Quantitative imaging of liver fat and fibrosis
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SUMMARY, DISCUSSION AND CONCLUSIONS
SUMMARY

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

In Chapter 2, MR-based imaging techniques for evaluation of liver steatosis are discussed. MR-based methods are increasingly used for liver fat quantification. Dual-echo in-phase and opposed-phase (IP/OP) MR imaging is a fast and easily implemented technique, allowing fat quantification of the entire liver. Confounding influences of T2* and fat-fat interference are limitations. Multi-echo chemical shift techniques have a higher diagnostic accuracy than dual-echo IP/OP imaging since T2* and fat-fat interference effects are corrected for. However, these techniques require more complex post-processing. Frequency selective fat saturation imaging is less susceptible to T2* effects than dual-echo IP/OP imaging, but is very sensitive to B0 and B1 heterogeneities. Proton MR-spectroscopy (1H-MRS) has a high diagnostic accuracy for liver fat quantification, comparable with multi-echo chemical-shift techniques. A limitation of 1H-MRS is the fact that measurements are confined to a pre-selected volume (voxel) in the liver, which means that only a relatively small part of the liver is analysed.

Chapter 3 summarizes the available literature on the diagnostic accuracy of US, CT, MRI and 1H-MRS. In a pooled analysis of 46 included studies, sensitivity estimates were 73-91% for US, 46-72% for CT, 82-97% for MRI and 73-89% for 1H-MRS. Pooled specificity estimates were 70-85% for US, 88-95% for CT, 76-95% for MRI and 92-96% for 1H-MRS. These results show that MR-based techniques (MRI and 1H-MRS) have a higher diagnostic accuracy for the evaluation of liver steatosis compared to US and CT, and can be considered as the techniques of choice for non-invasive evaluation of hepatic steatosis.

Because of its low burden and cost, and high availability, US is the technique of choice for evaluation of liver steatosis in children, but how good is the diagnostic accuracy of US for detecting and staging steatosis in an obese paediatric population? Chapter 4 describes the results of a prospectively conducted study comparing US with 1H-MRS as the reference standard in a group of 104 severely obese children. The overall prevalence of any hepatic steatosis was 46% (including mild, moderate and severe steatosis). A positive liver US study for any hepatic steatosis was correct in 41 of 66 children (62%). A negative liver US study excluded the presence of any steatosis (82%). Moderate and severe (or substantial) steatosis was present in 15% of children. In this group, a positive liver US study was correct in only 12 of 23 children (52%). However, a negative liver US study was correct in the majority, 95%. We learned from these results that US has an acceptable accuracy to predict the absence of substantial hepatic steatosis in severely obese children. US, however, cannot be used to predict the presence and severity of hepatic steatosis in obese children. A positive US study therefore requires additional work-up, for instance MRI or 1H-MRS, to confirm the presence and to stage the severity of hepatic steatosis.

For MR elastography (MRE), as for any other new technique, it is critical to know what the expected variation between repeated measurements is, and what the threshold for a significant change is. Chapter 5 describes the results of a comprehensive reproducibility study in a group of 16 healthy volunteers, and 14 patients with viral hepatitis B or C and biopsy proven liver fibrosis. All participants underwent MRE twice while in the same position during the same
session (intra-scan reproducibility), once after repositioning (within-day reproducibility), and once more after 1-4 weeks (between-weeks reproducibility). Four different MRE parameters were analysed: elasticity, viscosity, attenuation parameter $\alpha$ and propagation parameter $\beta$. We learned from this study that the over-time changes in the liver as measured with MRE that would represent significant changes are: 22% for elasticity, 26% for viscosity, 27% for $\alpha$ and 10% for $\beta$. Patient repositioning had a significant effect on reproducibility. In addition to these (whole liver) reproducibility analyses, we performed a more detailed voxel-wise reproducibility analysis in relation to spatial resolution. Reproducibility decreases when spatial resolution increases. This means that when looking at a focal area of liver fibrosis, reproducibility will be lower than when looking at fibrosis in a large liver area. By means of Gaussian filtering, we defined the highest spatial resolution (in mm full-width half-maximum, FWHM) at which results were still acceptably reproducible. The optimal trade-off between spatial resolution and reproducibility was found at a filter size of 8 mm FWHM for elasticity and $\beta$ and at 16 mm FWHM for viscosity and $\alpha$. In conclusion, this study showed that propagation parameter $\beta$ is the most reliable MRE parameter, with an over-time threshold for significant change of 10% and the ability to reproduce viscoelasticity up to a resolution of 8 mm FWHM.

