.

CEUS

CEUS was introduced in our hospital as an additional radiologic modality. CEUS was performed by various sonographers, but all examinations were reviewed by a specialist with more than 20 y of experience in liver ultrasound and more than 9 y of experience in CEUS. The sonographers were blinded to the patients' pre-existing imaging (computed tomography [CT] and MRI) information. CEUS was performed using the Hitachi 900 and Hitachi Preirus ultrasound platforms (Hitachi Medical Systems, Tokyo, Japan) with real-time grayscale, contrast-tuned imaging and a 2.5- 25.0-MHz probe. The contrast agent used was SonoVue (Bracco Diagnostics, Monroe Township, NJ, USA; dose range 1.0– 2.4 mL; repeated if needed and flushed by isotonic saline).

Ultrasound examination was performed in a standardized fashion. First, all patients underwent unenhanced abdominal and hepatic sonography using the fundamental color/power Doppler technique. The location, number, size and sonographic features of the focal liver lesions were recorded. In case of significant hepatic steatosis, the identification of a specific liver mass with ultrasound (US) might be more difficult. However, when the examination was done using the CEUS mode, specific features appear, which can be used to differentiate the different liver masses. Three phases can be observed with CEUS because of the unique network of the hepatic artery and the portal vein [14-17]. CEUS was performed during the hepatic arterial (10–40 s post-injection), portal venous (40–120 s post-injection) and late parenchymal phases (120 s, bubble disappearance), according to the standardized EFSUMB protocol [18]. The vascularity and enhancement pattern of the lesions were compared with the adjacent liver parenchyma. Accordingly, CEUS was performed 5 min after application of the contrast agent. The flash-replenishment technique was applied when needed.

Still images and digital cineloops were saved and later reviewed. Central arteries were defined by the presence of enhanced central arteries with a spoke-wheel appearance. A central scar was defined as a central stellate hypoechogenic area without contrast enhancement in the portal venous phase. Necrosis or a thrombus caused by previous bleeding was defined as an irregular area without contrast filling. Late

contrast-enhancement (contrast agent retention) was defined as the presence of hyperechogenic filling (mostly fine) compared with adjacent liver parenchyma in the late portal phase.

STATISTICAL ANALYSIS

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

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

Next, a multivariable logistic regression analysis was performed to investigate the association between the different covariates and the definitive diagnosis of HCA. A stepwise regression model with backward elimination was used. All items included in the univariate analysis were initially included as a covariate in the initial model. After each step, the covariate with the worst predictive value was removed until the best-fit model remained. The classification cut-off for elimination was set as p 5 0.05. The coefficients of the final multivariate model were used to create a formula for the prediction of the probability that the lesion was HCA.

RESULTS

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

Steatosis in a nontumorous parenchyma was observed in 47% of the patients with HCA compared with 23% in patients with FNH. Necrosis or thrombus formation was observed in 18% of the patients with HCA compared with 3% necrosis or thrombus formation in the patients with FNH. No acute bleeding or thrombus formation was observed.

Results from the univariate analysis of possible patient-related predictors combined with features on CEUS between the group of patients with HCA and FNH are shown in Table 1. HCA and FNH patients differed significantly with respect to age

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

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

HCA = hepatocellular adenoma; FNH = focal nodular hyperplasia; CEUS = contrast-enhanced ultrasound.

* Univariate analysis results for various comparisons for HCA and FNH. $p < 0.05$ was considered statistically significant.

Data were analyzed using a t-test or Pearson's c^2 test where appropriate. Values in parentheses are percentages unless otherwise noted.

and the CEUS features of steatosis, echogenicity, homogeneity, central scar, central artery, arterial enhancement pattern, necrosis or thrombus, and enhancement in the late portal phase (contrast agent retention).

In a multivariable analysis, the subsequent items were eliminated in the following order: homogeneity ($p = 0.888$), enhancement in the late venous phase (contrast retention) ($p = 0.797$), necrosis or thrombus caused by previous bleeding ($p = 0.527$), echogenicity ($p = 0.108$) and steatosis ($p = 0.193$). The regression coefficients of the final regression model are given in Table 2. Using these coefficients, the predicted probability of HCA was calculated using the following formula:

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

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

Table 2. Multivariable logistic regression analysis for the prediction of HCA based on patient characteristics and CEUS

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

HCA = hepatocellular adenoma; CEUS 5 contrast-enhanced ultrasound.
Central artery and enhancement pattern

DISCUSSION

Liver Steatosis

Currently, fatty liver disease is the most common chronic liver disease with an estimated incidence of 30% in Western countries [19]. Liver steatosis is often accompanied by obesity. HCA seems to also be associated with obesity, explaining why significantly more people with HCA have liver steatosis (47%) compared to patients with FNH (23%). Identifying a specific liver mass with US might be more difficult in obese patients. However, specific features can be depicted using CEUS, which can aid in differentiating the various liver masses.

HCA can be hyperechogenic in an otherwise normal liver during ultrasound without the use of contrast ("fat-containing HCA"); however, we also found that 7% of FNHs were hyperechogenic. This rare hyperechogenicity of FNH could be explained by the occasional presence of fat in FNH, which has been previously described and should be considered in ultrasound and imaging diagnostics [20]. When other classical FNH findings are seen, the presence of fat in the lesion can occasionally make the diagnosis less robust.

Central scar

A classic FNH is composed of nodules surrounded by radiating fibrous septa originating from a central scar [21] [8]. The central scar in FNH is a collection of blood vessels, bile ducts and fibrosis stroma [22]. With CT and MRI, the central scar has been reported in 22% – 85% of FNH cases [23-24]. On CEUS, in which the central scar appears as a hypoechogenic area in the delayed phase [16-17], or on B-scan ultrasound, where it appears as a white fibrotic stripe, we identified a central scar in 80% of the FNH cases. However, fibrotic stripes similar to a central scar have been observed in 24% of HCA cases as well. Recently, fibrotic scars have also been described in 21% of HCAs on MRI [9-10]. The central scar is characterized on MRI by a T2 weighted late enhancement in the delayed phase. As a central scar could also be visible in HCA: Differentiation between HCA and FNH should not be based on the rare, but can be present and is not exclusively diagnostic for HCA on US.

As expected, a central artery with centrifugal (stellate) filling was more common in FNH, and a petal appearance of the central scar alone, fibrotic stripes could have the same appearance.

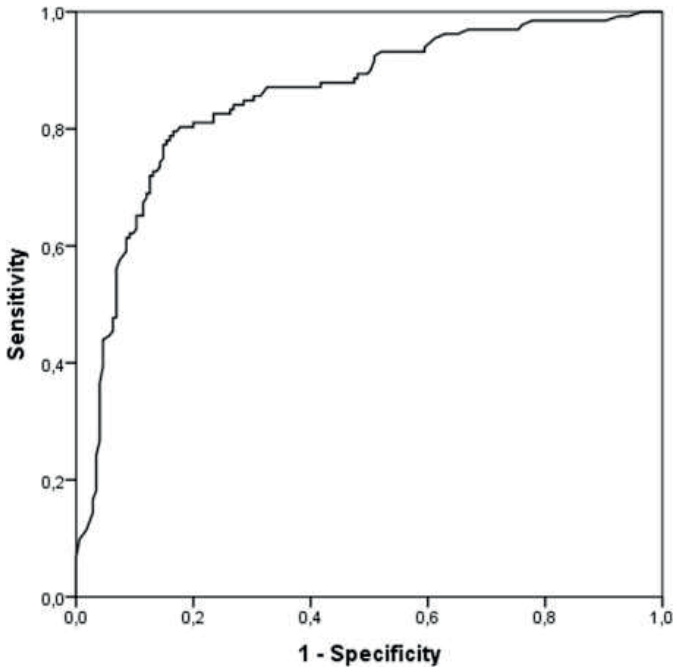

Fig. 1. ROC curve for the prediction of HCA using the formula 1/(1 1 e(0.778 1 (0.36 *Age)) 1 (central scar) (central artery) (enhancement mixed) (enhancement petal)), showing an area under the curve value of 0.854. ROC 5 receiver operating characteristic; HCA 5 hepatocellular adenoma.

Necrosis or thrombus

A thrombus caused by previous bleeding was present in 18% of the HCA and 3% of the FNH in this study. Thrombus caused by bleeding in HCA is fairly common, with an average overall frequency of 27.2% and a maximum reported frequency of 64% [25-26]. It should be noted that if an irregular area without contrast enhancement is observed on CEUS, no differentiation among necrosis, thrombus from an old bleeding, or a large central scar can be made [1-27]. The presence of an avascular area (necrosis/thrombus) in FNH is very rare, but can be present and is not exclusively diagnostic for HCA on US.

Central artery and enhancement pattern

As expected, a central artery with centrifugal (stellate) filling was more common in FNH, and a petal filling was most common in HCA [28]. Arteries in FNH can be abnormally large for the region of the liver they perfuse and in some nodules color Doppler examination can be diagnostic. It may sometimes be difficult to localize the central part of the arterial tree with single plane US because it can be located eccentrically and not centrally. A subset of patients has not one, but two or more centers with stellate arterial projections. In these cases, the centers probably tend to be faint and not robust. Further technological development, such as the regular use of 3-D CEUS, could be of benefit here.

Contrast-agent retention in the late portal phase

This US sign (contrast-agent retention in the late portal phase) was confirmed to be predominant in FNH but was found only in 40% of FNH patients. This level was statistically significant but not exclusive; up to 14% of adenomas were hyperechogenic in the late phase. Contrast-agent retention can be confusing because 25% of adenomas are already hyperechogenic on B-scan US. The sonographer should carefully differentiate between an already hyperechogenic tumor background that already exists and an influence of the presence of contrast agent. Some atypical FNHs may show a washout-like image in late phase — in our series 4%, which is usually seen in hepatocelullar carcinoma and also in some HCAs (15% in this study).

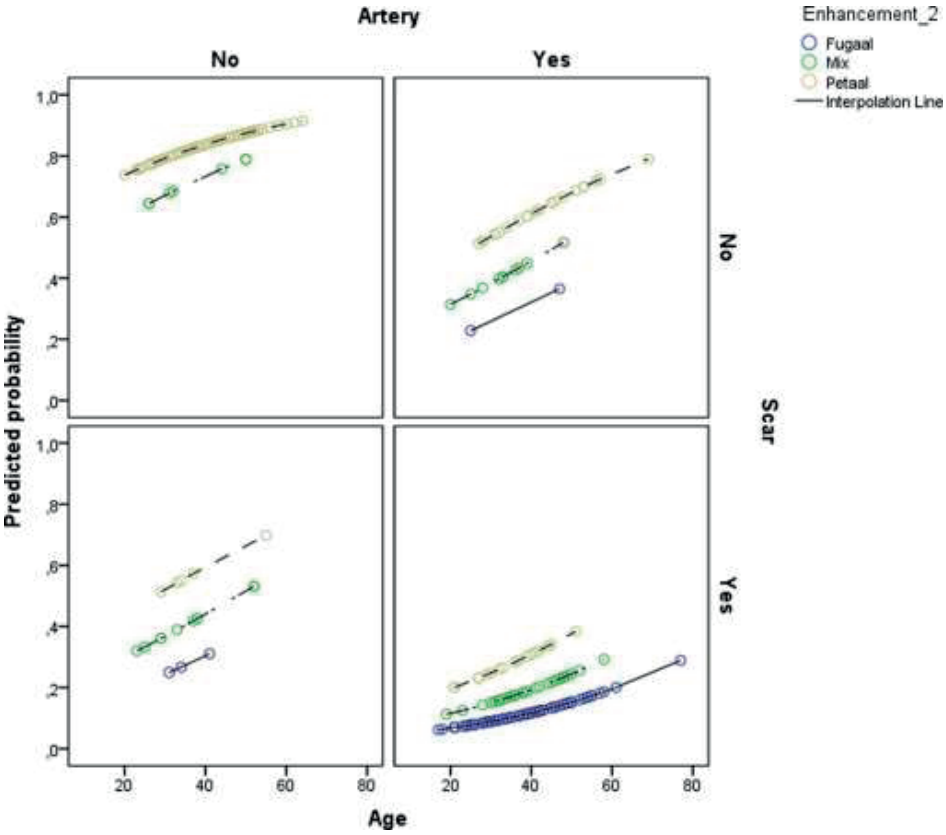

Fig. 2. HCA diagnosis. The predicted probability that the definitive diagnosis is HCA. The visualization of a central scar and a central artery on CEUS indicates which quadrant of the figure should be used. The colored lines in each quadrant represent the various enhancement patterns (blue, fugal; green, mixed; yellow, petal). By using the age of the patient at the time of diagnosis on the corresponding colored line, the predicted probability that the definitive diagnosis is HCA can be determined. HCA = hepatocellular adenoma; CEUS = contrast-enhanced ultrasound.

Limitations

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

The aim of this study was to differentiate FNH and HCA, two solid benign liver tumors. In clinical practice, it is also of the utmost importance to exclude hepatocellular carcinoma. The most important feature to differentiate benign and malignant liver lesions on CEUS is the presence of washout [29], [4]. However, this feature showed no additional value in differentiating between FNH and HCA. It is essential to first rule out a malignancy before using this model, which givesinsight intothe predicted probability of HCA.

CONCLUSIONS

In conclusion, increased age and CEUS features of liver steatosis, tumor echogenicity, homogeneity, thrombus presence, filling pattern and central scar, central artery, arterial enhancement pattern and absence of enhancement in the portal venous phase were found to be predictive in distinguishing between HCA and FNH. A reliable model using age and the presence of a central scar, central artery and enhancement pattern can predict the probability that the definitive diagnosis is HCA. If the diagnosis of HCA or FNH is equivocal on MRI, CEUS can be used to differentiate the two lesions, as a combination of the two methods provides the highest diagnostic accuracy [30]. This study gives insight about the reliability of the features on CEUS and helps clinicians to decide whether further liver mass biopsy is needed.

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CHAPTER 6

Liver contrast-enhanced ultrasound improves detection of liver metastases in patients with pancreatic or periampullary cancer

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Ultrasound in Medicine and Biology 2015

ABSTRACT

The aim of this study is to provide a diagnostic performance evaluation of contrast-enhanced ultrasonography (CEUS) in detecting liver metastases in patients with suspected of pancreatic or periampullary cancer. Computed tomography (CT) is often insufficient for detection of liver metastases, but their presence plays a crucial role in the choice of therapy. Eighty-nine patients with suspected pancreatic or periampullary cancer were included in this prospective study with retrospective analysis. Patients underwent an abdominal CT and CEUS. Fifteen patients had liver metastases. The CT sensitivity was 73.3% (11/15), the specificity 93.2% (69/74), the positive predictive value (PPV) 68.8% (11/16) and the negative predictive value (NPV) 94.6% (69/73). Based on CEUS, the sensitivity was 80% (12/15), specificity 98.6% (73/74), PPV 92.3% (12/13) and NPV 96.1% (73/76). CEUS improved characterization of liver lesions in patients with suspected pancreatic or periampullary cancer compared with CT. CEUS can better detect benign liver lesions and distinguish false-positive or indeterminate CT results.

INTRODUCTION

Pancreatic adenocarcinoma is one of the deadliest types of cancer. The overall US mortality rate for cancer has declined by 20% since 1991, according to the [1]. In contrast to many other malignancies, pancreatic cancer is a grim exception, and its death rate is slowly increasing. For all stages combined, the 5-y relative survival rate is only 6%, and most patients will die within the first year of diagnosis. In particular, pancreatic cancer metastasizes frequently, particularly to the liver. Complete surgical resection combined with adjuvant chemotherapy is currently the only potentially curative treatment. The 5-y overall survival in patients undergoing pancreatectomy is 15% – 25% compared to 5% without surgical treatment [2]. However, after a tumor is diagnosed, surgery is an option in only 15% – 20% of all patients. Most tumors are diagnosed at an already non-resectable stage due to late detection and early metastases, particularly in the liver [3-4].

Surgery will not improve survival if metastases are present in the liver. The presence or absence of liver metastases plays a pivotal role in the choice of therapy. It is therefore crucial to have accurate pre-operative methods for the detection of liver metastases [5].

Computed tomography (CT) is currently the reference method for the detection of metastases and is used in combination with clinical and histologic data. Patients suspected of having pancreatic cancer generally receive only an abdominal CT as a first-line investigation. The CT scan has two objectives: evaluation of the tumor's local resectability and exclusion of distant metastases. Although the quality of the CT scan has improved in recent years, the sensitivity for the detection of liver metastases is suboptimal and ranges from 38% to 73% [6-8]. A CT scan alone is often not enough for the detection of metastases, and surgeons frequently encounter metastatic liver lesions during an explorative laparotomy; metastases smaller than 1 cm can be frequently missed.

CT-related radiation exposure should also be taken into account. Magnetic resonance imaging (MRI) is another method for identifying liver metastases, but MRI is usually not part of the routine screening protocol. Despite advances in modern imaging techniques, assessing the presence of liver metastases remains challenging.

