Quantitative imaging of liver fat and fibrosis
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INTRODUCTION

In Europe, liver disease is estimated to affect approximately 6% of the population, and is the fifth most common cause of death. Liver related mortality is associated with the incidence and prevalence of cirrhosis and primary liver cancer, both representing end-stage liver pathology. Viral hepatitis B and C, alcohol consumption and non-alcoholic fatty liver disease (NAFLD) are the leading causes of both entities in Europe. An estimated 170,000 and 47,000 European die of cirrhosis and primary liver cancer each year, respectively [1]. The main indications for liver transplantation in Europe are cirrhosis and liver cancer. Figure 1 illustrates the relative contribution of different primary liver diseases to the total number of liver transplantations in Europe.

Over the last decades the contribution of NAFLD to the total burden of chronic liver disease has increased. This increase is largely explained by the growing prevalence of obesity, insulin resistance and type 2 diabetes, with which NAFLD is associated [2, 3]. A key component of NAFLD is excessive fat accumulation in the liver, called hepatic steatosis or fatty liver. The prevalence of NAFLD is 20–44% in the general adult European population, and even higher in people with type 2 diabetes: 43–70% [1]. The progressive form of NAFLD is called non-alcoholic steatohepatitis (NASH), a condition in which inflammation leads to the formation of liver fibrosis [3–5]. Liver fibrosis is the result of a sustained wound healing response to chronic liver injury and plays a central role in chronic liver disease in general. If untreated, fibrosis can progress to cirrhosis, liver dysfunction, portal hypertension and hepatocellular carcinoma (HCC) [6, 7]. In viral hepatitis B and C, fibrosis and even early cirrhosis can reverse after successful antiviral treatment [8–10].

Liver biopsy

Liver biopsy is considered the reference standard for the evaluation of both liver steatosis and fibrosis. Pathologists interpret disease severity qualitatively or semi-quantitatively based on
validated staging systems [11, 12]. The most commonly used staging systems for liver fibrosis and for hepatic steatosis are outlined in Table 1. Liver biopsy, however, has some drawbacks. First, a small tissue sample of 2-3 cm in length is biopsied, representing approximately 1/50,000 of the total liver [13]. Due to the inhomogeneous nature of liver fibrosis and steatosis, this small sample is prone to sampling error [14–16]. Second, liver biopsy is an invasive procedure. Severe complications occur in 0.57% and the mortality rate is approximately 1 in every 10,000-12,000 biopsies [13, 17]. Third, inter- and intraobserver variation plays a role in the interpretation of liver biopsy samples [14, 18].

These limitations have initiated the search for non-invasive alternatives for the evaluation of liver fibrosis and steatosis. Ideally, such a method should be easy to perform, reliable and inexpensive. In addition, the technique should have a low inter- and intra-observer variability, so that it can be used to monitor patients over time and evaluate treatment response.

Therefore, a variety of imaging methods have been proposed as alternatives to liver biopsy. For liver steatosis, imaging methods include ultrasonography (US), computed tomography (CT), magnetic resonance imaging (MRI) and proton magnetic resonance spectroscopy (1H-MRS). For evaluation of liver fibrosis, imaging methods include ultrasound based transient elastography (TE) and magnetic resonance elastography (MRE).

### Non-invasive imaging techniques for liver steatosis

**Ultrasonography:** US is widely used in clinical practice for the evaluation of hepatic steatosis, as it is a safe and inexpensive examination method. Criteria for steatosis assessment with ultrasound include: liver echogenicity, echotexture, visibility of the diaphragm and large vessels, and beam attenuation (Figure 2) [19]. However, evaluation of steatosis with ultrasonography is qualitative and not quantitative in nature. Due to this qualitative nature, the technique is not suitable to detect subtle changes in liver fat content over time.

