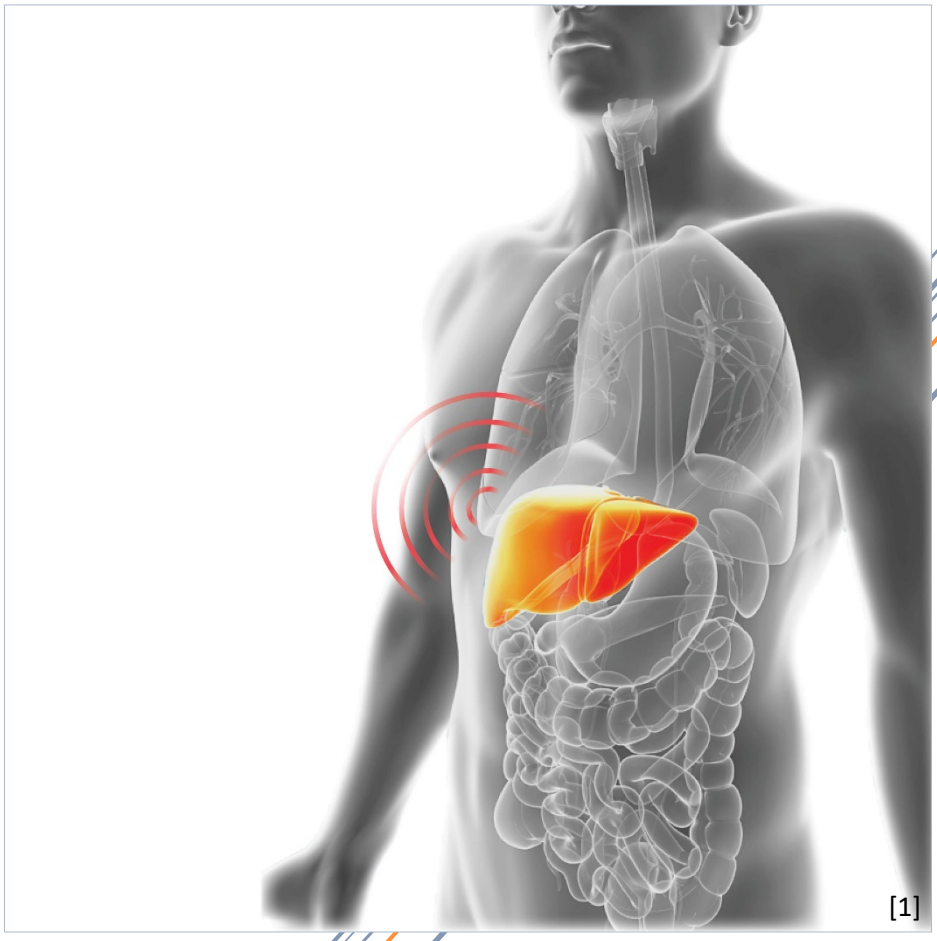
Current techniques and future directions of liver fibrosis imaging

Liver fibrosis imaging techniques for theranostics



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Abstract

Chronic liver disease is responsible for over 2 million deaths every year, with half of those deaths being caused by cirrhosis. Liver fibrosis is characterized by excessive collagen production and extracellular matrix (ECM) deposition, and can have a wide variety of causes. Fibrosis is divided into five stages, depending on the fibrotic state. Accurate diagnosis and staging are key in the prognosis and monitoring of the disease progression. The current golden standard for the diagnosis and staging of liver fibrosis is a biopsy. However, due to its invasive nature and the risks involved, there is a need for a non-invasive fibrosis imaging technique. Ultrasound (US) and magnetic resonance imaging (MRI) are imaging techniques currently used in the clinic, but have limited accuracy, especially in the earlier stages of fibrosis. US-based transient elastography (TE) has shown potential for the diagnosis and staging of early stages of liver fibrosis, but lacks in accuracy. MRI-based magnetic resonance elastography (MRE) has shown greater potential, as it can diagnose and stage liver fibrosis with high accuracy, even the earlier stages of liver fibrosis. The focus of this essay is to determine which imaging technique is the best for the diagnosis and staging of liver fibrosis, and whether it can be applied for theranostics.