Contrast-enhanced ultrasonography (CEUS) is an evolving technology that has been developed over the last decade. With this type of ultrasonography, it is possible to better characterize focal liver lesions [9]. CEUS has repeatedly shown to have high accuracy in diagnosing liver tumors [10-12] and can differentiate between benign and malignant focal liver lesions with a diagnostic accuracy of approximately 87% and a lesion type accuracy of approximately 78% [13-14]. Several limitations of CEUS do exist: The method can be performed only for a short period of time, usually

3–5 min, is operator dependent and the examination may be challenging in obese patients [15]. Usually only one lesion can be evaluated during each contrast-agent-usage period (unless multiple lesions are located in the same segment), but examination can be repeated to evaluate other lesions. Still, CEUS is flexible, inexpensive, lacks radiation risk and is suitable for use in patients with renal insufficiency or allergies to CT contrast agents.

Thus far no study has evaluated the performance of CEUS in assessing liver metastases of patients with suspected pancreatic or periampullary tumors. The aim of this study was to evaluate the diagnostic value of screening CEUS compared to CT scan in the detection of liver metastases in patients suspected of having pancreatic or periampullary cancer.

MATERIAL AND METHODS

Study design

In this single-center, prospective study with retrospective analysis, CEUS was compared to contrast-enhanced CT for the detection of liver metastases. The results of other diagnostic tests, such as MRI, biopsy, laparoscopy or open surgery, as well the outcome of clinical follow-up, were used to define the final diagnosis. In cases where no metastases were observed during surgery or by biopsy, there was a follow-up period consisting of a CT scan at least 3 mo after surgery.

Patients

Between March 2008 and September 2011, 111 patients were enrolled. The inclusion criteria were presence of suspected pancreatic or periampullary cancer. The exclusion criteria were an age <18 y, concomitant serious illness (ie. recent cardiac infarct) and known hypersensitivity to contrast agents to preclude CT or CEUS scanning. All patients were assessed for possible liver metastases. Informed consent from study participants was signed. The procedures were conducted according the ethical standards of the Committee on Human Experimentation of the institution and with the ethical standards of the Helsinki Declaration of 1975.

Methods and procedures

CEUS.

Ultrasonography was performed by various sonographers, but all examinations' cineloops were reviewed by one examiner (PT) with more than 20 y of experience in liver ultrasound and more than 8 y of experience in CEUS. The sonographers were blinded to the patient's pre-existing CT results, but were informed about

suspected pancreatic neoplasm and the need to exclude possible liver metastases. CEUS was performed using the Hitachi 900 and Hitachi Preirus ultrasound platforms (Hitachi Medical Systems, Japan) with real-time grayscale, contrast-tuned imaging and a 2.5–5.0 MHz probe. The contrast agent used was SonoVue (Bracco, Italy; range 1.5–2.4 mL; repeated if needed and flushed by isotonic saline). Ultrasound examination was performed in a standardized fashion. First, all patients underwent unenhanced abdominal and hepatic sonography using the fundamental color/power Doppler techniques, and the location, number, size and sonographic features of the focal liver lesions were recorded. CEUS was performed during the hepatic arterial, portal venous and late parenchymal phases, according to the standardized EFSUMB protocol [16]. The vascularity and pattern of enhancement of the lesion compared with the adjacent liver parenchyma were evaluated for diagnosis by CEUS for up to 4 min after the application of the contrast agent. If multiple suspected lesions (more than 8–10) were found, for the arterial phase only the dominant lesion was selected. If one or two lesions were found, then all were checked in the arterial phase. In one case, all three suspected metastases were located close together in adjacent segments and only one arterial phase was performed, but all lesions checked. Still images and digital cineloops were saved and reviewed.

CT scan.

Patients analyzed at Erasmus MC were scanned with a Siemens helical multi-detector CT scanner according to the local triple-phase pancreatic cancer protocol [17], which included noncontrast-enhanced and contrast-enhanced scans in the pancreatic phase after 40 s (2.5- to 3-mm slices) and in the portal phase after 80 s (3–5-mm slices). CT scans performed at other hospitals included triple phase, biphasic (arterial or pancreatic phase and venous phase) or single phase (venous phase) protocols, with 3–5-mm slices.

All scans were evaluated or reassessed (in case of a CT scan from another hospital) by several abdominal radiologists as part of the normal pre-operative clinical workup. Radiologists were aware of all relevant clinical information and made a standard radiology report regarding the tumor's local resectability and the presence or absence of distant metastases.

For the purpose of this study, only the scans of 18 patients suspected of having liver metastases on either CT or CEUS were re-reviewed by a single radiologist (NK) with 5 y dedicated experience in abdominal radiology.

Final reference diagnosis. The final reference diagnosis was obtained by surgery and/or histology plus all available clinical information. Biopsies were taken before or during laparoscopy when suspected metastases were observed. The blood results,

such as CEA and CA19-9, as well as size and compartment of the lesions, were assessed, but did not contribute to the final evaluation in this study.

Statistical analysis

Statistical analysis was performed using SPSS version 22.0 (SPSS Inc., Chicago, Illinois). Sensitivity, specificity and kappa (overall agreement) were calculated for the detection of metastases. Comparisons between groups were performed using the McNemar test. Further logistic regression was applied to compare CT and CEUS as predictors of metastases.

RESULTS

Study population

For the purposes of the study, 111 patients were enrolled. All patients were suspected of having pancreatic or periampullary cancer. Over the course of the study, 14 patients were excluded. Six patients were excluded because they had only undergone an MRI and not a CT scan, and eight patients were excluded because their CT and/or CEUS scans were not performed according to protocol (most frequently, an overly long time interval elapsed between CEUS and CT). Another eight patients were excluded from analysis because their final diagnosis turned out not to be a pancreatic or periampullary adenocarcinoma (autoimmune pancreatitis in three patients, neuroendocrine tumor in two patients, intra-ductal papillary mucinous neoplasm in two patients and pancreatic cyst in one patient).

The total study population consisted of 89 patients, comprising 55 men and 34 women. The age range was 31–87 y. Pancreas head adenocarcinoma was diagnosed in 66 (74.1%) patients, periampullary adenocarcinoma in 21 (23.9%) patients and 2 (2.2%) patients had adenocarcinoma in the pancreas tail.

CEUS was performed before or after CT; mean interval between CT and CEUS was 27 d (range 0–73). We do not consider the mean interval too long, but there are no reliable guidelines to follow and our patients were treated according to current clinical practice in a tertiary center.

The patients presented with various indications and symptoms. The main symptom was obstructive jaundice (32/89, 35.9%). Furthermore, certain patients presented with symptoms of weight loss, pain, feelings of malaise and gastric outlet obstruction. In three patients, pancreatic carcinoma was discovered during other diagnostic investigations. In seven patients, the main symptom was not clear or non-specific.

In 15 (16.9%) patients, at least one liver metastasis was diagnosed during the diagnostic procedures, at laparotomy or during follow-up.

No adverse events were reported, and no patients with ultrasound or CT contrast agent allergy were encountered.

Computer tomography (CT)

In 40 (44.9%) patients, various focal lesions were observed by CT, and 16 (17.9%) patients were suspected of having liver metastases. In 24 (26.9%) patients, other focal lesions were visualized but were not suspected for metastatic disease. Forty-nine (55%) patients had no focal lesions observed on CT. These results are summarized in Table 1. Furthermore, in two (4.1%) patients with no suspected lesions, fatty liver (steatosis) was present, and in one (2%) patient, calcification of the liver was seen on CT.

Of the 16 patients who were suspected of having liver metastases based on the CT scan, 11 had histologically proven metastases. Therefore, the positive predictive value (PPV) for the detection of liver metastases with CT was 68.8%. A false-positive diagnosis of metastasis was given in five patients (average 11 mm, range 8–15 mm). These lesions were finally diagnosed as cysts (n = 2) or non-specific lesions that could not be characterized further on CT (n = 3). Of the three non-specific lesions, one was due to suboptimal CT quality (5-mm slices, only venous phase), one was a hypervascular lesion to be determined as a shunt on MRI and one involved focal bile duct dilation as shown by CEUS.

In four patients with proven metastasis, non-malignant focal lesions were seen on CT (one patient had 19-mm metastasis and three patients multiple small ones).

Seventy-three patients were not suspected of having liver metastases based on CT. In total, 69 of these patients had no liver metastases. Therefore the negative predictive value for the detection of liver metastases by CT was 94.6%.

The sensitivity of CT in the diagnosis of metastasis in a patients suspected of malignant pancreatic tumor was 73.3% (11/15), its specificity was 93.2% (69/74) and its kappa was 0.655 (SE 0.11).

Contrast-enhanced ultrasound CEUS

In 36 (40.4%) patients, focal lesions were identified by CEUS, and 13 (14.6%) patients were suspected of having liver metastases (Fig. 1). In 23 (25.8%) patients, other focal lesions were noted but were not suspected of being metastases. In 53 (59.6%) patients, no focal lesions were observed by CEUS. These results are summarized in Table 2.

Of the 13 patients who were suspected of having liver metastases based on CEUS, 12 were confirmed.

One false-positive diagnosis of liver metastasis was made in an obese patient with fatty liver (steatosis) and a 23-mm, hypoechogenic lesion. Focal non-steatosis lesion

was incorrectly diagnosed in the differential diagnosis as possible metastasis. Therefore, the PPV for the detection of liver metastases by CEUS was 92.3% (12/13). In total, 76 patients' liver metastases were not seen on CEUS, and 73 of these patients actually had no liver metastases, meaning that metastasis was undetected by CEUS in three patients who actually had metastasis. The same three malignant lesions were also undiagnosed by CT, and were only correctly diagnosed during surgery. Therefore, the NPV for the detection of liver metastases by CEUS was 96.1% (73/76).

Table 1. Focal lesions found by CT

Lesions	Frequency (n)	Percentage of n = 40
Malignant		
Metastases	9	22.5%
Metastases and cysts	1	2.5%
Metastases and hemangioma	3	7.5%
Metastases and steatosis	1	2.5%
Metastases, steatosis and hemangioma	1	2.5%
Metastases and abscess	1	2.5%
Total malignant	*16*	*40.0%*
Benign		
Cysts	13	32,5%
Cysts and hemangioma	4	10%
Cysts and steatosis	1	2.5%
Hemangioma, steatosis and calcifications	1	2.5%
Abnormalities too small or nonspecific to characterize	5	12.5%
Total non- malignant	*24*	*60.0%*
Total lesions	40	100%

The sensitivity of CEUS in the diagnosis of pancreatic tumor metastasis was 80% (12/15), its specificity was 98.6% (73/74) and its kappa was 0.833 (SE 0.08).

Computed tomography versus contrast-enhanced ultrasound

In total, 18/89 (20.2%) patients were suspected of having liver metastases on imaging. In suspected lesions, 11/18 (61.1%) patients were identified by both CT and CEUS, 2/18 (11.1%) patients only by CEUS and 5/18 (27.8%) patients only by CT.

Actual liver metastases were proven in 15 patients. In 11 (73.3%) patients, the suspected lesions were observed by both CT and CEUS. In one (6.7%) case, the metastasis was observed only by CEUS, whereas no patient metastases were diagnosed only by CT. Thus 12/15 (80%) patients had correctly suspected metastasis on an imaging (CEUS and/or CT) and 3/15 not suspected.

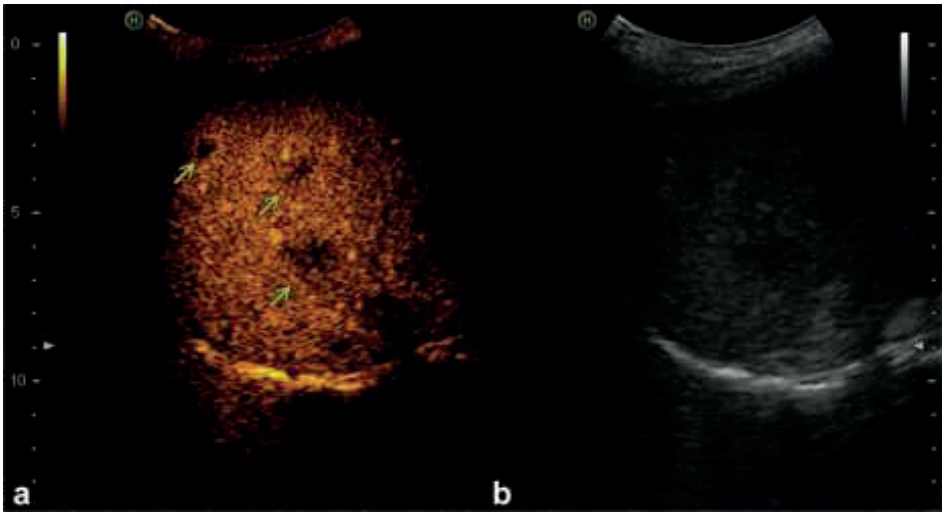

Fig. 1. Metastases detected by contrast-enhanced ultrasound. (a) Significant washout in multiple focal lesions 83 s after contrast IV injection. (b) Control native, non-enhanced ultrasound. Liver parenchyma heterogeneity can be easily misinterpreted.

From the 12 patients with metastasis, there were six patients with only one metastasis (diameter: 8, 12, 15, 18, 23 and 31 mm), one patient with two metastases (max 30 mm), one patient with three metastases (max 18 mm) and four patients with multiple metastases (8, 9, >10 and >10).

In three (20%) metastatic patients, no suspected lesions were found by either CT or CEUS. In these three patients with metastases diagnosed during operation, all were located on the liver surface, seen or palpated during open laparotomy. The average diameter cannot be correctly calculated (one was only 8 mm in diameter, and two had more than one metastasis with diameters not specified by the surgeon). Those three patients also had a longer mean interval between imaging and operation (CT 60 d, CEUS 31 d).

There was no significant difference between the sensitivities of the methods (73.3% vs. 80%, p = 1.00).

It would be interesting to investigate the performance for number and size of lesions; however, our study was too low-powered to test the answer, as the numbers were too small for a proper analysis (7/15 had only one metastasis [diameter 16.4 mm; 6 detected, 1 undetected by imaging], 2/15 had two or three metastases [max diameter 24 mm, all detected] and 6/15 had multiple [four detected, two undetected by imaging]). The sensitivity of different imaging methods relative to lesion number and size is as follows:

CEUS: one lesion, 85.7% (6/7); two to three lesions, 100% (2/2); and more than three lesions, 66.7% (4/6); <1 cm, 50.0% (1/2); 1–2 cm, 100% (6/6); and >2 cm, 71.4% (5/7).

CT: one lesion, 86.7% (6/7); two to three lesions, 100% (2/2); and more than three lesions, 50.0% (3/6); <1 cm, 50% (1/2); 1–2 cm, 83.3% (5/6); and >2 cm, 71.4% (5/7).

It could be suggested that larger and multiple lesions would be easier to detect as opposed to smaller individual ones. In our study, the one undetected lesion was only 8 mm and located on the liver surface, which made it even more difficult to visualize on any imaging technique. However, one third (2/6) of multiple metastases were missed. It is possible that other factors played a role, such as the previously mentioned interval (31 d between CEUS and operation); however, this interval did not differ from other studies in detection of liver metastasis from gastrointestinal cancer (4 wk in paper by Piscaglia [18]. The real importance of a shorter interval stays open and will be a topic for further clinical discussion.

Table 2. Focal liver lesions found by CEUS

Lesions	Frequency (n)	Percentage of n = 36
Malignant		
Metastases	7	19.4%
Metastases and cysts	2	5.5%
Metastases, cysts and hemangioma	1	2.8%
Metastases, hemangioma and steatosis	1	2.8%
Metastases and steatosis	2	5.5%
Total malignant	*13*	*36.1%*
Benign		
Cysts	16	44.4%
Hemangioma	1	2.8%
Cysts and steatosis	1	2.8%
Hemangioma and steatosis	1	2.8%
Abscess (bacterial)	1	2.8%
Abscess (amoeba)	1	2.8%
Focal non-steatosis	2	5.5%
Total non-malignant	*23*	*63.9%*
Total lesions	36	100%

CT and CEUS were both significant univariate predictors for the presence of liver metastasis in patients with pancreatic cancer. To compare CT and CEUS as predictors of liver metastasis, the results of both imaging methods were simultaneously entered into a logistic regression model. CEUS remained significant ($p < 0.001$), whereas CT did not add any additional information ($p = 0.292$).

A Venn diagram (Fig. 2) shows results of all possible metastases outcomes between both imaging methods (CT and CEUS) and the gold standard. Of notice is a large red area representing false-positive CT results.

Biliary drainage, stents and surgery

During CEUS, 6/89 (6.7%) patients had a percutaneous transhepatic cholangiography drain, 61/89 (68.5%) had a biliary stent and 4/89 (4.5%) had both a stent and a drain.

A total of 80 patients underwent surgery/diagnostic laparoscopy, whereas surgery was not performed in 17 patients. However, six (35.2%) of these patients did not undergo surgery because both CT and CEUS had already visualized suspicious lesions. In those patients, the lesions were proven to be malignant during further clinical observation.

Six patients who underwent surgery had actual liver metastases, diagnosed by perioperative biopsy. In three (50%) of those patients, the suspected lesions had already been observed by CEUS (CEUS alone in one patient; by both CEUS and CT in two patients). These lesions could not be histologically confirmed pre-operatively due to small size.