The study presented in Chapter 6 prospectively compares the diagnostic accuracy of transient elastography (TE) and MRE for the non-invasive evaluation of liver fibrosis in a cohort of 103 patients with hepatitis B or C. Liver biopsy was used as the reference standard. Data of 85 patients were analysed (65 hepatitis B, 19 hepatitis C, 1 co-infected). Fibrosis stages were F0 (no fibrosis) in 3 patients, F1 (mild fibrosis) in 53, F2 (moderate fibrosis) in 15, F3 (severe fibrosis) in 8 and F4 (cirrhosis) in 6 patients. An important clinical event in hepatitis B and hepatitis C is the development of significant fibrosis (stage F2 or higher). From this stage, the risk of progression to cirrhosis and subsequent development of hepatocellular carcinoma increases, and antiviral treatment needs to be considered to slow down or reverse progressive liver fibrosis or cirrhosis. The diagnostic accuracy of both techniques for detecting stage F2 or higher were comparable. We defined lower and upper cut-off values for both techniques, which we used to retrospectively select those patients who would or who would not have needed a liver biopsy, based on TE and/or MRE results. We employed both a single technique strategy as a conditional technique strategy (MRE after inconclusive TE and vice versa). Based on this strategy, cut-off values of <5.2 and ≥ 8.9 kPa for TE and <1.66 and ≥ 2.18 kPa for MRE diagnosed 64% and 66% of patients correctly as F0-F1 and F2-F4, respectively. A conditional strategy, by employing MRE in case of an inconclusive TE result and vice versa (TE result between 5.2 and 8.9 kPa, or MRE result between 1.66 and 2.18 kPa), increased the diagnostic yield to 80%. This means that based on this strategy, 80% of patients could have correctly been diagnosed as F0-F1 or F2-F4 fibrosis, obviating the need for a liver biopsy.

Chapter 7 describes the results of a pilot study in which the possible use of MRE, TE and liver iron measurements of the liver with MRI was studied in patients with type 1 Gaucher Disease (GD). Long-term liver-related complications in patients with type 1 GD include fibrosis and an increased incidence of hepatocellular carcinoma. Splenectomy, formerly the only treatment option in GD, has been implicated as a risk factor for the development of liver pathology in GD. Also, excess liver iron storage may play a role in the increased risk of GD patients to develop HCC. In this pilot study, comprising 14 type-1 GD patients (7 splenectomized, 7 non-splenectomized) and 7 healthy controls, we demonstrated that liver stiffness values, as measured by Transient Elastography and MR-Elastography,
were significantly higher in splenectomized GD patients when compared with non-splenectomized GD patients. Liver iron concentration was elevated in 4 GD patients of whom 3 were splenectomized. No significant relationship was found between liver stiffness and liver iron concentration. Liver disease appeared more advanced in splenectomized than in non-splenectomized patients. Based on the results of this pilot study, we can only hypothesize a relationship between liver pathology and excessive hepatic iron accumulation in splenectomized patients.

DISCUSSION

Why imaging of steatosis and fibrosis?
The drawbacks of liver biopsy have initiated the search for non-invasive alternatives. These alternatives need to be able to accurately represent the information on the condition of the liver that is obtained by liver biopsy, including (1) the presence and stage of fibrosis, (2) the presence and amount of steatosis, (3) the presence of iron and (4) the presence of necro-inflammatory activity.