### Table 1. Staging systems for liver steatosis and liver fibrosis

<table>
<thead>
<tr>
<th>Steatosis*</th>
<th>Parenchymal involvement by steatosis</th>
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</thead>
<tbody>
<tr>
<td>Grade 0 – none</td>
<td>&lt;5%</td>
</tr>
<tr>
<td>Grade 1 -mild</td>
<td>5-33%</td>
</tr>
<tr>
<td>Grade 2 -moderate</td>
<td>33-66%</td>
</tr>
<tr>
<td>Grade 3 - severe</td>
<td>&gt;66%</td>
</tr>
<tr>
<td>*Steatosis staging system according to Kleiner et al. [11].</td>
<td></td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Fibrosis*</th>
</tr>
</thead>
<tbody>
<tr>
<td>F0 – none</td>
</tr>
<tr>
<td>F1 – mild</td>
</tr>
<tr>
<td>F2 – moderate</td>
</tr>
<tr>
<td>F3 – severe</td>
</tr>
<tr>
<td>F4 - cirrhosis</td>
</tr>
<tr>
<td>*Fibrosis staging system according to the METAVIR cooperative study group [12].</td>
</tr>
</tbody>
</table>
Computed Tomography: CT beam attenuation in the liver is related to liver fat content, and therefore CT allows for quantitative evaluation of hepatic steatosis (Figure 3). Drawbacks of using CT for the evaluation of liver fat content include radiation exposure and susceptibility to confounding effects, for instance the presence of cirrhosis or the accumulation of glycogen, iron or amyloid [20].

Magnetic Resonance Imaging and Magnetic Resonance Spectroscopy: MRI and proton MR spectroscopy (1H-MRS) are considered the most accurate non-invasive imaging methods...
for liver steatosis quantification. Several acquisition methods have been studied, including chemical shift imaging and frequency selective fat saturation for MRI \[21–26\] and point-resolved spectroscopy (PRESS) and stimulated-echo acquisition mode (STEAM) sequences for $^1$H-MRS \[27–29\]. The mechanisms behind calculating liver fat content with MRI and $^1$H-MRS are described in detail in chapter 2 - including figures.

**Non-invasive imaging techniques for liver fibrosis**

Several non-invasive imaging techniques make use of the fact that the liver becomes stiffer as fibrosis progresses \[30–36\]. The technique that has been most extensively studied, and that is currently used in clinical practice is **ultrasound based transient elastography (TE)** or **Fibroscan®** (Echosens, France) \[31, 33, 37\].

**Transient elastography** measures the propagation speed of a shear wave in liver tissue. The corresponding stiffness is calculated and expressed in kilopascals (kPa). The stiffer the tissue, the faster the shear wave propagates. Shear waves are generated by a vibrating device that is attached to an ultrasound probe (Figure 4). The propagation speed of the shear waves is evaluated by the Doppler effect, and is related to the stiffness of the tissue. The liver tissue volume that is measured with transient elastography approximates a cylinder of $1 \times 4$ cm. This volume is 100-200 times bigger than a biopsy sample. In a meta-analysis on the diagnostic accuracy of transient elastography, sensitivity and specificity for stage F2 fibrosis were 0.79 (95% CI 0.74–0.82) and 0.78 (95% CI 0.72–0.83), and 0.83 (95% CI 0.79–0.86) and 0.89 (95% CI 0.87–0.91) for cirrhosis (stage F4) \[38\]. Limitations of transient elastography include the fact that the penetration of shear waves within tissues is limited, hampering evaluation of obese patients. Moreover, shear waves do not penetrate through liquids; therefore the technique cannot be performed in patients with ascites. Finally, TE is less reliable in case of liver inflammation or shortly after a meal \[39–43\].