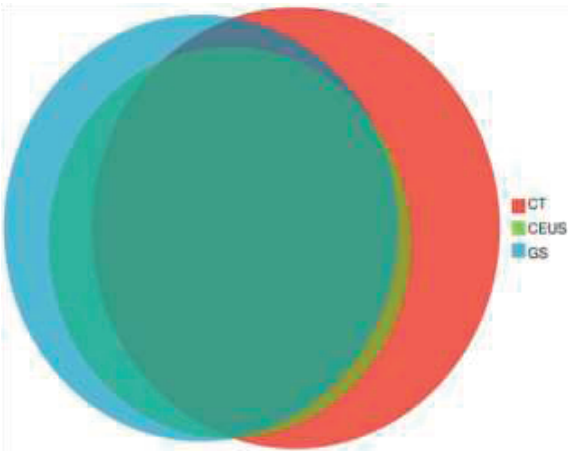

Fig. 2. Venn diagram showing results of all possible metastases outcomes between both imaging methods (CT and CEUS) and the gold standard. Of notice is a large red area representing false positive CT results. CT = computer tomography; CEUS = contrast-enhanced ultrasound; GS = gold standard.

DISCUSSION

The good performance of CEUS in detecting liver metastasis is already well known and established [19]. Still, in most studies, the patient population had liver metastases from various primary extra-hepatic tumors or only from colorectal cancer. Here, we present the first study in which CEUS was performed to assess the detection of liver metastases only in patients suspected of having pancreatic or periampullary carcinomas.

According to the results of this study, we can conclude that CEUS has a comparable sensitivity to CT in the detection of liver metastases (80% CEUS v. 73.3% CT, p = 1.00 by exact test) from suspected pancreatic or periampullary cancer. As already stated in a previous study [18], several metastatic lesions were detected by only one of the techniques, CT or CEUS, and this fact reflects complementarity of both methods with possible clinical implementation of both modalities in clinical practice. Sensitivity of CEUS and CT in detecting metastases was somehow lower in our study compared to previous reports (CEUS: 80% – 95%, CT 76% – 91% [20]); whether this reflects different characteristics of pancreatic adenocarcinoma remains an open question.

However, the main difference between CT and CEUS in our study was the ability of CEUS to differentiate between metastasis and accidental liver lesions that were later shown to be benign (cysts in two patients and lesions that were difficult to correctly characterize on CT in three patients, one due to suboptimal scan quality, one hypervascular lesion that turned out to be shunt [on MRI] and one lesion thought to be a metastasis or a focal bile duct dilation that turned out to be the latter on CEUS). In all these cases, CT yielded a higher number of false-positive results and thus a lower PPV (68.7% vs. 92.3%).

In a logistic regression model, CEUS remained significant ($p < 0.001$), whereas CT did not provide any additional information.

CEUS is accurate in distinguishing between malignant and benign liver lesions. We should keep in mind that benign liver lesions can be found at the same frequency in patients with liver metastases as in the healthy population. In our study, 29% of the patients had benign focal lesions in their livers that were unrelated to pancreatic cancer. CEUS may thus reduce the number of unnecessary imaging procedures and invasive examinations and even prevent unnecessary laparotomies. Thus far, only a few false-positive malignant liver findings have been observed by CEUS, mainly due to abscesses, necrosis, focal scar tissue, sarcoidosis or inflammatory pseudotumors [21].

Several studies have evaluated the costs of liver CEUS compared with CT. The main conclusion of those studies was that CEUS is a cost-efficient method for the detection of focal liver lesions during first-line diagnosis compared with first-line contrast CT or first-line contrast MRI [22-23]. CT cannot be replaced by CEUS and is still necessary to define the local resectability of the primary tumor, thus the comparison of cost efficiency between first-performed diagnostic CT and CEUS does not arise. Still, the costs of unnecessary laparotomies and further imaging can be prevented when CEUS is adopted as an indispensable part of the diagnostic algorithm, perhaps in combination with percutaneous biopsy to characterize unclear lesions.

Even for multi-detector CT, the ability to detect small hepatic metastases is approximately 75% [24-25]. CT has difficulty in differentiating small hypo-vascular liver

metastasis from small benign lesions that also appear hypovascular, such as cysts and hemangiomas. The sensitivity of CEUS in detecting liver metastasis is generally approximately 86%–94% [26]. During CEUS, the metastasis typically appears as a very short arterial enhancement (peripheral or diffuse) or remains isoechogenic, followed by rapid washout in the portal phase. The primary advantages of CEUS are its realtime scanning capability and high temporal resolution. A CT obtained at a single time-point can miss shorter, early enhancements. Additionally, in contrast to CT, rapid and complete washout in the portal phase is an invariable characteristic of metastasis during CEUS [27].

In primary pancreas tumor staging, CT is mandatory and cannot be replaced by CEUS. However, complementary CEUS after CT scan can rule out both false-positive (i.e., small or unclear lesions) and false-negative CT findings in the liver, and so CEUS can hold an important role in the patient's workup.

The limitations of this study include small sample size. Whether a longer interval between imaging and operation can play a role in false-negative CT and CEUS is an open question, as our performance results come from current clinical practice. It would be also nice to include size and number of lesion study performance; however, our study power was too low.

Several limitations of ultrasound also remain, such as its operator dependence and the limited visibility of certain portions of the liver, especially in an obese patient with liver steatosis. In our series, the one false-positive diagnosis of metastasis was due to liver steatosis in which an area of focal non-steatosis mimicked a hypoechogenic tumor. This lesion was observed but was incorrectly interpreted. In a case of doubt we can increase CEUS performance by repeating contrast agent injection(s) and/or with ultrasound setting adjustment.

CONCLUSION

CEUS can improve the detection of liver metastases in patients with suspected pancreatic cancer in comparison with CT. Our data suggest that CEUS can improve the determination of benign versus malignant liver lesions, detect false-positive or indeterminate CT results and spare the patient unnecessary diagnostic procedures. Therefore, CEUS could be recommended as a part of screening protocol before pancreatic resection.

Acknowledgment— The study was partially funded by ZonMW, The Netherlands, dossier number 170991010. Period 2009–2011.

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CHAPTER 7

Characterization of focal liver lesions by contrast-enhanced ultrasonography: economic evaluation

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Submitted 2020

ABSTRACT

Objectives: Liver imaging techniques aim to correctly characterize focal lesions and influence choices of therapeutic strategies. The objective of this study was to compare diagnostic efficacy and direct medical costs of contrast-enhanced ultrasonography (CEUS) to magnetic resonance imaging (MRI) or computed tomography (CT) in the characterization of focal liver lesions (FLL).

Methods: Prospective study enrolled 170 patients. Two diagnostic algorithms were compared to standard reference: (1) Ultrasonography (US) followed by MRI/CT and, (2) US followed by CEUS. In the economic evaluation, the perspective of the healthcare sector in the Netherlands was used. Clinical outcomes were 'correctly/incorrectly characterized' benign and malignant lesions and life-years (LY). Model inputs were taken from the hospital database, literature and publicly available sources. Univariate and probabilistic sensitivity analyses were performed.

Results: CEUS was able to identify benign and malignant FLLs with a sensitivity of 96.9% and specificity of 92.3%. For correct tumor subgroup characterization, sensitivity and specificity were 86.2% and 85.6%, respectively. Base-case results of the economic evaluation revealed that the CEUS strategy had similar effectiveness compared to the MRI/CT strategy (incremental effects of 0.002 LYs) and resulted in cost-savings of €452 per patient. The cost-savings for diagnostic phase and treatment phase were €160 and €292, respectively. The main drivers of variation were sensitivity, specificity and cost of the diagnostic tests. Results robustness was confirmed by probabilistic sensitivity analysis.

Conclusions: CEUS is a highly accurate and cost-saving alternative compared to the traditional procedures and should be considered as the front-line option in the characterization of FLLs.

INTRODUCTION

Abdominal ultrasonography (US) is the most common baseline imaging modality for patients with liver disease. However, US findings do not provide sufficient specificity to differentiate benign from malignant focal liver lesions (FLL) and is now regarded as inadequate in their characterization [1-2]. The reported specificity range for unenhanced or baseline US in characterizing FLLs is between 23% and 68% [3].

Contrast-enhanced ultrasonography (CEUS) is increasingly accepted in clinical settings for diagnostic imaging of FLLs [4-8].

Other imaging modalities such as contrast-enhanced computed tomography (CT) and magnetic resonance imaging (MRI) are performed to detect and characterize hepatic lesions [9]. But, there are risks associated with both CT and MRI technologies. The contrast agent used in MRI/CT could be harmful in patients with renal failure or for those allergic to iodinated agents. Similarly, there are risks, including cancer, associated with repeated use of both CT and MRI [10-11]. In addition, the use of MRI excludes identification of patients with claustrophobia, leads to disproportionally long waiting times, and high costs.

The European Federation of Societies for Ultrasound in Medicine and Biology (EFSUMB) published guidelines and recommendations for the use of contrast-enhanced ultrasonography in the characterization of FLLs [12]. Recent studies showed that CEUS and MRI had comparable diagnostic performance in the characterization of FLLs and numerous multicenter and single center publications reported no significant difference between the diagnostic efficacy of CEUS, MRI and/or CT [13-17]. A meta-analysis also confirmed high pooled sensitivity and specificity of CEUS and reported no significant difference compared with contrast-enhanced CT and contrast-enhanced MRI [14].

In addition to the diagnostic performance, cost-effectiveness considerations of health care interventions are important due to rising costs. Economic evaluations together with clinical analyses provide the foundation for allocation of various resources including new technologies, treatments and procedures. Economic analyses indicated that CEUS was a cost-effective replacement for CT and MRI in various clinical situations [18]. Therefore, the objective to be addressed in this study was to compare diagnostic performance and direct medical costs of CEUS with MRI or CT in the characterization of FLLs in the Netherlands.

METHODS

Clinical Evaluation

I. Patients and Procedure

Total 199 patients were eligible to participate in this prospective single center study, at the Erasmus MC, Rotterdam, the Netherlands. 29 patients were excluded because of absence of control imaging and/or informed consent. The study population consisted of 170 prospectively enrolled patients (66 men and 104 women), with a mean age of 50.5 yrs (19-86 yrs, SD 15.8) with at least one focal liver lesion detected with baseline ultrasonography. Exclusion criteria were age <18 years, pregnant or lactating women, contraindication to any of the contrast agents, and/or inability to provide informed consent. All subjects underwent CEUS examination, MRI and/or CT for further characterization of their lesions. Patients with benign lesions were followed for at least six months. Patients with malignant lesions received curative treatment or palliative care according to current clinical recommendations. Treatment strategies included in the analysis were related to the primary liver disease only. Clinical and economic outcomes for secondary metastatic sites were excluded. The protocol of the study was approved by the local medical ethical committee. All patients provided written informed consent to participate in the study, which was performed according to the guidelines provided in the Declaration of Helsinki.

II. Data Collection

Data collection for the clinical analysis was performed at the single-patient level (bottom up approach) and patients were used as their own control. In addition to the traditional diagnostic protocol for FLL characterization, in which MRI or CT followed unenhanced US, CEUS was performed in all subjects. Two experienced sonographists who were blinded to the results of other techniques performed diagnostic examinations. The sonographists were informed about age and sex of each patient, as it represents obvious patient characteristics during examination.

III. Imaging Technologies

Ultrasonography was performed with Hi-Vision Preirus equipment (Hitachi Medical Systems) using contrast agent (SonoVue, Bracco, Italy). Only dominant FLLs were selected for ultrasound image evaluations. Diagnostic criteria for FLLs were followed according to published EFSUMB guidelines [12] [19-20].

Magnetic resonance imaging (MRI) examinations were performed by 1.5 Tesla (Philips Medical Systems, the Netherlands or General Electric, WI, USA), by using a body-array coil, with identical scan protocols. Contrast agent was either non-liver specific gadolinium-chelate (Magnevist, gadopentate-dimeglumine, Schering,

Germany) or liver specific (Multihance, gadobenate-dimeglumine, Bracco, Italy). Computed tomography (CT) examinations were performed with a 64-section scanner (Siemens, Somatom Sensation 64).

The typical appearances of the lesions on MRI and results of histopathology obtained from biopsy or surgical specimens and judgments of clinicians were used as reference standard for diagnosis and characterization. Due to ethical reasons, biopsy samples were not taken without clinical indication. Therefore, it was not feasible to use pathology results from all patients as the reference standard. Biopsy was taken only when deemed absolutely necessary for the diagnosis, and was thus performed according to current guidelines and good clinical practice. CT served as a second reference imaging method when MRI was inconclusive, in patients with claustrophobia or when histology examination was not applicable.

IV. Diagnostic Performance

All imaging results were interpreted in a blind manner by two experienced sonographists and consensus was achieved. At the time of analysis, the examiners were unaware of the final diagnosis and the results of other techniques.

Statistical analysis was performed using SPSS software (SPSS Inc, Chicago, IL, USA). Sensitivity of each diagnostic imaging technology was calculated as the percentage of true malignant lesions out of the total number of true malignant and false benign lesions. Specificity was calculated as the percentage of true benign lesions out of the total number of true benign and false malignant lesions.

Diagnostic performance of CEUS, MRI and CT were compared to identify FLLs as benign, malignant and further characterize them into subgroups (i.e. adenoma, FNH, hemangioma, hepatocellular carcinoma (HCC), cholangiocellular carcinoma (CCA), metastasis, other benign and other malignant).

Economic Evaluation

I. Decision Analytic Model

A decision analytic model was developed using TreeAgePro 2009 Software Inc. (Williamstown, MA, USA) to estimate incremental costs and effectiveness of diagnostic imaging technologies from the healthcare perspective in the Netherlands. The results of the clinical evaluation were used as base-case inputs for the economic model. The diagnostic performance of CEUS was compared to MRI/CT. Reference standard comprised imaging (MRI or CT-in case MRI was not applicable), histology and judgments of clinicians. The clinical outcomes were expressed in life-years (LYs). Health related quality of life measures were not available. Figure I. shows the schematic representation of the economic model. The first three branches that originate from the decision node represent the choices between CEUS, MRI/CT and standard

reference. The branches emanating from each chance node represent the probability that a patient is identified correctly or incorrectly with a benign or malignant lesion (with further subgroup characterization). Each subsequent branch emanating from the second chance node reflects the possible outcomes that may occur after diagnosis. Outcomes considered in the model for malignant group were curative treatment after diagnosis or palliative care. Outcomes for benign group were follow-up after diagnosis or no follow-up.

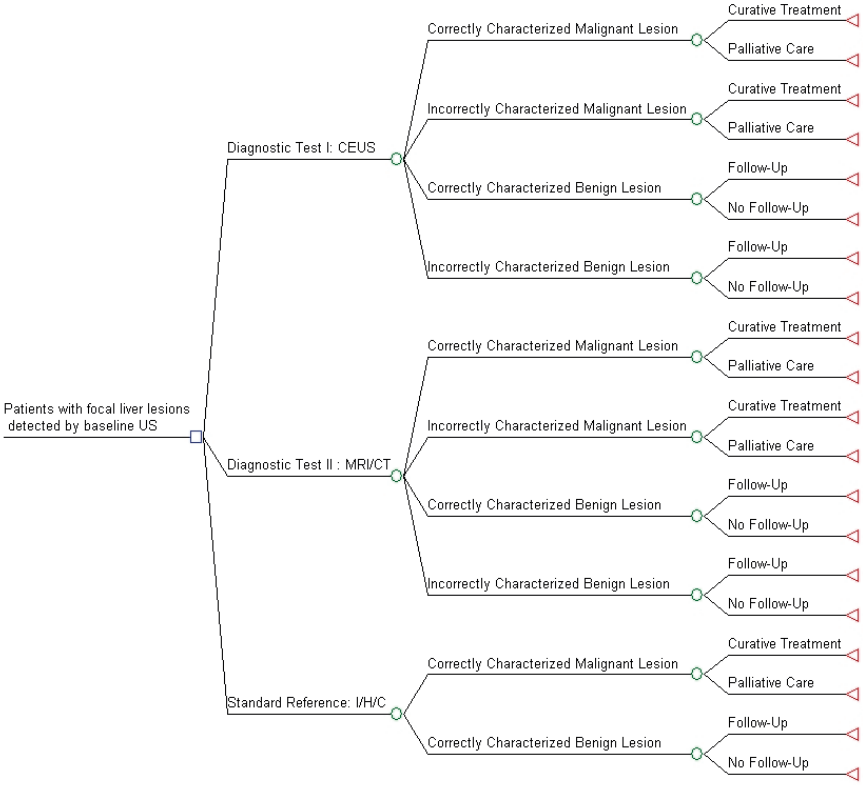

FIGURE 1: The decision tree. The decision tree represents the population of patients with focal liver lesions detected by baseline US. Subgroups defined by CEUS diagnostic strategy were then defined as patients with true positive/ false positive (malignant) and true negative/false negative (benign) tumors. Patients in the MRI/CT diagnostic strategy arm were also defined as having true positive/false positive (malignant) and true negative/false negative (benign) tumors. Malignant and benign tumors were characterized further into subgroups (HCC, CCA, Metastasis & Hemangioma, Adenoma, FNH). The branch defined as the Standard Reference: I/H/C strategy represents patients based on typical appearances of their lesions (true positive (malignant) and true negative (benign) after imaging or results of histopathology/FNAB obtained from biopsy or surgical specimens as well as judgments of clinicians. Outcomes were defined as curative treatment after diagnosis or palliative care for malignant lesions. For benign lesions, outcomes were follow-up after diagnosis or no follow-up.