Abbreviations

AASLD	American Association for the Study of Liver Diseases
AFRI	Acoustic radiation force impulse imaging
ALT	Alanine aminotransferase
APASL	Asia-Pacific Association for the Study of the Liver
APRI	Aspartate-to-platelet ratio index
BMI	Body Mass Index
CT	Computed tomography
EASL	European Association for the Study of the Liver
ECM	Extracellular matrix
ELF	Enhanced Liver Fibrosis
EPI	Echo planar imaging
FDA	Food and Drug Administration
Gd	Gadolinium
Gd-EOB-DTPA	Gadolinium ethoxybenzyl dimeglumine (Primovist®)
HA	Hyaluronic acid
HCV	Hepatitis C virus
HSC	Hepatic stellate cell
HVPG	Hepatic venous pressure gradient
kPa	kilopascal
MNP	Magnetic nanoparticles
MRE	Magnetic resonance elastography
MRI	Magnetic resonance imaging
NAFLD	Nonalcoholic fatty liver disease
NASH	Nonalcoholic steatohepatitis
OI	Optical imaging
PET	Positron emission tomography
PIIINP	Amino-terminal propeptide of type III procollagen
ROI	Region of interest
SPECT	Single-photon emission computed tomography
SWE	Shear wave elastography
TE	Transient elastography
TIMP-1	Tissue inhibitor of metalloproteinase 1
US	Ultrasound
WHO	World Health Organization

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1.0 Introduction

Liver disease is responsible for approximately 2 million deaths per year worldwide, of which 1 million deaths are due to complications of cirrhosis [2]. Cirrhosis is an advanced state of liver fibrosis, and is described as the development of regenerative nodules surrounded by fibrous bands [3]. Liver fibrosis results from chronic liver injury, and is characterized by the accumulation of extracellular matrix (ECM) proteins. This, in turn, leads to disruption and distortion of the tissue architecture and function [4]. The main causes of liver fibrosis include chronic hepatitis C virus (HCV) infection, alcohol abuse, autoimmune reactions, generation abnormalities, and nonalcoholic steatohepatitis (NASH) [4-5]. These conditions result in inflammation, hepatic stellate cell (HSC) activation, pathological angiogenesis, overproduction of cytokines, and ECM deposition [4]. Also, there is an increase in fibronectin osteopontin, hyaluronan, proteoglycans, laminins, and different collagen subtypes [4].

The cell types involved in the regulation of ECM homeostasis are fibroblasts and other mesenchymal cell types [17]. Responsible for the activation and proliferation of fibroblasts are different cytokines such as IL-1, tumor necrosis factor (TNF), and transforming growth factor-ß (TGFß), but also other growth factors including platelet-derived growth factor (PDGF) [17]. The proteins produced by the fibroblasts can promote the differentiation of profibrotic myofibroblasts during fibrosis, via positive feedback regulation [18].

There are five different stages of fibrosis: F0 (no fibrosis), F1 (minimal scarring), F2 (significant fibrosis; scarring has occurred and extends outside the liver area), F3 (severe fibrosis; fibrotic tissue spreading and forming bridges with other fibrotic liver areas), and F4 (cirrhosis; advanced scarring) [11]. Stage 2 fibrosis and above is an indication of chronic liver fibrosis [19]. However, when the underlying cause is treated, almost all stages of liver fibrosis are reversible.

Accurate diagnosis and staging of fibrosis is key in determining the proper prognosis and monitoring the disease progression. The golden standard for staging and diagnosis of liver fibrosis is a biopsy. However, due to its invasive nature and limited representativeness, there is a need for a non-invasive technique that allows for diagnosis, staging and treatment monitoring [4]. Non-invasive imaging techniques such as computed tomography (CT), magnetic resonance imaging (MRI), optical imaging (OI), positron emission tomography (PET), single-photon emission computed tomography (SPECT), and ultrasound (US) have all been studied for this purpose.