![Figure 4. Left: TE machine (Fibroscan); Right: positioning of the TE probe against the right side of the chest. Images: Sandrin et al. [53].](image-url)
Magnetic resonance elastography (MRE) uses MR imaging for the evaluation of wave propagation within tissue. The main principle of MRE is comparable to that of transient elastography, namely, measuring tissue stiffness (viscosity and elasticity). However, MRE differs from transient elastography in several ways. First, mechanical waves are sent into the liver by a portable transducer that is positioned to the right side of the chest. By sending waves in continuously, a steady-state condition is created. In transient elastography, only short pulses of shear waves are sent into the liver. Second, MRE uses compression (or longitudinal) waves, where TE makes use of shear (or transverse) waves. The advantage of compression waves is the good penetration through tissue and water. This allows for analysis of a much larger portion of the liver with MRE compared with TE. Also, patients with ascites can be examined with MRE, which is not possible with TE. At tissue interfaces, mode conversion of compression waves occurs, which leads to the generation of shear waves perpendicular to the direction in which the compression wave is travelling. A motion-sensitive MRI sequence measures the tissue displacement in three orthogonal directions. From these three-dimensional displacement maps, elasticity and viscosity values can be calculated (Figure 5) [44–46]. The elasticity with

**Figure 5.** MRE examination. **A:** positioning of the transducer against the right side of the chest, with which the patient is positioned in the MRI scanner. **B:** anatomical image of the liver (transverse view). **C:** MR phase images representing mechanical waves travelling though the liver, evoked by the vibrating transducer. **D:** the final elastogram. Voxels are colour-coded, and represent elasticity in kilopascals (kPa). A region of interest (red) is drawn in the liver to calculate the mean elasticity value in the liver.
MRE is, just like TE, also expressed in kPa. Its magnitude is by rule 1/3 of the stiffness value measured with TE [32]. Several studies have shown the high accuracy of MRE for determining the stage of liver fibrosis [32, 47–52]. Until now, only one study compared the diagnostic accuracy of MRE with that of TE in a group of 96 patients with different stages of fibrosis, and showed that the accuracy of MRE was higher than that of TE [32].

**THESIS OUTLINE**

This thesis focuses on the application of non-invasive imaging techniques for evaluation of liver steatosis and liver fibrosis. Several aspects of different techniques are discussed such as feasibility, reproducibility and diagnostic accuracy, based on both existing literature and original data.

**Hepatic steatosis**

Chapter 2 gives a concise overview of how hepatic steatosis is measured with different MRI and ¹H-MRS methods. Furthermore, the available literature on the diagnostic accuracy of MR-based methods is reviewed.

Besides MRI and ¹H-MRS, CT and Ultrasonography can also evaluate hepatic steatosis. In the meta-analysis presented in Chapter 3, the available literature on the diagnostic accuracy of all four modalities is analyzed to answer the following question: which modality performs best?

In obese children, ultrasonography is the technique of choice to examine the presence and severity of hepatic steatosis. But how well can ultrasonography detect hepatic steatosis?

Chapter 4 describes the results of a clinical study in a group of 104 severely obese children. Liver ultrasonography was performed with ¹H-MRS as the reference standard. Subsequently, the diagnostic accuracy and inter-observer agreement of ultrasonography was evaluated.

**Hepatic fibrosis**

Magnetic resonance elastography is an emerging technique for the evaluation of liver fibrosis. Besides aspects such as diagnostic accuracy, success rate and patient acceptance, a key feature of a new technique is its reproducibility. The reproducibility of MRE of the liver and the relationship between reproducibility and spatial resolution was studied in a group of 32 patients and healthy volunteers. The results of this study are described in Chapter 5.

In Chapter 6 we compare the diagnostic accuracy of MRE with transient elastography (Fibroscan) in a group of 103 patients with viral hepatitis B or C, with liver biopsy as the reference standard. Which technique is best, and what is the additional diagnostic yield of a conditional strategy?

In Gaucher Disease (GD), a lysosomal storage disorder, long-term liver complications include fibrosis, cirrhosis and development of hepatocellular carcinoma (HCC), especially in those patients who underwent a splenectomy in the past as part of their treatment. Also, excess liver iron storage may play a role in the increased risk of GD patients to develop HCC. Chapter 7 describes a study in which we investigated whether type 1 GD patients with and without a splenectomy differed with respect to liver stiffness and liver iron content, which would support the hypothesis that the increased HCC risk in splenectomized GD patients is related to the presence of fibrosis and iron.

In Chapter 8 we summarize our main findings and discuss the findings of this thesis.
REFERENCES