II. Resource Use and Unit Costs

Direct medical costs were considered. The following hospital costs were included in the analyses: costs of diagnostic strategies such as MRI, CEUS, CT and 79MBq TC^{99M}, liver biopsy, laboratory tests, intensive care days, hospital days, outpatient visits and costs of several treatment strategies such as radiofrequency ablation (RFA), transarterial chemo-embolization (TACE), surgical resection, chemotherapy, palliative care, targeted therapy and screening for liver transplantation. Resource use and unit costs are presented in Table I. For CEUS, the only additional cost compared to the baseline US was the price of the contrast agent. The volume of the cost items was obtained from the Erasmus University Medical Center database and patient files. The costs of the medications were based on the prices listed on the Dutch Health Care Insurance Board (Pharmacotherapeutic Compass – CVZ) [101]. For items with low cost prices (i.e. laboratory tests) Dutch tariffs and the hospital data were used as proxies of the real values. Time horizon was two years. Costs and effects beyond first year were discounted at a rate of 4% and 1.5% respectively, in abidance with the Dutch guidelines (CVZ) for cost-effectiveness analyses [102]. The base year was 2010 (in Euros). All costs were inflated to 2010 values using Statistics Netherlands (CBS) website [103].

III. Model Assumptions

Based on available data, the following assumptions were made in the economic analysis: (a) Diagnostic costs were assumed to include the cost of the imaging tests, one outpatient visit and one consultation by phone. (b) Outpatient visits were assumed to be on average three visits per benign and six visits per malignant groups. (c) Average hospital duration in case of complications was assumed to be seven days. (d) Mean laboratory tests were assumed to be €5559 per malignant group and €656 per benign group, based on the patient data. (e) Cost of diagnostic test for CEUS was assumed to be the total of the cost of baseline ultrasonography and the contrast agent SonoVue. (f) Treatments for HCC included in the study were liver resection, RFA, TACE and screening for liver transplantation. Patients with advanced tumor stage and poor liver function were eligible for palliative care. (g) Palliative care was assumed to last for four months. At the end of four months patients were assumed to be deceased. (h) Palliative care was assumed to include general practitioner visits, day-care and medication. Mean costs for palliative care patients were calculated as €12,000. (i) Gemcitabine (1000mg) and cisplatin (250mg) were assumed to be chemotherapy agents for eligible patients. Administration of doublet chemotherapy was on average six cycles. (j) Costs and effectiveness calculations for secondary metastatic sites were excluded. (k) Patients eligible for targeted therapies were assumed to receive sorafenib (400mg, Nexavar, Bayer). Based on the results

Table 1: Resource Use and Unit Costs.
Direct medical costs from the hospital perspective were considered and were obtained from the hospital database and patient files. The base year was 2010. The costs of the medications and laboratory tests were based on the prices listed on the Dutch Pharmacotherapeutic Manual (Pharmacotherapeutic Compass – CVZ), Dutch tariffs and Erasmus Medical Center (EMC) data. Time horizon was two-years. Costs and effects beyond first year were discounted at a rate of 4% and 1.5% respectively, in abidance with the Dutch guidelines (CVZ) for cost-effectiveness analyses.

Resource Use	Unit Cost*	Source
Diagnostic Imaging		
MRI abdomen with contrast†	€ 250	NZA
CT abdomen with contrast†	€ 216	NZA
US abdomen without contrast†	€ 77	NZA
US abdomen with contrast (CEUS)	€ 154	NZA/EMC data‡
79 MBq TC-99M (Bridatec)	€ 313	NZA
Procedures & Treatments		
Liver Biopsy†	€ 186	NZA
Surgical Resection	€ 9,637	NZA
RFA- US guided ¶	€ 950	EMC data‡
RFA- CT guided §	€ 1,655	EMC data‡
TACE ¢	€ 1,355	EMC data‡
Chemotherapy (Gem 1000mg-Cis 25mg)	€ 372	F. Kompas (CVZ)
Targeted Therapy (Sorafenib 200mg)	€ 305	F. Kompas (CVZ)
Screening for Liver Transplantation	€ 31,362	NZA
Palliative Care		
Mean GP visits/4 months	€ 751	CVZ
Mean Care Day /4months	€ 11,005	CVZ
Mean Medications/4 months	€ 34	F. Kompas (CVZ)
Furosemid (80mg)	€ 1	F. Kompas (CVZ)
Spironolactone (300mg)	€ 4	F. Kompas (CVZ)
Propanolol (80mg)	€ 3	F. Kompas (CVZ)
Laboratory Tests & Other Resource Use		
Mean Lab Tests for malignant group	€ 5,559	EMC data‡
Mean Lab Tests for benign group	€ 656	EMC data‡
Inpatient Hospital Day	€ 669	van Gils et. al 2010¥
Outpatient Visit	€ 127	van Gils et. al 2010¥
Intensive Care Day	€ 2,120	van Gils et. al 2010¥
Consultations by telephone	€ 14	Handleiding voor Kostenonderzoek (CVZ)

* All values were reported in Euros (2010). Decimal digits were rounded to the nearest whole number.
† Including specialist fees
¥ Pilot outcomes research: Effects and costs of oxalipatin stage III colon and metastatic colorectal cancer. (July 2010)
‡ Erasmus University Medical Center
¶ Radio Frequency Ablation -Ultrasound Guided
§ Radio Frequency Ablation -Computed Tomography Guided
¢ Transarterial chemoembolization

of recent trials, sorafenib is currently recommended by the U.S. Food and Drug Administration (FDA) for the management of advanced HCC patients. In the model, administration of sorafenib was assumed to be for four months. (l) Time horizon was assumed to be two-years. (m) Life expectancy of benign group was assumed to remain unchanged in the model. (n) Twenty percent of HCC patients were assumed to be deceased at the end of two-years. (o) Metastasis, cholangiocarcinoma and other malignant groups were assumed to survive for twelve months. (p) Screening for liver transplantation was included in the analysis. Two-year time horizon did not capture patients undergoing liver transplantation. (r) Costs and probabilities for treatment, follow-up and no follow-up phases were assumed based on the reference standard.

IV. Uncertainty Assessment

The uncertainty and robustness of the model were evaluated by univariate sensitivity analysis. In addition, probabilistic sensitivity analysis was performed using Monte Carlo Simulation (30). In this method, the chosen parameter estimates in the model were randomly drawn from probability distributions based on many simulations. Triangular distributions for costs, beta distributions for diagnostic accuracy inputs and Dirichlet distributions for probabilities were applied. The results of these simulations were then used to generate probabilities. The cost-effectiveness plot indicates the confidence limits that can be placed around the base case.

RESULTS

Clinical Evaluation

I. Baseline Patient Characteristics

The study included 170 patients (19-86 yrs) with average age of 50.5 yrs (SD 15.8) and a male: female ratio of 66:104 (38.8%:61.2%). Average age for patients with benign lesions was 44.4 yrs (SD 13.3) and for malignant lesions 60.1 yrs (SD 14.6).

Mean lesion diameter was 54.3 mm (8-180 mm, SD 33.8, median 46.0 mm). Benign lesions were present in 104 (61.2%) and malignant in 65 (38.2%) patients. No tumor was finally detected in one patient (false positive findings). Liver cirrhosis was present in 41 patients (24.1%). In cirrhotic patients the diagnosis of lesion was predominantly malignant (35/41 cirrhotic subjects, 85.4%) whereas, in non-cirrhotic patients the diagnosis was mainly benign (98/129 non-cirrhotic subjects, 75.9%).

CEUS was performed in all patients (100%) in repeated 2 ml boluses. No adverse reactions were observed. At least one MRI examination was performed in 114 patients (67.1%), and CT examination in 111 patients (65.3%). In 55 patients both MRI and CT were performed. The total number of MRI and CT examinations performed

in all patients was 242 (mean: 1.42 exam/patient). In one subject, a ^{99m}Tc scan was also performed.

II. Diagnostic Performance

CEUS was able to identify benign and malignant FLLs with a sensitivity of 96.9 % and specificity of 92.3%. The overall diagnostic accuracy was 94.1%. MRI/CT combined strategy was able to identify benign and malignant FLLs with a sensitivity of 95.4% and specificity of 90.4%. The overall diagnostic accuracy was 92.3%.

In the clinical analysis, focal lesions characterized as benign were further divided into four subgroups, which were defined as hemangioma, adenoma, focal nodular hyperplasia (FNH) and other. Similarly, FLLs characterized as malignant were further divided into four subgroups, which were defined as hepatocellular carcinoma (HCC), cholangiocarcinoma (CCA), metastasis and other.

For correct subgroup characterization, the results were somehow lower: the sensitivity, specificity and diagnostic accuracy of CEUS were 86.2%, 85.6% and 85.8%, respectively. MRI/CT combined strategy was able to characterize subgroups with the sensitivity, specificity and diagnostic accuracy of 86.2%, 86.6% and 86.4%, respectively. The results were similar when detailed subgroup characterization was performed.

Table II. shows incorrectly characterized focal lesions including subgroups by CEUS and MRI/CT strategies. Category I represents correctly identified lesions (majority) by both technologies. Category II indicates incorrectly characterized focal lesions by MRI/CT strategy only. (i.e. a patient with HCC was incorrectly characterized as having adenoma by MRI/CT). Patients in this category were correctly identified by CEUS according to the reference standard. Category III shows incorrectly characterized focal lesions by the CEUS strategy only. (i.e. a patient with HCC was incorrectly characterized as having CCA by CEUS). Patients in category III were correctly identified by MRI/CT strategy according to the reference standard. Category IV shows incorrectly characterized lesions by both CEUS and MRI/CT strategies (the overlap of mistakes resulted by both CEUS and MRI/CT). (i.e. a patient with FNH was characterized as having HCC by MRI/CT and characterized as having adenoma by CEUS.) Histology examinations (except for one benign case) and judgments of clinicians were referred to establish the reference standard.

Economic Evaluation

I. Base Case Results

Base case results of the economic evaluation revealed that total discounted per patient costs associated with CEUS and MRI/CT strategies were estimated by the model to be €8,309 and €8,761, respectively. The costs of diagnosis and treatment

Table II: Incorrectly characterized focal liver lesions by CEUS and MRI/CT.

		MRI/CT	MRI/CT
		Correctly Identified FLL	Incorrectly Identified FLL
CEUS	Correctly Identified FLL	Category I: AGREEMENT	Category II: Incorrect by MRI/CT
CEUS	Incorrectly Identified FLL	Category III: Incorrect by CEUS	Category IV: OVERLAP

Category II	**CEUS**	**MRI/CT**	**Gold Standard**	**Remarks**
4 Patients (malignant)	HCC	Adenoma	HCC	Surgery
	CCA	Metastasis	CCA	Biopsy
	Metastasis	Other Benign	Metastasis	Clinical Judgement
	Metastasis	Other Benign	Metastasis	Biopsy
6 Patients (benign)	Hemangioma	Other Benign	Hemangioma	Clinical Judgement
	Hemangioma	CCA	Hemangioma	Surgery
	Adenoma	Other Malignant	Adenoma	Clinical Judgement
	Other Benign	HCC	Other Benign	Clinical Judgement
	Other Benign	Metastasis	Other Benign	Clinical Judgement
	Other Benign	FNH	Other Benign	Clinical Judgement

Category III	**CEUS**	**MRI/CT**	**Gold Standard**	**Remarks**
4 Patients (malignant)	CCA	HCC	HCC	Clinical Judgement
	Other Malignant	CCA	CCA	Biopsy
	Other Benign	Metastasis	Metastasis	Clinical Judgement
	Other Benign	Metastasis	Metastasis	Clinical Judgement
7 patients (benign)	Other Malignant	Hemangioma	Hemangioma	Clinical Judgement
	Other Malignant	Adenoma	Adenoma	Clinical Judgement
	Adenoma	FNH	FNH	Clinical Judgement
	Adenoma	FNH	FNH	Clinical Judgement
	Adenoma	FNH	FNH	Clinical Judgement
	HCC	Other Benign	Other Benign	Clinical Judgement
	No tumor found	Other Benign	Other Benign	Clinical Judgement

Category IV	**CEUS**	**MRI/CT**	**Gold Standard**	**Remarks**
5 Patients (malignant)	Meta/HCC	HCC/Meta	HCC/CCA	Biopsy
	Other Malignant	Other Benign	Metastasis	Biopsy
	Other Malignant	CCA	Other Malignant	Biopsy
	CCA	CCA	Other Malignant	Surgery
	Other Malignant	HCC	Other Malignant	Biopsy
6 Patients (benign)	Other Malignant	Other Malignant	Hemangioma	Biopsy
	Other Malignant	CCA	Hemangioma	Surgery
	Adenoma	HCC	FNH	Biopsy
	Other Benign	CCA	Other Benign	Clinical Judgement
	Metastasis	Metastasis	Other Benign	Biopsy
	Other Malignant	Nonspecific Tumor	Other Benign	Biopsy

Table shows incorrectly characterized lesion in subgroups. Category I represents correctly identified lesions (majority) by both imaging modalities and is not show in the table. Category II indicates incorrectly characterized FLL's by MRI/CT strategy only. Patients in this category were correctly by CEUS. Category III shows incorrectly identified lesions by CEUS strategy only and these patients were correctly diagnosed by MRI/CT strategy. Category IV shows incorrectly characterized by both imaging methods. Histology examinations (except for one benign case) were used as reference golden standard.

per branch were estimated by multiplying the resource use of each patient with the estimated average unit prices of diagnostic tests and therapeutic interventions. The incremental cost advantage of -€452 per patient was predicted for CEUS strategy. It was further estimated that -€160 was a cost advantage for diagnostic phase and -€292 was a cost advantage for treatment phase.

Life expectancy in the form of years saved or gained was the effectiveness endpoint of the study. Total discounted per patient life years (LYs) were 1.538 for CEUS

Table III: Parameters and distributions used for the sensitivity analysis:

	Model Input	Lower Limit -30%	Upper Limit 30%	Distribution
Cost of Diagnsotic imaging				
CEUS	€ 295	€ 207	€ 384	Triangular
MRI/CT combined	€ 455	€ 319	€ 592	Triangular

	Model Input	Lower Limit -30%	Upper Limit 30%	Distribution
Assumption Costs Per Subgroup				
HCC	€ 10,377	€ 7,264	€ 13,490	Triangular
CCA	€ 5,611	€ 3,928	€ 7,294	Triangular
Metastasis	€ 2,735	€ 1,915	€ 3,556	Triangular
Other Malignant	€ 6,236	€ 4,365	€ 8,107	Triangular
Hemangioma	€ 679	€ 475	€ 883	Triangular
Adenoma	€ 889	€ 622	€ 1,156	Triangular
FNH	€ 789	€ 552	€ 1,026	Triangular
Other Benign	€ 837	€ 586	€ 1,088	Triangular
Costs of Palliative Care	€ 12,000	€ 8,400	€ 15,600	Triangular

	Model Input	Lower Limit -30%	Upper Limit 30%	Distribution
Treatment Costs Per Subgroup				
HCC Treatment	€ 11,673	€ 8,171	€ 15,175	Triangular
CCA treatment	€ 12,423	€ 8,696	€ 16,150	Triangular
Metastasis Treatment	€ 5,562	€ 3,893	€ 7,231	Triangular
Other Malignant Treatment	€ 11,483	€ 8,038	€ 14,928	Triangular

	Model Input	Lower Limit -30%	Upper Limit 30%	Distribution
Follow Up Costs Per Subgroup				
Hemangioma	€ 3,900	€ 2,730	€ 5,070	Triangular
Adenoma	€ 2,269	€ 1,588	€ 2,950	Triangular
FNH	€ 305	€ 214	€ 397	Triangular
Other Benign	€ 270	€ 189	€ 351	Triangular

	Model Input	Lower Limit of 95% CI	Upper Limit of 95% CI	Distribution
Diagnostic Performance				
Sen CEUS	0.862	0.757	0.925	Beta
Spec CEUS	0.856	0.776	0.911	Beta
Sen MRI/CT	0.862	0.757	0.925	Beta
Spec MRI/CT	0.865	0.787	0.918	Beta

Probabilities	Model Input	Lower Limit of 95% CI	Upper Limit of 95% CI	Distribution
pHCC	0.68	0.556	0.778	Drichlet
pCCA	0.09	0.043	0.187	Drichlet
pMetastasis	0.17	0.097	0.278	Drichlet
pOther Malignant	0.06	0.024	0.148	Drichlet
pHemangioma	0.17	0.112	0.257	Drichlet
pAdenoma	0.36	0.27	0.451	Drichlet
pFNH	0.28	0.202	0.371	Drichlet
pOther Benign	0.19	0.213	0.279	Drichlet

Table shows input parameters for deterministic sensitivity analyses with lower and upper limits In order to identify model drivers and examine key areas of uncertainty, one-way deterministic sensitivity analyses were performed. For the efficacy parameters, 95% confidence intervals were used. Resource use and unit costs data were tested by varying the costs by +30% and -30% from the mean.

and 1.536 for MRI/CT, representing similar effectiveness results (incremental effects of 0.002 LYs) for both strategies. The incremental cost of -€452, for an incremental advantage of 0.002 LYs provided a favorable cost-effectiveness ratio (ICER) for CEUS, showing that CEUS strategy achieved slightly higher LYs for lower total per patient costs.