Some liver diseases require assessment of a subset of these parameters. In viral hepatitis B and C for instance, information on the stage of fibrosis and amount of inflammatory activity comprise important parameters with respect to patient prognosis. In hepatitis C, steatosis is associated with more severe fibrosis and rapid disease progression [1, 2]. In case of liver transplantation, macrovesicular liver steatosis of the graft of more than 30% is a significant risk factor for primary graft non-function [3–5]. In Gaucher Disease, we have shown that information on liver fibrosis and iron accumulation is relevant.

Imaging of fibrosis: What technique to use?
We compared two imaging techniques for fibrosis: MR-elastography (MRE) and transient elastography (TE). Although a previous study by Huwart et al. [6] found a higher diagnostic accuracy for MRE compared with TE for the assessment of liver fibrosis, we were not able to confirm these results in our cohort: we found no significant difference between the accuracies of both techniques. As discussed in that chapter, possible explanations include the skewed distribution of the fibrosis stages in our population (most patients had stage F1 fibrosis in our population) or the quality of the liver biopsies (not all biopsies met the required 11 portal tracts). Also, a different MRI field strength and MRE sequences were used in our work versus their work: 3T vs. 1.5T MR field strength and a spin-echo echo-planar imaging (EPI) sequence versus a spin-echo MR sequence. By using a spin-echo EPI sequence instead of a spin-echo sequence, the acquisition time of a single MRE examination was shortened from 20 minutes to 70 seconds [7]. Since EPI sequences are more sensitive to field inhomogeneities, which could possibly have led to less stable results, this time advantage may have been at the cost of accuracy. To overcome this disadvantage, a more robust MRE sequence making use of fast-field echo imaging with fractional encoding has been developed [8].

Assuming that TE and MRE also have a similar diagnostic accuracy, what would be the reason to use MRE? Drawbacks are the fact that MRE is probably more expensive than TE, more time-consuming and not suitable for every patient. However, the theoretical advantages of MRE over TE include: (1) analysis of a larger liver volume. Averaging over a larger volume
will decrease the risk of sampling error; (2) focal liver lesions can be assessed [9]; (3) MRE is feasible in obese subjects and patients with ascites; (4) measurement of both elasticity and viscosity, which may be beneficial in the differentiation of liver tumours [9]; (5) Multifrequency MRE allows studying the frequency-dependent behaviour of tissue. This additional parameter might give additional information on the micro-architecture of tissue [10]; and finally (6) the possibility to combine MRE with other MR examinations such as fat and iron quantification, and detection of focal lesions (one-stop-shop MRI).

Other techniques for imaging fibrosis and steatosis

Acoustic radiation force imaging (ARFI) is an ultrasound-based elastography method for the evaluation of liver fibrosis [11]. ARFI is integrated in a conventional ultrasound machine. In contrast to TE, the exact measurement site can be localized. Other theoretical advantages of ARFI relative to TE include better tissue penetration and penetration of the ultrasound waves through ascites. Currently, ARFI has shown similar accuracy, but better feasibility compared with TE in patients with liver fibrosis [12].

Controlled attenuation parameter (CAP) is a novel parameter that is measured using the latest TE systems (Fibroscan®). Liver steatosis is measured by analysing the decrease in amplitude of the ultrasound waves that travel through the liver [13]. So far, CAP seems to be a promising tool for the evaluation of liver steatosis [14–17]. To our knowledge, no studies have yet been published that compare CAP with MRI or 1H-MRS.

Non-alcoholic fatty liver disease

There is one entity, that has been discussed briefly in the introduction of this thesis: non-alcoholic fatty liver disease (NAFLD). In NAFLD steatosis, fibrosis and inflammation all play a role. The disease is considered to be the hepatic manifestation of the metabolic syndrome, and insulin resistance is regarded the main pathophysiological hallmark [18, 19]. The growing epidemic of obesity has led to a rapid increase of NAFLD prevalence [20]. The inflammatory form of NAFLD, non-alcoholic steatohepatitis (NASH) is characterized by inflammation and liver-cell injury. As a result, fibrosis develops [21]. About 9% to 20% of patients with early stage NASH will progress to cirrhosis over a period of 5–10 years [22]. In contrast, patients with simple steatosis tend to be stable over time [23]. It is therefore important to know whether a patient has simple steatosis or NASH, and whether fibrosis is present.