II. Univariate Sensitivity Analysis

In order to identify model drivers and examine key areas of uncertainty, univariate sensitivity analyses were performed. Ranges for effectiveness inputs and probabilities were based on 95% confidence intervals. Costs were varied between plus and minus thirty percent. Table III. shows input parameters for univariate sensitivity analyses with lower and upper limits.

The results of the sensitivity analyses indicated that the primary drivers of variation were the sensitivity and diagnostic cost of CEUS, specificity of MRI/CT and CEUS, treatment costs of HCC, diagnostic cost of MRI/CT and treatment costs of CCA (Figure II.). The most influential variable was the sensitivity of CEUS.

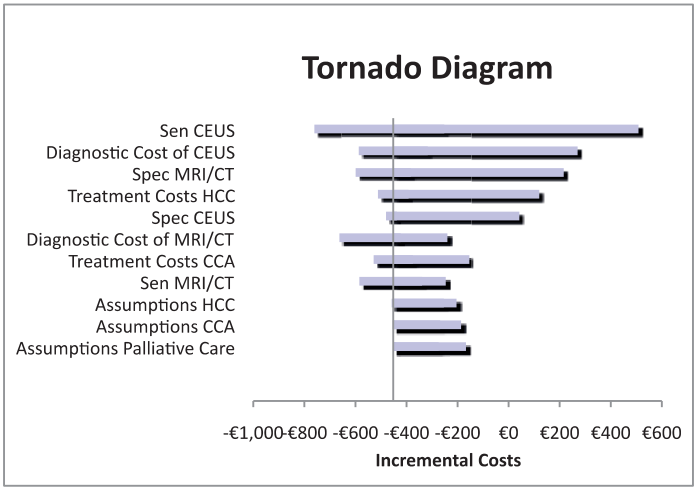

Figure 2: Tornado Diagram showing the primary drivers of variation. Tornado diagram represents the incremental costs of one-way sensitivity analysis of CEUS versus MRI/CT testing strategies. The horizontal line indicates the incremental costs under base case conditions. The results of the deterministic sensitivity analyses indicated that the most influential variable was the sensitivity of CEUS.

II. Probabilistic Sensitivity Analysis

Probabilistic sensitivity analysis (PSA) was performed using Monte Carlo Simulation [21]. The input parameters and corresponding distributions are presented in Table III. PSA results showed that, in the majority of the scenarios (90% of simulations),

the CEUS strategy was below the €20,000 acceptability threshold. Incremental costs per LYs were grouped below the origin suggesting cost-savings for the CEUS strategy (89.99% of simulations). Given the relatively small differences in estimated incremental LYs and costs for two strategies, the observed level of uncertainty around the incremental cost-effectiveness ratio was expected. Figure III. depicts cost-effectiveness scatter plot based on 100,000 simulations.

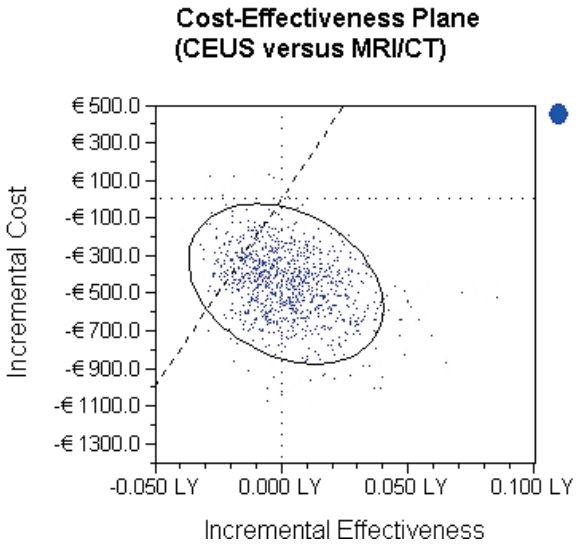

Figure 3: Incremental cost-effectiveness scatter plot of CEUS versus MRI/CT testing strategies. Probabilistic sensitivity analysis was performed by using Monte Carlo
Simulation and is based on 100,000 simulations. The incremental costs per LYs were grouped below the origin suggesting cost-savings for the CEUS strategy. Given the relatively small differences in estimated incremental LYs and costs for two strategies, the observed level of uncertainty around the incremental cost-effectiveness ratio was expected.

DISCUSSION

Base case results for CEUS and MRI/CT diagnostic strategies showed that CEUS was a valuable diagnostic tool in the management of focal liver lesions (FLLs) and it was cost-saving alternative compared to the current front-line diagnostic imaging work-up in the Netherlands. The cost-savings for diagnostic phase were comparable to other already published studies [22-28]. In one study, CEUS determined a change in the diagnostic workup [29]. But the main advantage of our study consists of adding a treatment phase. CEUS economic impact of diagnostic and treatment phase combined was to be found much higher and reached 452 €.

The proportion of correctly diagnosed benign or malignant lesion and characterization of different diagnostic subgroups was accurate and were similar to MRI/CT results.

It is believed that replacing MRI or CT for preoperative assessment of patients with liver tumors is unlikely. MRI and CT offer a more comprehensive assessment

of the liver parenchyma and the abdomen, which is mandatory to properly plan and stage any kind of surgical or interventional procedure. Furthermore, the panoramic pictures obtained by CT or MRI are considered necessary for correct staging and detection of lesions at sites other than liver. This CEUS drawback can be reduced by up-to-date abdominal ultrasound technologies, better operator training and by improvement in accompanying CEUS software and protocols.

In this study, one may argue that a single diagnostic imaging method should have been performed after baseline US. However, it would not be ethical to do a fully randomized study in which a treatment choice is based solely on one of the diagnostic methods to which patients are randomly allocated [30]. Instead, patients should be diagnosed with all necessary methods for correct tumor characterization. For each diagnostic method, the results will then be allocated anonymously to external experts for clinical evaluation [31]. We also used Sonovue as a contrast agent, instead Sonazoid could be another option [32].

In the clinical design of this study, the actual treatment of choice was based on all necessary methods; namely the standard reference (gold standard) results. In the economic evaluation, a treatment decision was based on the characterization of lesions according to each method. Thus, in the decision analytic model a patient was considered for a follow-up according to CEUS, who had in reality received surgical treatment according to the reference standard. The penalty of incorrect characterization of lesions was calculated based on reference standard results.

Characterization and treatment of malignant liver lesions in the economic evaluation model were based only on the primary liver site. Costs and effectiveness calculations did not capture secondary metastatic sites. Different patient characteristics with regard to other metastatic sites (lung, colorectal, brain and others) will likely yield more uncertainty in the outcomes.

Liver transplantation as a first-line treatment in eligible patients is shown to improve survival and is potentially cost-effective in selected groups when compared to other therapies [33-35]. In practice, some of medically eligible patients (with early stage small HCCs) never receive a transplant because the number of potential recipients continues to outnumber the number of donor organs or patients will continue to another treatment option [36]. In this study, a two-year time horizon is not long enough to capture the implications of the liver transplantation.

Guidelines for economic evaluations recommend use of a time horizon that allows inclusion of all relevant costs and effects [102]. In this study, we restricted the time horizon to two years, mainly because of the uncertainty in determining the long-term costs and life expectancy of cancer patients whose primary tumor sites were not liver. On the other hand, inclusion of a longer time horizon would not affect the overall conclusions presented in this study. The main objective of the performed

economic evaluation was to assess the incremental differences between different diagnostic imaging strategies. Hence, marginal differences in costs and effects based on correct diagnosis are more important than the absolute values in each strategy.

Last but not least, one may argue that there was a selection bias in this study. The presence of an FLL detected by US examination was one of the inclusion criteria. Therefore, our study may exclude some patients whose lesions were missed by baseline US. Nevertheless, this bias does not affect the conclusions of the present study but reflects the limitations of unenhanced US in general.

CONCLUSIONS

CEUS is a highly accurate and cost-saving alternative compared to the traditional diagnostic procedures and should be considered as one of the first line options in the characterization of FLLs. Further research should explore diagnostic performance and cost consequences that would result with the application of CEUS in other clinical settings.

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CHAPTER 8

Inflammatory and multiple hepatocellular adenoma are associated with a higher BMI

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Eur J Gastroent 2017

ABSTRACT

Aim: To identify patient and lesion characteristics associated with the occurrence of single or multiple hepatocellular adenoma (HCA)

Patients and methods: Using a tertiary centre database, we retrospectively collected information on patient and lesion characteristics, management and follow-up of all patients with HCA included between 2001 and 2016. Patients were classified into three groups; patients with a single HCA, 2–9 HCA and at least 10 HCA

Results: A total of 458 patients were diagnosed with HCA, including 121 (26.4%) with single HCA, 235 (51.3%) with 2–9 HCA and 102 (22.3%) with at least 10 HCA. Significant differences in the mean BMI were found, with the highest BMI in patients with more than 10 HCA ($P< 0.05$). The mean BMI was significantly higher in patients with inflammatory HCA compared with steatotic HCA (31 vs. 26, respectively, $P< 0.05$). Steatotic HCA were more often single lesions (22/55, 40%), whereas patients with inflammatory HCA were often diagnosed with multiple lesions (122/166, 73%).

Conclusion: Our series show a significantly higher BMI and frequency of inflammatory HCA in patients with multiple HCA compared with single HCA.

INTRODUCTION

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

All these different subtypes may present as a solitary lesion on imaging. A small minority of patients with HCA presents with liver adenomatosis (LA), defined by Flejou et al. [7] as the presence of more than 10 adenoma lesions in an otherwise normal liver parenchyma. Only several case reports and small case series with patients with more than 10 HCAs have been described [8]. However, as estimation of the exact number of HCAs appears to be difficult, the term liver adenomatosis has been replaced by multiple HCAs [3]. Multiple HCAs have been described to be present in ~50% of all HCA.

Studies describing risk factors of HCA are mainly based on analysis of a solitary HCA and include the long-term use of oestrogen-containing oral contraceptives, female sex and obesity [4] [9-10]. It has yet to be studied whether risk factors for multiple HCA differ from single HCA.

It may be questioned whether patients with multiple HCAs must be treated according to the same guidelines as those with solitary lesions. The EASL guideline on the management of benign liver tumours suggests treatment of these patients on the basis of the size of largest nodule as the risk of complications is not related to the number of HCA [3-11]. However, this might be challenging if there are multiple HCAs more than 5 cm in size. With the availability of advanced imaging techniques and their increased use, liver lesions, including multiple adenomas, seem to be diagnosed more often. The management of these lesions may be a challenge for physicians as the guidelines may not always be applicable.

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

PATIENTS AND METHODS

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

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

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

Patients were subdivided into three groups: single HCA, multiple (2–9) HCAs [multiple adenoma (MA)] and more than 10 HCAs or liver adenomatosis (LA). Noninvasive MRI diagnosis of HCA was made on the basis of the typical features including results of using liver-specific contrast agents. HCA subtypes (H-HCA, I-HCA, β-HCA, β-IHCA and U-HCA) were based on immunohistochemistry as described by the Bordeaux-group [12] or on typical MRI features: H-HCA diffuse and homogenous fat signal, IHCA hyperintensity on T2-weighted images and T1-hyperintensity on the delayed phase or atoll sign on T2-weighted images. β-HCA and U-HCA have no validated specific sign [13] [14].

DATA ANALYSIS

All analyses were carried out using the statistical package for the social sciences (SPSS) (Released 2013, IBM SPSS Statistics for Windows, version 22.0; IBM Corp., Armonk, New York, USA). Differences between groups were assessed using a one-way ANOVA for continuous variables or the χ^2-test for categorical variables. Statistical significance was considered at a P-value of less than 0.05.

RESULTS

Overall, 458 patients were included and 121 (26.4%) were found to have a single HCA, 235 (51.3%) had multiple HCAs and 102 (22.3%) had liver adenomatosis. Baseline characteristics are presented in Table 1. The median age at presentation was 39 (interquartile range: 15–78) years. Most patients were women (n=451, 98%), with 12 (2.6%) female patients having no history of oral contraceptive use. There were six male patients, all of whom were diagnosed with a single HCA. One was found to have an H-HCA, four were found to have an I-HCA and one had a U-HCA. The median follow-up period of all patients was 34 (interquartile range: 17–49) months. No malignant transformation of any HCA into a hepatocellular carcinoma was found in this period.

Table 1. Patient characteristics (N = 458)

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

This table shows characteristics of patients with single hepatocellular adenoma, multiple hepatocellular adenomas and liver adenomatosis.
HCA, hepatocellular adenoma; LA, liver adenomatosis; MA, multiple adenomas; OC, oral contraceptive. [a]Data are presented as median with the range in parentheses.
[b]Data are presented as n (%).
P values below 0.05 were considered statistically significant.

Comparison between these three groups showed a significant difference in BMI (kg/m^2), with a median of 27.7 in patients with a single HCA, 30.4 in patients with MA and 31.2 in patients with LA (Fig. 1). Pairwise post-hoc analysis showed a significant difference in BMI between single HCA and MA and HCA and LA. No difference was observed between MA and LA. Female sex and the use of oral contraceptives were significantly different between groups. A pairwise post-hoc analysis showed a difference between single HCA and MA and single HCA and LA. Oral contraceptive (OC) use was significantly higher in MA compared with single HCA and LA. There was no difference in age or bleeding of adenomas between groups.

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

was the most common subgroup in single HCA as well as MA (63%). The median BMI in I-HCA was found to be 30.9 compared with a median BMI in H-HCA of 26.0 and 29.7 in U-HCA 29.7 in β-HCA. Additional analyses were carried out in patients in whom the largest lesion exceeded 50 mm as this specific group should be considered for resection or other curative treatment as described in the EASL Clinical Practice Guidelines on the management of benign liver tumours [3]. Larger lesions were found in 56 (46%) single HCAs, 109 (46%) MAs and 54 (52%) LAs. There were significant differences in intervention between the three groups (Table 3). More patients with a single HCA underwent resection if the lesion exceeded 50 mm compared with patients with larger lesions in MA or LA

Table 2. Bordeaux classification

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

DISCUSSION

In this study, we describe the largest series of patients with HCA, MA and LA with a follow-up of more than a decade. A review by Veteläinen et al. [8] described 94 patients from case reports and case series with LA. They reported abdominal ultrasound to be the initial imaging in all 94 patients, but confirmation of the diagnosis using highly advanced imaging modalities such as MRI, with or without contrast, or a contrast-enhanced ultrasonography was often missing. Currently, in our hospital, all patients with a suspected benign hepatic tumour will receive an MRI in at least four phases (precontrast, arterial, portal and delayed) after administration of an intravenous bolus nonliverspecific gadolinium chelate or a liver-specific contrast agent (Gadoxetate disodium, Primovist; Bayer Healthcare, Berlin, Germany or Gadobenate dimeglumine, Multihance; Bracco Imaging, Milan, Italy). Furthermore, patients are assessed by contrast-enhanced ultrasonography using a second generation contrast agent Sonovue (2.4–4.8ml, intravenous; Bracco Ltd., High Wycombe, UK). Both imaging methods provide additional information that improves differentiation of liver lesions [15] [16].

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

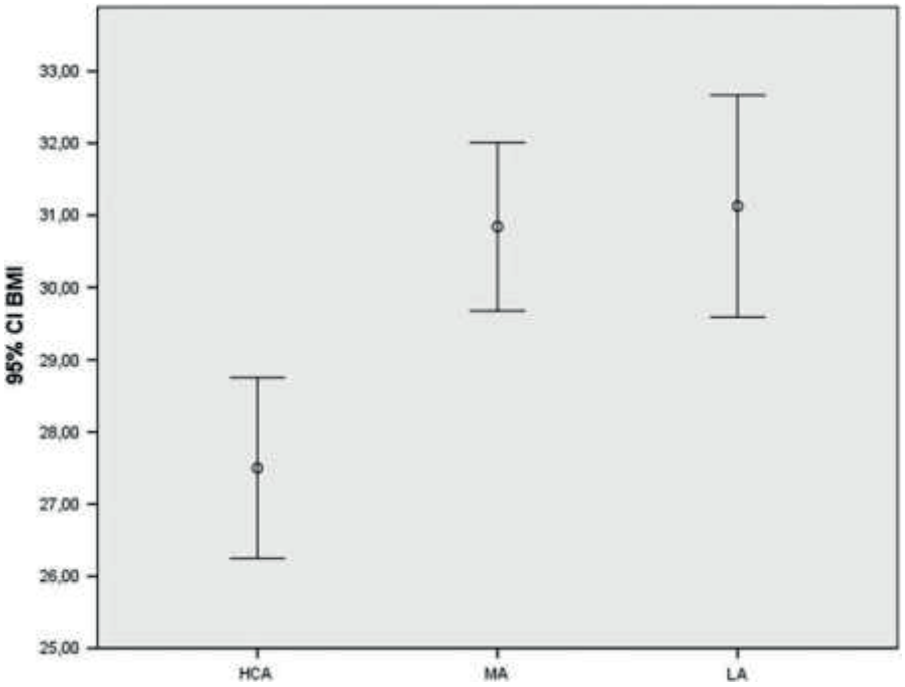

Fig. 1. BMI and number of adenomas. This figure shows BMI of patients with a single hepatocellular adenoma, multiple liver adenomas and liver adenomatosis.
CI, confidence interval; HCA, hepatocellular cellular adenoma; LA, liver adenomatosis; MA, multiple adenomas.
This table shows the Bordeaux classification the adenoma of patients with a liver adenomatosis, multiple liver adenomas and a single hepatocellular adenoma. β-HCA, hepatocellular cellular adenoma with mutations of the β-catenin gene; HCA, hepatocellular cellular adenoma; H-HCA, steatotic hepatocellular cellular adenoma; I-HCA, inflammatory hepatocellular cellular adenoma; LA, liver adenomatosis; MA, multiple adenomas; U-HCA, unclassified hepatocellular cellular adenoma without markers.

The aetiology and pathogenesis of HCA is unknown, although an association with the use of oestrogens was described in 1973 [17]. In the following years, many authors confirmed the hypothesis of an association between oestrogen-containing contraceptives and HCA [18-22]. Withdrawal of oral contraceptives in these patients will usually lead to regression of HCA [5]. However, we have yet to discover the physiological explanation for the association between oestrogen and HCA. The data on sex steroid receptors are rare, inconsistent and some of them used outdated techniques [23]. The largest study that used immunohistochemical analysis found an oestrogen and progesterone receptor in 26% of the HCA [24]. However, they did not draw any conclusions on the correlation between the number of HCA and the presence of the sex steroid receptors. New steroid hormone receptors have been identified in recent years, but have not yet been tested on HCA tissue [25-27].

Oestrogens are mostly known to be produced by the ovary. However, adipose tissue can contribute significantly towards the pool of oestrogens [25] [28]. Previous studies showed that obese patients have higher oestrogen levels compared with healthy individuals [28-29]. This could explain the relation between BMI and the number of HCA in this group of patients. In 2012, Rui et al. [29] carried out a study in which they found that high BMI had a significant positive association with the risk of liver tumours. Bioulac-Sage et al. [30] first suggested a connection between overweight and HCA. Bunchorntavakul et al. [31] found 23 cases of MA in obese patients and suggested a correlation between MA and obesity.

We describe a significant difference in BMI between single HCA, MA and LA. The median BMI is the highest in the group of patients with LA. We confirmed the suggested association between the number of HCA and BMI in a large group of patients. It has been suggested that HCA could decrease or disappear if patients lose weight [9]. The decrease could be attributed to a lower concentration of hormones because of weight loss [32].

Another explanation could be less inflammation because of weight loss as enhanced inflammation in the metabolic syndrome allows cell growth to develop HCA [30-31]. Currently, all patients with HCA are advised to stop the use of OC as well as lose weight. Therefore, it is not always clear whether the regression is caused by the withdrawal of OC or by the weight loss.

The Bordeaux subtype classification was introduced and included in our data. Subclassification of the largest HCA was performed in 267 patients. The incidence of the subgroup H-HCA has been reported previously to be 30–40% of all HCA [33]. Patients with H-HCA and thereby germline mutations of HNF1A are predisposed to develop LA [33]. However, in our cohort, only eight patients with LA were classified as H-HCA. In patients with I-HCA, obesity is a known risk factor. Furthermore, the presence of I-HCA is associated with MA as well [8] [30-31] [34-35]. I-HCA and a high BMI seemed to cause LA in our cohort as well. The BMI in the patients with I-HCA was significantly higher compared with the patients with H-HCA and LA.

Table 3. Management of patients with a single hepatocellular adenoma, multiple liver adenomas and liver adenomatosis, in which the largest lesion was at least 50 mm

Management	HCA (N= 56) [n (%)]	MA (N= 109) [n (%)]	LA (N= 54) [n (%)]
Conservative	18 (32)	64 (59)	36 (67)
Surgery	33 (59)	36 (32)	14 (26)
RFA	3 (5)	0 (0)	2 (4)
Embolization	2 (4)	8 (7)	0 (0)
Surgery and RFA	0 (0)	2 (2)	1 (2)
RFA and embolization and surgery	0 (0)	0 (0)	1 (2)

HCA, hepatocellular adenoma; LA, liver adenomatosis; MA, multiple adenoma; RFA, radiofrequency ablation. P< 0.001.

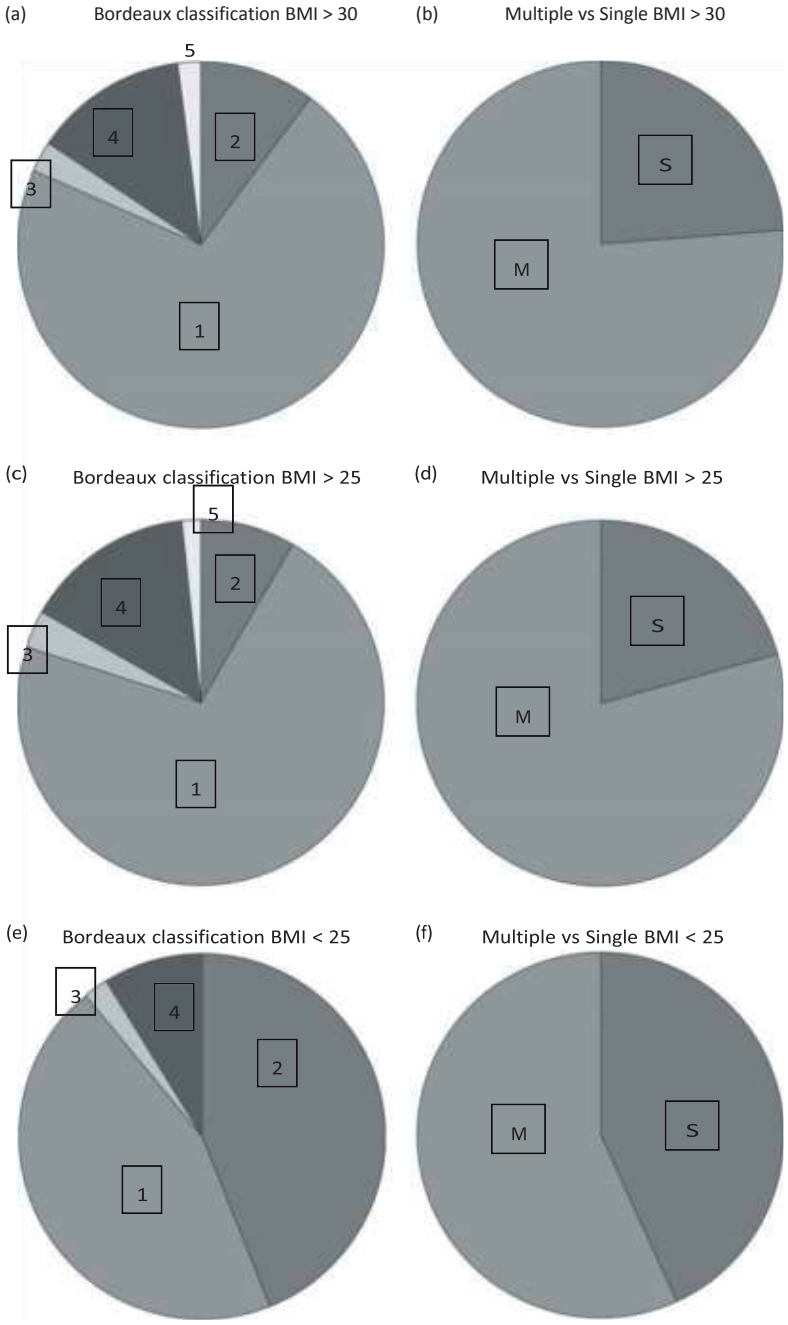

Fig. 2. Multiple and subtype distribution of HCA depending on BMI. a,c,e; 1 H-HCA; 2 I-HCA, 3 B-HCA; 4 U-HCA, IB-HCA (Inflammatory and B-cat positive HCA) b,d,f; S; single HCA, M: multiple HCA. HCA, hepatocellular cellular adenoma; β-HCA, hepatocellular cellular adenoma with mutations of the β-catenin gene; H-HCA, steatotic hepatocellular cellular adenoma; I-HCA, inflammatory hepatocellular cellular adenoma; U-HCA, inflammatory hepatocellular cellular adenoma.

In our cohort, the incidence of I-HCA is much higher compared with the distribution between the different subgroups described by the Bordeaux group [33]. This could be explained by the rapidly increasing incidence of obesity in women worldwide [36]. The increasing incidence of obesity could lead to a shift towards I-HCA, which will be observed more frequently. Furthermore, the prevalence of obesity in women is higher in the Netherlands (46.1%) compared with France (36.9%) [36].

We acknowledge that our study has a limitation. The final diagnoses were not all histologically proven. In those cases, combined imaging was used as the reference method for the final diagnosis, which was done after consensus in our multidisciplinary tumour board committee. In the early years, diagnoses of HCA have been differentiated from FNH with at least a conventional MRI. According to signal intensity and dynamic vascular patterns after an intravenous aspecific gadolinium injection, the different benign liver tumours are differentiated [13]. However, during the inclusion period of this study, specific hepatobiliary contrast agents were introduced and the differentiation is now more specific in challenging cases [37].

Biopsy is only approved in our hospital if there is doubt on the diagnosis or radiological examinations are not in agreement. However, MRI yields a highly accurate diagnosis, with a sensitivity of 91–100% and a specificity of 87–100% for differentiating HCA from FNH [38]. The Bordeaux subtype classification was performed on MRI in most patients; it should be noted that the MRI features of β-HCA are not completely defined [39]. Therefore, there could have been false negatives on MRI, possibly resulting in an underestimation of β-HCA.

Further research on the role of obesity in HCA and the effect of weight loss needs to be carried out. Because of the higher risk of surgery and the comorbidities of fatty liver, we suggest starting with weight reduction in all obese patients [3]. If follow-up indicates no decrease in the HCA, treatment should be decided depending on the anatomic location and the steatosis of the remaining liver tissue.

The management should be discussed by a multidisciplinary committee and strategies may be individualized.

CONCLUSION

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

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CHAPTER 9

Discussion and future perspectives

DISCUSSION AND FUTURE PERSPECTIVES

When a patient has diagnosed a focal liver lesion (FLL) for the first time, the main question is whether the lesion is benign or malignant. Due to widespread availability of modern radiological imaging, the incidence of detected liver tumors is increasing. Approximately 30% of general population has some type of focal lesion [1]. The majority of these incidentaly detected lesion are benign (cysts, hemangioma, adenoma, focal nodular hyperplasia), but malignancy (such as metastasis) should be always ruled out. Precise imaging diagnosis is of high importance, as their management and prognosis varies greatly. For liver imaging, the impact of the increased interest in liver diseases has been significant [2-4]. In general, unenhanced liver ultrasound (B-mode US) has not played a significant role in specific tumor diagnosis. Information concerning tissue perfusion was always missing. The advent of microbubble imaging has changed this concept. As contrast agents used in US (CE-US) move completely to the intravascular compartment, we can visualize perfusion in tissues, and information can be obtained in real-time. The role of contrast agents in US are somehow similar to the role they play in CT or MRI. Without intravenous contrast agents, these imaging modalities would also lack most of their diagnostic power. In a case of CE-US, we retain one of most important qualities of ultrasound that is its flexibility in everyday clinical practice. To date we have not defined specific role of CE-US in diagnostic work-up of liver tumors [5-6]. One may question how many patients with liver lesion suspected to be benign may by finally diagnosed by the means of CE-US only or how many and under which conditions they should be send to additional diagnostics (MRI, CT)? In this thesis, the author examined the position of CE-US and its role in management in the field of focal liver lesions (FLL).

CT or MRI is the most commonly imaging used for characterizing focal hepatic lesions [7]. However, the position of liver CEUS is now more settled. According to current WFSUMB guidelines – update 2020 [8] – is CEUS recommended in patients in the non-cirrhotic liver with inconclusive findings at CT or MRI imaging or inconclusive focal liver lesion biopsy. Contrast-enhanced CT and/or MRI are preferred modalities. Sometimes they are even contraindicated. Guidelines also stated, if CEUS has definitely characterized a benign FLL, further investigations are not necessary to confirm the diagnosis. Our study suggests a more restricted approach.

Liver adenoma (HCA) and focal nodular hyperplasia (FNH) are two most important benign lesions. FNH and HCA are two lesions that are sometimes mutually misdiagnosed, but their management and prognosis differs. It is of utmost importance to learn specific features and real-time dynamics of both of these lesions. At present, both CE-US and MRI are best regarded as complementary for diagnosing HCA and FNH [9]. In our large study, CE-US resulted in a conclusive diagnosis (HCA versus

FNH) in 92% of the cases, whereas MRI correctly diagnosed 99% of cases. In discordant cases, CE-MRI is highly accurate and superior to CE-US in histopathology-confirmed diagnoses [10]. We conclude that CE-US is less suitable as a stand-alone imaging modality for the final diagnosis of HCA of FNH. It has an adjunct role in discordant cases in patients in whom a quality MRI cannot be obtained, or reliably interpreted and in which liver biopsy is contraindicated. Perhaps in some less developed countries, where proper CT and MRI are not available.

What are these specific HCA and FNH features visualized by CE-US? In general, different benign tumours show different enhancement patterns. Benign liver lesions are mainly characterized by traits in the arterial phase. Portal or late phases are not so important. By comparison, some of patterns cannot be properly appreciated on CT or MRI scans because patterns change very rapidly. Using CE-US, we display microbubbles filling in real-time, and we use a frequency around 20 pictures per second (Hz) [11]. Our study in 324 patients with HCA or FNH described various more or less specific diagnostic features of the lesions [12]. Finally, we composed a calculus of predicted HCA probability using those various features. Approximately, 20% of FNH lesions lack typical CE-US morphological or perfusion features and thus can decrease proper diagnosis based on contrast agent perfusion characteristics.

Compared to men, women are more frequently diagnosed with these tumors, namely HCA and FNH. This phenomenon is probably related to higher estrogen levels [13]. In case of an HCA, the influence of oral contraception is also suspected as well [14-15]. Another putative reason for the rising HCA incidence is the increase in overweight/obesity in the population [16]. Significantly higher BMI is related to multiple adenomas [17]. Our findings, as well as reports from other authors indicate that in an obese patient, US is often difficult to perform. CE-US can provide some additional information about the nature and number of lesions and influence the decision as to surgical or non-surgical treatment. In a patient in whom physicians are reluctant to perform a lesion biopsy to clarify diagnosis and exclude hepatocellular carcinoma or metastasis, CE-US can often rule out malignancy.

FNH is known to contain centrally located fibrotic tissue (so called central scar), HCA tissue does not have a substantial fibrotic component and more closely resembles normal liver parenchyma. The current standard diagnostic workup includes MRI with adjunct role of CE-US. Larger lesions (> 35 mm) reduce diagnostic accuracy of CE-US [18]. FNH's fibrotic scar is stiffer than surrounding normal liver parenchyma, and this characteristic could be used for improving the diagnosis by focused elastography. Current methods (point Shear Wave Elastography, pSWE) use a US pulse released to a region of interest (ROI). Once there, it generates a perpendicular mechanical wave propagation through the liver tissue, and its velocity can be reliably measured. Such measurements can be also used to check stiffness of focal liver

abnormalities. pSWE of FNH and adenoma looks like a promising modality, and several preliminary studies suggest it may be beneficial in FLL diagnosis. To combine CE-US and local elastography appears to be an even more promising diagnostic step [19]. pSWE could improve CE-US diagnostic performance in equivocal cases in one US session. However, results for current small studies are dubious, and our study could not confirm the claimed diagnostic improvement. Interpretation of focal stiffness measurements should be done with caution [20].

Not all focal liver lesions are benign and management of malignant liver tumors varies substantially. Surgery will improve the survival of the patients if malignant lesions are not disseminated in the liver. Precise pre-operative method for their detection is therefore crucial. The exclusion of liver metastases in a patient with an already known extrahepatic tumour is nowadays suboptimal. Currently the reference method is computed tomography (CT), but metastases smaller than 1 cm can be missed or mistaken for another entity. The sensitivity and specificity of detecting metastases in the liver is low (for colorectal cancer metastases 82.1%, resp. 73.5%) [21]. CE-US presents good performance in differentiating benign versus malignant liver lesions, including metastasis. As our study confirmed, CE-US has a comparable sensitivity to CT for liver metastases detection in pancreatic cancer. We found that the main advantage of CE-US is in its capability to detect false-positive or clarify indeterminate CT results [22]. These results are promising and should be further explored in various screening programs.