Non-invasive imaging of NASH: what is missing? Is it possible?

From this thesis, and from the information given above, it becomes clear that if we want to use imaging to diagnose NASH non-invasively, one critical parameter is still missing. While we can use imaging techniques to diagnose and stage liver steatosis and fibrosis, we also need information on the inflammatory status of the liver. If the necro-inflammatory processes that are typical for NASH can be detected non-invasively, this may well be the key to differentiating simple steatosis from NASH. Over the last few years, several MR-based imaging techniques have been proposed that might be able to fill this gap.

Phosphorus MR Spectroscopy (31P-MRS) measures the MR signal originating from phosphorus atoms that are present in liver metabolites, such as adenosine triphosphate (ATP), phosphomonoesters
Superparamagnetic particles of iron oxides. In the healthy liver, superparamagnetic iron oxide (SPIO) particles accumulate in the liver through phagocytosis by Kupffer cells (the macrophages of the liver). Since SPIO reduces signal intensity of the liver on T2 and T2* weighted MR sequences, the particles can be used as an MRI contrast agent [26]. In NASH, a decrease in the phagocytic activity of hepatic Kupffer cells is thought to play a crucial role in its pathogenesis [27–29]. This led to the hypothesis that a decrease in the amount of SPIO particles taken up into the liver would be a sensitive marker for NASH when using MRI. So far, some small studies in humans have been conducted, and have shown that indeed SPIO-enhanced MRI may be helpful for identifying NASH among patients suspected of having NAFLD [26, 30].

Molecular MR-imaging of myeloperoxidase. Besides the involvement of Kupffer cells, neutrophil accumulation is a prominent feature of the inflammation in NASH [31, 32]. These phagocytes can induce tissue damage through the generation of aggressive oxidants, which is mediated by the enzyme myeloperoxidase (MPO) [33, 34]. A recent study conducted in a NASH model with MPO deficient mice confirmed the important role of MPO in the development of NASH [35]. Another group used MPO-gadolinium, an MRI contrast agent that interacts with MPO [36], in a NASH mouse model and demonstrated that MPO-Gd enhanced molecular MRI could reliably distinguish NASH from steatosis [37].

In summary, although there are promising techniques with respect to imaging liver inflammation, all are still in an early phase, and their accuracy and clinical applicability will have to be confirmed in much larger (human) populations.

CONCLUSIONS

As demonstrated in this thesis, accurate non-invasive imaging techniques exist that can be used for the evaluation of liver fibrosis and steatosis, each with their own advantages and disadvantages. When looking at steatosis, ultrasound is cheap and widely available. Disadvantages are its qualitative nature and interobserver variability. Compared to other available techniques, the diagnostic accuracy of ultrasonography for steatosis evaluation is relatively low for mild and moderate steatosis. CT can quantify the amount of steatosis, however the main drawback of CT is the ionizing radiation. Like ultrasonography, the accuracy of CT for mild steatosis is limited. MRI and 1H-MRS have a high accuracy for measuring steatosis over its entire range. Drawbacks however included costs and availability. For detection and staging of fibrosis, transient elastography (TE) and MR elastography (MRE) have been studied and discussed. TE is a rapid procedure with low intra- and interobserver variability. Drawbacks include the one-dimensionality of the measurement (no image of the liver available), and failure in obese patients and in patients with ascites. MRE overcomes these limitations, and evaluates a much larger portion of the liver. However, due to attractive alternatives with comparable accuracy, MRE may not become the first-line examination to screen for liver fibrosis.
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