In addition to diagnostic performance, the cost-effectiveness evaluations of healthcare interventions are important due to the rising costs of healthcare. Economic considerations together with clinical analyses provide the foundation for allocation of various resources. Several studies have shown that CE-US is a valuable cost-saving alternative compared to the current front-line diagnostic imaging. Expert MRI for FLL has better diagnostic results, but CE-US is cheaper. Studies exploring CE-US performance and cost consequences in various clinical situations, different countries, and healthcare settings calculated only direct diagnostic costs. Our study was the first one which included also treatment phase. It is of importance to also add a treatment phase to cost analysis studies and thus, combine diagnostic and treatment phases to evaluate the real impact of CE-US in the management of FLL.

One might assume that in the future, we can reasonably expect the availability of more specific microbubbles. The era of clinically available targeted CE-US may happen; as an example, bubbles coupled with antigens or receptors on their phospholipid surfaces that can attach to specific structures on tumor vasculature could be used. Such modified bubbles can improve tumor characterization. Targeted CE-US is not a surrogate for histological examination, but in some patients, we can avoid the need to perform a biopsy, and we can repeat measurements noninvasively,

check the tumor as a whole not only in the biopsy needle specimen, thus avoiding sampling error. The strict intravascular distribution of contrast agent is decisive for evaluating the antiangiogenic effects of new drugs. Furthermore, emergence of targeted CEUS allows not only more specific diagnostics but also targeted therapy. In addition, artificial intelligence (AI), particularly deep learning (DL) algorithms, is a significant yet emerging technological innovation in radiology. The power of DL devices lies in the capability to imitate neuronal cell activity, and they were developed to perform tasks specific for the human neocortex, such as learning and recognizing specific patterns of digital images. Also, AI-powered ultrasound is going to be more mature. It is approaching routine clinical application [23]. US examination includes operator-, patient-, and scanner-dependent parts [24]. In the future, AI can support human work, extend it, and also replace it. The projected impact on sonographers' work can be very profound [25]. But we are not there yet.

Furthermore, an area of future research involves the use of liver-specific microbubble contrast agent such as Sonazoid (perfluorobutane) [26]. Sonazoid is intracellular Kupffer phase agent and has limited reports yet. Kupffer phase starts at about 10 minutes and can last up to 120 min, allowing scanning of the whole liver in detail.

Current body of evidence is limited by small single-center studies of mostly retrospective design. Large multicenter studies would be helpful to clarify the usefulness of CEUS for indeterminate lesions on CT or MRI. Considering the difference of method of image acquisition and contrast material between CEUS and CT and MRI, combined assessment potentially improves accuracy.

Conclusion

In summary, there is still much to be investigated before we are able to effectively use CEUS in clinical practice. However, given the many endeavours by scientists, clinicians and radiologists all over the world, the future appears bright and hopeful.

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CHAPTER 10

English summary
Nederlandse samenvatting

ENGLISH SUMMARY

In this thesis, we focused on the various aspects of performing contrast-enhanced ultrasound (CEUS) of various liver focal lesions. Its position in relation to other imaging methods, the characteristic of the method and its financial aspects.

In **chapter 2,** we performed an up-to-date review of current status of contrast enhanced US in benign liver lesions. It has been used in several countries for more than a decade but still awaits more general acceptance in clinical practice. All clinically approved contrast agents (UCA) are microbubbles. There is a widespread availability of general abdominal ultrasound for a significant proportion of examined patients, in whom for whatever reason, have incidentally discovered one or another FLL, most of which are benign. Nevertheless, we should be able to precisely characterize these lesions. And CE-US appears to be a handy tool to achieve this goal. In this chapter, we describe dynamics of the method, physical, and technological background, its safety, technique of examination and limitations. In a more specialized section we described different benign FLLs, both cystic and solid ones. We describe CE-US features with emphasis on hemangioma, liver adenoma, and FNH.

In **chapter 3,** we compared the diagnostic performance of CE-US with CE-MRI using gadobenate dimeglumine for diagnosing FNH and HCA in prospective work-ups. A total of 182 patients were included, and the final diagnosis was considered when results of CE-US and CE-MRI were conclusive. Lesion biopsy assesment followed in cases of discrepancy between both imaging methods. The reference standard was established within a multidisciplinary hepatobiliary team, and in cases in which the biopsy was contraindicated, CE-MRI was considered as the reference method for final diagnosis. The sensitivity and specificity of CEUS were 83% and 72%, respectively. Agreement between CE-US and CE-MRI was fair for diagnosis of FNH and HCA. In a case of disagreement among imaging nethods, CE-MRI demonstrated the advantage in a more accurate diagnosis.

Chapter 4 assessed the potential of pSWE (poit shear wave elastography) for differentiating HCA from FNH. Differentiation between these two lesions is important because their therapeutic options differ signficantly FNH with its scar tissue is expected to produce higher stiffness results than adenoma. Eighty-eight patients were included, and two experienced observers blinded for the clinical data did the studies. We determined the median FNH and HCA stiffness values per observer, for both examiners the median FNH stiffness value was significantly higher than the HCA stiffness value (7.01 versus 4.98 kPa [P = 0.017] and 7.68 versus 6.00 kPa [P =0.031], respectively). Receiver operating characteristic (ROC) analysis for lesion pSWE values showed the area under the ROC (AUROC) for differentiating FNH from HCA was 0.67 (resp. 0.69 for observer 2). We were unable to determine an acceptable

cut-off point for differentiation between HCA and FNH due to great variability in pSWE values.

In 15 patients in whom CE-US versus MRI or lesion biopsy did not agree, the stiffness result range was also too variable to add any significant diagnostic information.

The second parameter we followed was the question of inter- and intra-observer reliability of pSWE in the examination of lesions and native liver tissue (NLT). Remarkably, we found poor to moderate intra- and interobserver reliability, which could indicate that the performance of the present methodology is insufficient.

In **chapter 5,** we described the imaging and clinical characteristics of FNH and HCA in a large cohort of patients. A total 324 contrast-enhanced examinations were performed, and we determined the presence of specific imaging features (central scar, central artery, enhanced pattern in the late phase) and clinical characteristics (age of patient). Furthermore, we developed a diagnostic model for prediction of HCA using CE-US:

Predicted Probability (P) = 1/(1+e[0.778+{0.36 *Age} + [−1.251*central scar] + [−1.198*central artery]+[0.541*enhancement mixed] + [1.157*enhancement portal]).

Using this formula, the presence of an HCA can be predicted correctly in 0.854 cases via the use of CE-US.

Chapter 6 addressed the implication of CE-US in protocols related to screening liver metastasis in patients with a suspected pancreas adenocarcinoma. In contrast to many other malignancies, the number of pancreatic cancer patients is slowly increasing with five-year survival only around 6%. Surgery, which is the only curative option, is feasible in only a minority of these patients. The presence of liver metastases has a crucial role in the choice of therapy for pancreatic cancer patients. CT is currently the reference method. We performed CT and CE-US in eighty-nine patients. The CT sensitivity for detecting liver metastasis was 73.3% and specificity 93.2%, whereas CEUS showed a sensitivity of 80% and specificity of 98.6%. The main difference between CT and CE-US was the capability of CE-US to differentiate between true metastasis and accidental innocent liver lesions. False positive findings were reduced by CE-US (PPV was lower in the CT group 68.7% versus 92.3% in the CE-US group) and in a logistic regression model, CE-US remained significant ($p < 0.001$), whereas CT did not provide any additional information.

In **chapter 7,** we reported a cost-effectiveness analysis of CE-US examination in various FLLS. The study population consisted of 170 prospectively enrolled patients with at least one lesion. A decision analytic model was developed to estimate incremental costs and effectiveness of diagnostic technologies from the healthcare perspective in the Netherlands and the diagnostic performance of CE-US was compared to MRI/CT. The main advantage of our study was the addition of a treatment phase

to the evaluation. The impact of CE-US on the combined diagnostic and treatment phase was higher compared to other studies and reached 452 €.

Chapter 8 continued to assess patients with HCA who are registered in our database (N= 458). We studied patients who are at risk for multiple HCA and whether patient and lesion characteristics between single and multiple HCAs differ. Patients were subdivided into three groups: 1) single HCA, 2) multiple 2–9 HCAs, and 3) adenomatosis > 10. In this large series we found a significantly higher BMI and frequency of inflammatory HCA in patients with multiple HCAs compared with single HCA. Our study suggests that not only cessation of oral contraception but also body weight reduction can decrease the size of HCA.

In the final chapter, **chapter 9**, we discussed and reviewed our diagnostic and economic work-up related to CE-US. Future perspectives are highly important, and antigen specific microbubbles (targeted imaging) could be one of them. Diagnostic work-up can be personalized, but also therapeutic CE-US (targeted therapy) is considered and has been perforemd at the level of laboratory animals.

NEDERLANDSE SAMENVATTING

In dit proefschrift hebben we ons gericht op de verschillende aspecten van het uitvoeren van contrastversterkte echografie (contrast-enhanced ultrasonography, CEUS) van verschillende focale lever laesies. We bestudeerden de positie ten opzichte van andere beeldvormende methoden, de kenmerken van de methode en de financiële aspecten ervan.

In **hoofdstuk 2** hebben we een actuele beoordeling uitgevoerd van de huidige status van contrastversterkte echografie bij goedaardige leverlaesies. Het wordt al meer dan een decennium in verschillende landen gebruikt, maar wacht nog steeds op meer algemene acceptatie in de klinische praktijk. Alle klinisch goedgekeurde contrastmiddelen (UCA) zijn gebaseerd op microbelletjes. Er is een wijdverbreide beschikbaarheid van algemene echografie, bij een aanzienlijk deel van de onderzochte patiënten is om welke reden dan ook incidenteel een of andere focale leverlaesie ontdekt, meestal volkomen goedaardig. Desalniettemin moeten we deze laesies nauwkeurig kunnen karakteriseren. CEUS is dan een handig hulpmiddel om te helpen. In dit hoofdstuk beschrijven we de dynamiek van de methode, de fysische en technologische achtergrond, de veiligheid, de onderzoekstechniek en de beperkingen. In een meer gespecialiseerde sectie doorlopen we verschillende goedaardige focale leverlaesies, zowel cysteuze als solide. We beschrijven CEUS-kenmerken, met de nadruk op hemangioom, adenoma en focale nodulaire hyperplasie.

In **hoofdstuk 3** vergeleken we de diagnostische prestaties van CEUS met CE-MRI met behulp van gadobenaat dimeglumine voor de diagnose FNH en HCA in een prospectieve work-up. In totaal werden 182 patiënten geïncludeerd, de definitieve diagnose werd overwogen als de resultaten van CEUS en CE-MRI overtuigend waren. Biopsie van de lesie volgde in gevallen van discrepantie tussen beide beeldvormings methoden. De referentiestandaard werd vastgesteld binnen een multidisciplinair hepatobiliair team, als biopsie gecontra-indiceerd was, werd CE-MRI beschouwd als de referentiemethode voor de uiteindelijke diagnose. De gevoeligheid en specificiteit van CEUS was respectievelijk 83% en 72%. Overeenstemming tussen CEUS en CE-MRI was redelijk voor de diagnose van FNH en HCA. In het geval van discrepantie tussen de beeldvormende methoden, toonde CE-MRI een voordeel.

Hoofdstuk 4 onderzocht het potentieel van point shear wave elastography (pSWE) om hepatocellulair adenoom (HCA) te onderscheiden van focale nodulaire hyperplasie. Differentiatie tussen deze twee laesies is belangrijk omdat hun therapeutische opties aanzienlijk verschillen. FNH met zijn littekenweefsel zal naar verwachting hogere stijfheids resultaten opleveren dan adenoom. Achtentachtig patiënten werden geïncludeerd door twee ervaren waarnemers geblindeerd voor de bekende klinische gegevens. We bepaalden de mediane FNH- en HCA-stijfheidswaarden per

waarnemer, voor beide onderzoekers was de mediane FNH-stijfheidswaarde significant hoger dan de HCA-stijfheidswaarde (7,01 vs. 4,98 kPa (P = 0,017) en 7,68 vs. 6,00 kPa (P = 0,031) ROC-analyse voor pSWE-waarden van laesie toonde AUROC voor differentiatie FNH van HCA van 0,67 (resp. 0,69 voor waarnemer 2). We konden geen acceptabel afkappunt bepalen voor differentiatie tussen HCA en FNH vanwege de grote variabiliteit in pSWE-waarden.

Bij 15 patiënten, waar CEUS versus MRI of de uitslag van de histologie niet overeenkwamen, was het bereik van de stijfheidsresultaten ook te variabel om significante diagnostische informatie toe te voegen.

De tweede vraag die we onderzochten was de vraag naar de interobserver- en intraobserver betrouwbaarheid van pSWE bij het onderzoeken van laesies en normaal leverweefsel (NLT). Opvallend was dat we een slechte tot matige intra- en interobserver betrouwbaarheid vonden, wat erop zou kunnen wijzen dat de methodologie nog onvoldoende ontwikkeld is.

In **hoofdstuk 5** beschrijven we de beeldvorming en klinische kenmerken van focale nodulaire hyperplasie (FNH) en hepatocellulair adenoom (HCA) in een groot cohort van patiënten. Er werden in totaal 324 contrastversterkte onderzoeken uitgevoerd en we bepaalden de aanwezigheid van specifieke beeldkenmerken (centraal litteken, centrale slagader, aankleurings patroon in de late fase) en klinische kenmerken (leeftijd van de patiënt). Verder ontwikkelden we een diagnostisch model voor HCA-voorspelling: voorspelde waarschijnlijkheid (P) = 1 / (1 + e (0.778+ (0.36 * leeftijd) + (-1.251 * centraal litteken) + (- 1.198 * centrale ader) + (0.541 * verbetering gemengde fase) + (1,157 * verbetering portale fase)) Met deze formule kan met CEUS een HCA correct worden voorspeld in 0,854 gevallen.

Hoofdstuk 6 behandelt de implicatie van CEUS in protocollen die verband houden met het screenen op levermetastasen bij patiënten met een vermoedelijk adenocarcinoom van de pancreas. In tegenstelling tot veel andere maligniteiten, neemt het aantal alvleesklier kanker patiënten langzaam toe, met een overleving na vijf jaar slechts rond de 6%. Chirurgie - de enige genezende optie - is haalbaar bij slechts een minderheid van de patiënten. De aanwezigheid van levermetastasen speelt een cruciale rol bij de keuze van de therapie. CT is momenteel de referentiemethode. We hebben CT en CEUS uitgevoerd bij negenentachtig patiënten, de CT-gevoeligheid bij het detecteren van levermetastase was 73,3% en specificiteit 93,2%, terwijl CEUS een gevoeligheid van 80% en specificiteit 98,6% vertoonde. Het belangrijkste verschil tussen CT en CEUS was het vermogen van CEUS om onderscheid te maken tussen een echte metastase en een accidentele onschuldige leverlaesies. Vals-positieve bevindingen werden verminderd door CEUS (PPV was lager in CT-groep 68,7% vs. 92,3% in CEUS-groep) en in een logistisch regressiemodel bleef CEUS significant (p <0,001), terwijl CT geen aanvullende informatie leverde.

In **hoofdstuk 7** rapporteren we een kosteneffectiviteitsanalyse van CEUS-onderzoek bij verschillende focale leverlaesies. De studiepopulatie bestond uit 170 prospectief ingeschreven patiënten met ten minste één laesie. Er is een beslissingsanalysemodel ontwikkeld om de incrementele kosten en effectiviteit van diagnostische technologieën vanuit het perspectief van de gezondheidszorg in Nederland te schatten en de diagnostische prestaties van CEUS werden vergeleken met MRI / CT. Het belangrijkste voordeel van ons onderzoek was het toevoegen van een behandelingsfase aan de evaluatie. De CEUS-impact van de gecombineerde diagnose- en behandelingsfase was hoger in vergelijking met andere onderzoeken en bereikte 452 €.

Hoofdstuk 8 gaat door met het beoordelen van patiënten met HCA geregistreerd in onze database (N = 458), we hebben onderzocht welke patiënten risico lopen op meervoudige HCA en of de kenmerken van patiënten en laesies tussen enkelvoudige en meervoudige HCA's verschillen. Patiënten werden onderverdeeld in 3 groepen (1 HCA, meerdere 2-9 en adenomatose> 10). In deze grote serie vonden we een significant hogere BMI en frequentie van inflammatoire HCA bij patiënten met meerdere HCA vergeleken met enkelvoudige HCA. Onze studie suggereert dat niet alleen stopzetting van OC (orale anticonceptie), maar ook de vermindering van het lichaamsgewicht de omvang van een HCA kan verminderen.

In het laatste hoofdstuk, **hoofdstuk 9**, bespreken en beoordelen we onze diagnostische en economische werkzaamheden met betrekking tot contrastversterkte echografie. Toekomstige ontwikkelingen zijn daarbij van groot belang en bieden ook perspectief: antigeen specifieke microbellen (gerichte beeldvorming) zouden daar een van kunnen zijn. Diagnostische evaluatie kan gepersonaliseerd worden, maar ook therapeutische CEUS (gerichte therapie) wordt overwogen en al op in proefdieren onderzoek uitgevoerd.

Appendices

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LIST OF PUBLICATIONS:

Publications related to this thesis:

Bröker MEE*, **Taimr P***, de Vries M, Braun LMM, de Man RA, IJzermans JNM, Dwarkasing RS. Performance of Contrast-Enhanced Sonography versus MRI with a Liver-Specific Contrast Agent for Diagnosis of Hepatocellular Adenoma and Focal Nodular Hyperplasia. Am J Roentgenol 2020; 214(1):81-89. ** Shared first authorship*

Taimr P, Klompenhouwer AJ, Thomeer MGJ, Hansen BE, IJzermans JNM, de Man RA, de Knegt RJ. Can point shear wave elastography differentiate focal nodular hyperplasia from hepatocellular adenoma? J Clin Ultrasound 2018; 46(6):380-385.

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Polak WG, Fondevila C, Karam V, Adam R, Baumann U, Germani G, Nadalin S, **Taimr P**, Toso C, Troisi RI, Zieniewicz K, Belli LS, Duvoux C. Impact of COVID-19 on liver transplantation in Europe: Alert from an early survey of European Liver and In-

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PHD PORTFOLIO

Name PhD Student:	Pavel Taimr
PhD Period:	2017-2020
Erasmus MC Department:	Hepatology and Gastroenterology, Surgery
Promotors:	Prof. Dr. Robert A. de Man
	Prof. Dr. Jan N. M. IJzermans
Research School:	Faculty of Medicine

1. PhD Training	**Year**	**Workload (ECTS)**
General Courses		
Research Integrity, Erasmus MC, Rotterdam	2017	0,3
Courses of the Fundamentals of Scientific Work at the CAS	2017	
Introduction to the methodology of science	2017	0,1
The ethics of scientific work	2017	0,1
The fundamentals of bioethics	2017	0,1
The fundamentals of rhetoric and the techniques of speech	2017	0,2
Communication in science (written genres)	2017	0,1
Academic writing techniques	2017	0,1
Publishing in journals in terms of editorial work	2017	0,1
The fundamentals of scientometry	2017	0,2
Intellectual property rights	2017	0,1
The financing research, the grant systems	2017	0,1
Presentation of scientific results, posters	2017	0,1
Teaching and presentation skills	2017	0,2
Postdoc interviews	2017	0,1
Modern electronic resources for research and education	2017	0,1
Academic writing in English: Writing for the reader	2017	0,1
The analysis and assessment articles, poster etc	2017	0,1

In-Depth Courses	**Year**	**Workload (ECTS)**
Cursus Abdominale Sonografie DLW	2017	1
Transplantation course, Parma	2017	1

Presentations at (inter-) national conferences	**Year**	**Workload** (ECTS)
Cursus Abdominale Sonografie DLW Leiden	2017	1
Abdominal ultrasound courses, Bratislava, Slovakia	2017-2019	3
Cursus Abdominale Sonografie DLW, Rotterdam	2018	1
International Abdominal Ultrasound course, Prague	2018	1
Contrast-enhanced Ultrasound, IKEM, Prague	2018	1
Benign liver tumors, CEUS, EASL Abd.ultrasound course, Rotterdam	2018	1
Ultrasound course (hepato-gastro), Banska Bystrica, Slovakia	2019	1
II. Workshop Ultrasound Elastography, Prague	2019	1

Attendance at (inter-) national Conferences	**Year**	**Workload (ECTS)**
The Liver meeting, AASLD, Washington	2017	1
Erasmus Liver Day, Rotterdam	2017	1
ESOT, Barcelona	2017	1
The Liver meeting, AASLD, San Francisco	2018	1
The International Liver Congress, EASL, Paris	2018	1
The Transplantation Society Congress, Madrid	2018	1
ILTS, Toronto	2019	1

2. Teaching

Supervising Practicals and Excursions, Tutoring	**Year**	**Workload (ECTS)**
MDL Minor teaching	2017	1
Supervising Students	2017-2020	2
Examination Basic Life Support	2016-17	1
Lectures in liver disease, 3rd Medical Faculty, Prague	2017-2020	5
Lecturing Ultrasound workshops, Czechie	2019-2020	3

DANKWOORD

Er is hiermee een einde gekomen aan een mooie, lange en bijzondere periode in mijn leven. Zoals in veel proefschriften, gaat ook dit boekje over verandering, over tijd en over hoop. Dit proefschrift was nooit tot stand gekomen zonder de hulp, inzet en aanmoediging van vele patiënten, collega's, vrienden en familie. Ik heb het geluk gehad om met een groep geweldige mensen samen te mogen werken en daarnaast veel lieve mensen om me heen te hebben die het leven zo mooi maken. Daarom wil ik iedereen die op welke manier dan ook aan het tot stand komen van dit proefschrift heeft bijgedragen bedanken. De volgende personen wil ik graag in het bijzonder bedanken voor hulp, advies, steun en gezelligheid.

Allereerst mijn promotor, beste professor De Man, beste Rob, bedankt voor je begeleiding tijdens het promotietraject. De afgelopen jaren heb ik veel van je geleerd en me op wetenschappelijk gebied kunnen ontplooien. En niet alleen medisch-inhoudelijk, maar ook over samenwerking, het opzetten van studies en beslisbomen. Ook de bemoedigende woorden in de afrondende fase. Je bent een ontzettend fijne supervisor: enthousiast, gedreven en makkelijk benaderbaar. Je bent een voorbeeld voor iedereen die gedegen methodologisch onderzoek wil combineren met de klinische praktijk. Heel erg bedankt!

Mijn andere promotor, beste professor IJzermans, dank voor het vertrouwen! Dank voor uw kritische blik en uw vlotte commentaar op de manuscripten. Uw nuchtere en resultaatgerichte aanpak in het laatste jaren hebben geleid tot afronding van dit proefschrift.

Geachte leden van de kleine commissie, prof. dr. Metselaar, prof dr. Schouten en prof. dr. Van Delden, dank voor uw interesse en uw beoordeling van het manuscript. Tevens wil ik de overige leden bedanken voor de bereidheid om als opponent deel te nemen in de grote commissie.

Prof. Metselaar, beste Herold, ik wil je bedanken dat ik deel mocht nemen aan het klinische team van levertransplantatie afdeling. Als clinicus ben je heel goed door een brede blik, directe en waardevolle commentaren en veel kennis waar ik veel respect voor heb. Later gaf je me de mogelijkheid om levertransplantatie traject verder te bewandelen waarvoor ik je nog steeds erg dankbaar ben.

Aan alle MDL echografisten – dr. de Knegt, prof. Schouten, dr. Sprengers, drs. Coenen, drs. Polak en dr. den Hoed. Beste Rob, Jeoffrey, Dave, Sandra, Alexandra en Caroline, dank voor jullie hulp, kennis, enthousiasme en pure plezier! En niet alleen bij echo-onderzoek! Ontzettend bedankt voor alle kansen en de ruimte die jullie me hebben geboden om mezelf te ontwikkelen. Lieve Rob, Jeoffrey en Dave, dank voor jullie morele support en fantastische vriendschap. Onze gezamenlijke passie (echo) konden we met elkaar delen, de nodige frustraties maar ook successen

op buitenlandse cursusdagen en congressen. Onze meetings waren altijd gezellig, wat hebben we veel gelachen, zeker tijdens alle echo- tripjes. Ik hoop dat we onze samenwerking nog lang mogen voortzetten.

Dr. Dwarkasing, beste Roy, dank voor je inzet en hulp bij alle radiologische aspecten van de artikelen in dit proefschrift. Bedankt voor je inbreng, bijdragen en fijne samenwerking, een monsterklus!

Dr. Hořejšová en dr. Skála, MDL-echographie, Praag. Beste Milena, het begon eigenlijk allemaal toen ik onder jouw begeleiding mocht werken. Ik kan me onze eerste meeting in echokamer afdeling geneeskunde B3, nog goed herinneren. Begin december 1991, toch! Je had in een mum van tijd alle pijnpunten van abdominale echo onderzoek op papier blootgelegd en ik mag deze tekeningen tot nu met mijn studenten gebruiken. Beste Ivo, dank voor je kritische blik en waardevolle commentaren. Je bent een ontzettend fijne supervisor.

Dr. Zelinková, MDL- echographie, Bratislava. Beste Zuzana, darm echo-ethousiast! Bedankt voor samenwerking en enthousiasme!

Beste co-auteurs, dank voor jullie hulp en begeleiding, bij de totstandkoming van de manuscripten. Dr. Klompenhouwer en dr. Bröker, beste Julia en Mirelle, jullie wil ik in het bijzonder bedanken. Jullie enthousiasme en doortastendheid heb ik wel eens gemist tijdens het afronden van dit boekje.

Mw. Hoogendoorn, beste Marion, het is al vaak gezegd maar zonder jou zou er toch een hoop mislopen. Al sinds 2004 en niet alleen tijdens mijn promotietraject, je bent altijd enthousiast en vriendelijk, bewaart het overzicht en bent bereid net dat beetje extra te doen. Ook heel erg bedankt!

Prof. Kuipers, beste Ernst, bedankt voor alle mooie Rotterdamse jaren, dat u me wegwijs hebt gemaakt in het Erasmus MC. Daarnaast was u altijd geïnteresseerd in mij als persoon, dank u wel!

Familie Polak, beste Alexandra, het geeft me een trots gevoel om jou zoveel te zien bereiken en te zien staan waar je nu staat. Beste Wojciech, dank voor jouw hulp, vriendschap en expertise bij de levertransplantaties, ik hoop dat we nog vaak kunnen samenwerken in de toekomst.

Dr. Darwish Murad, beste Sarwa, bedankt voor plezierige samenwerking! Daarnaast speciale dank voor levertransplantatie secretariaat - Lara, Sandra, Miranda, Catelijne, Carola, Wendy en de andere (ex-) coördinatoren en assistenten! Bedankt voor alle moeite, grenzeloze steun en mentale ondersteuning die heb ik gehad in dit langlopend traject. Ik heb altijd heel erg genoten van het informele en vrolijke contact de afgelopen jaren! Jullie snoeppot is vast een stuk gevulder nu ik er niet ben! Ik wil ook Koekela graag bedanken voor alle taarten en cakes.

Prof. Špičák, beste Julek, ik wil jou bedanken dat ik deel mocht nemen aan het team van jouw Maag- Darm en Lever- afdeling IKEM. Jouw uitgebreide expertise

en onmisbare ondersteuning heeft geleid tot een hele mooie samenwerking waar onder andere ik de vruchten van mag plukken middels mijn PhD boekje.

Beste prof. Bruno, Henk, Janneke, Jan-Werner, Arjun, Wilmar, Manon, Lieke, Ester, Rachel, Marian, Vera, Lissete, Margot, José, Frank, Joyce, Aafke, Leonike, Daphne, Ad, Geerd, Vincent, Claudia, Edith (…) en andere lieve endoscopisten en (ex-) MDL-artsen (ook in opleiding) van Erasmus MC, veel heb ik van jullie mogen leren en het verbaasde me altijd hoe scherp iedereen was op ochtendbesprekingen, met de nodige dosis humor erbij.

Prof. Janssen en prof. Hansen. Beste Harry, bedankt voor de zeer waardevolle carrière adviezen! Ik heb bewondering voor je doorzettingsvermogen, kennis en motivatie. Beste Bettina, dankzij jou heeft dit boek zijn statistische robuustheid en sterkte gekregen. Elke analyse die je geduldig hebt uitgevoerd was voor mij net hocus pocus, bedankt!

Lieve collega's van de heelkunde, Jeroen, Diederik, Khé, Türkan, Bas en prof. Tilanus, de samenwerking met jullie op de afdeling heelkunde en daarbuiten zoals tijdens levertransplantatie besprekingen was de basis van mijn werk. En ik blijf jullie hier altijd dankbaar voor!

MDL-AIOS-en en onderzoekers, bedankt voor alle gezelige momenten tijdens borrels, congressen en ski-tripjes. Toch ben ik de eerste „Hero of the day" geweest!

Prof. Nico de Jong, ik heb veel aan onze gesprekken gehad, ik hoop dat we nog vaak van gedachten zullen wisselen over contrast-enhanced echografie, bedankt.

MRI team IKEM, Praag, beste Monika en beste Milan, ik ben geen hardcore wetenschapper maar jullie gelukkig wel. Doordat jullie in mij bleven geloven kon ik ook blijven geloven dat dit boek geschreven kon worden en kijk eens, een project welke jaren geleden begon komt nu tot zijn eind. Bedankt!

Last but not least, Spolek Oidipus, veel dank voor het stichten van orde in de chaos en jullie onvoorwaardelijke steun voor alle bizzare gedachten, psychiatrische, analytische, historische en culturelle discussies, opinies en heel, heel veel andere dingen in de afgelopen jaren! Wat een openminded berg van vriendschap zijn jullie, verander nooit!

De allerbelangrijkste personen in mijn leven wil ik als laatste bedanken.

Lieve Papa en Mama, ik krijg al tranen in mijn ogen als ik iets over jullie wil zeggen en wat jullie voor mij betekenen. Er zijn geen woorden en daden om mijn liefde voor jullie te beschrijven. Wat hebben jullie mij met liefde opgevoed en wat hebben jullie allemaal in de loop van de jaren opgeofferd voor mij en oh wat heb ik jullie lastig gevallen met mijn problemen in het leven. Jullie zijn onvervangbaar, ik houd van jullie.

Mijn schoonfamilie: František, Emilie en Petr. Dank u wel voor jullie warmte en dat ik zo hartelijk ontvangen ben in de familie. Dank voor gezelligheid en goede zorgen.

Beste kinderen, Martin en Anna, jullie waren onmisbaar, ik genoot van onze vele discussies samen en ik ben trots dat ik jullie vader ben. Zonder jullie zou het leven een grijze bladzijde zijn. Ook veel plezier en succes met jullie nieuwe uitdaging in Utrecht en Praag/Berlijn, op top universiteiten! … en misschien proberen een PhD traject wat vroeger beginnen dan ik… oké, just suggestion.

Mijn grootste liefde, Renata. Mijn vrouw, mijn vriend, mijn maatje, mijn soulmate, moeder van onze geweldige kinderen, Martin en Anna, mijn alles eigenlijk. Wat zou ik in het leven moeten zonder jou. Eerlijk gezegd zou mijn proefschrift ook niet af zijn zonder jouw ondersteuning. Jij bleef in mij geloven dat het mij zou lukken om deze promotie tot een goed eind te brengen in tijden waarin anderen mij uitlachten. Door jouw steun en begrip voelde ik me sterk en ben ik zo geholpen dat het me eindelijk gelukt is. Hoe jij alles tegelijk doet is onvoorstelbaar. Je bent mijn vrouw, moeder, vriendin en je bent arts en je combineert alles alsof het niks voorstelt. Je bent een supermens zoals geen ander.

CURRICULUM VITAE

Pavel Taimr werd op 4 maart 1964 geboren in Praag, Tsjechie. Hij groeide op in Praag (Tsjechoslowakie) en Waterloo-Kitchener (Ontario, Canada). In 1982 slaagde hij voor zijn eindexamen aan Gymnasium Modrany, Praag. Na 1 jaar Geneeskunde te hebben gestudeerd aan de Comeniusuniversiteit, Martin (Slowakie) werd hij toegelaten tot studie Algemene geneeskunde aan de Karelsuniversiteit Praag.

Na behalen van zijn arts-examen (1988) heeft hij als AIOS op de afdeling Geneeskunde gewerkt in het Thomayer Ziekenhuis (Praag). In 1994 was hij medeoprichter van een nieuw gevormd levertransplantatie team bij Institute for Clinical and Experimental Medicine (IKEM, Praag).

In 1996 legde hij het examen in Interne geneeskunde (gr.II., Tsjechie) met eervolle vermelding af, gevolgd door specialisatie als MDL-arts in Nederland in 2006. Zijn werkervaring als fellow levertransplantatie (1994-1999) omvat verschillende buitenlandse (levertransplantatie) centra waaronder Virchow (Prof. dr. P. Neuhaus, Berlin), Ichillov (Prof. dr. N. Arber, Tel Aviv), Mt. Sinai (Prof. dr. C. Miller, New York City) and Mayo Clinic (Prof. dr. R. Wiesner, Rochester, MN, USA).

In de jaren 2000-2001 ontving hij de Fulbright-studiebeurs en werkte als research fellow bij Center for Basic research in Digestive Diseases (Prof. dr. G. Gores), Mayo Clinic and Foundation, Rochester (MN, USA).

In 2005-2017 behaalde hij een positie als MDL arts bij MDL (Maag- Darm- Leverziekten) afdeling, Erasmus MC (Rotterdam, Netherlands, Prof. dr. E. J. Kuipers, Prof. dr. M. Bruno). In 2017 startte hij met zijn promotieonderzoek op de afdelingen MDL en Heelkunde (Prof. dr. R.A. de Man, Prof. dr.J.N.M. IJzermans). Bij zijn terugkeer (2017) naar Tsjechie werd hij benoemd als medisch directeur van het levertransplantatie programma van IKEM, Praag.