Besides imaging techniques, liquid-based biopsies may also be helpful for the assessment of liver fibrosis. Several serum biomarkers are associated with the stage of liver fibrosis, and testing these parameters is already applied in patients with chronic HPV infection [4]. One of the most commonly used tests for the assessment of liquid biomarkers, is the FibroTest®. The FibroTest® measures gamma-GT, total bilirubin, alpha-2-macroglobulin, apolipoprotein A1, and haptoglobin. This test is often combined with an ActiTest, which measures alanine aminotransferase (ALT) as a marker for inflammatory activity [6]. Both tests are recommended by the World Health Organization (WHO), American Association for the Study of Liver Diseases (AASLD), the European Association for the Study of the Liver (EASL), and the Asia-Pacific Association for the Study of the Liver (APASL) for the assessment of hepatitis fibrosis in patients with chronic hepatitis C [6]. However, the FibroTest® measures indirect fibrosis markers and is quite expensive. Also, the diagnostic accuracy does not exceed 80-85% [8].

The aspartate-to-platelet ratio index (APRI) is another test that is commonly used. Although a study found that APRI is an accurate test for the prediction of mild versus significant fibrosis, it is not an accurate predictor for cirrhosis [7].

The Enhanced Liver Fibrosis score (ELF) measures hyaluronic acid (HA), amino-terminal pro-peptide of type III procollagen (PIIINP), and tissue inhibitor of metalloproteinase 1 (TIMP-1) [9]. Although widely used in the clinic, this test also has its shortcomings. HA is not specific to the liver and only gives global information about ECM metabolism [10]. PIIINP is also not specific to the liver, and its serum levels may also be increased due to degradation of collagen. Finally, TIMP-1 has also been shown to not be a specific marker to hepatic tissue, and the half-life and clearance of all three markers has not been studied enough [10].

In general, liquid-based biomarkers alone may not be organ-specific enough to give a proper indication on liver function, and also do not reflect fibrosis nor discriminate between the different stages of fibrosis. However, combining liquid biomarkers with other techniques such as imaging, may improve the accuracy of fibrosis diagnosis and staging.

Until now, only the non-invasive assessment of chronic hepatitis B and C infections is possible through the combination of two tests, i.e. serum biomarkers and transient elastography (TE) [4]. For diagnosis and staging of liver fibrosis, biopsies are still required. However, functional imaging of liver fibrosis can assess liver stiffness, which relates to the fibrotic state. Methods for elastography include TE, acoustic radiation force impulse imaging (ARFI), shear wave elastography (SWE), and MR elastography (MRE) [4].

The first approved TE device is FibroScan®, which measures the shear wave velocity generated on the skin [4,12]. The main downside of TE is that it does not directly measure fibrosis, which can result in over-estimation of the fibrotic state. Possible reasons for an over-estimation are liver inflammation, cholestasis, lesions in the liver, and liver congestion [12].

The ARFI method uses a gray-scale US image [14]. Short-duration acoustic push pulses travel along the US beam, inducing shear stress in the target tissue. The shear waves move away from the region of excitation, and can be detected with tracking US beams. The speed of these shear waves is related to the elasticity of the tissue. SWE is very similar to ARFI as it uses the same excitation pulse [15]. The shear waves are tracked and used to estimate the shear-wave speed.

Combining ARFI, SWE, or the FibroScan® with the aspartate aminotransferase (AST)/ALT ratio or APRI may increase diagnostic accuracy, and may have an advantage over using a single test, especially when a single test alone is inconclusive [12,13].

For the detection of fibrosis and differentiating the progression stages, MRE has a greater diagnostic performance than the US-based elastography techniques [4]. A recent study showed that MRE was more accurate than TE in patients with nonalcoholic fatty liver disease (NAFLD) [16]. MRE was also shown to be more accurate than TE for the diagnosis of any fibrotic stage except for cirrhosis.

Besides functional imaging, molecular imaging tools have also been proposed for assessing liver fibrosis. Targeted contrast agents for MRI enable the imaging of organ specific molecular processes. Gadolinium ethoxybenzyl dimeglumine (Gd-EOB-DTPA), also known as Primovist® (Europe), is a liver-specific contrast agent that is taken up and excreted by hepatocytes [4]. As the uptake and excretion of compounds is impaired in liver fibrosis, Primovist® enables the detection of cirrhosis and severe fibrosis.

Besides cellular uptake, ECM components like collagen and elastin can also be analyzed by MRI. This concept has already been proven in animal models, and was shown to be helpful in staging liver fibrosis and enabled therapy monitoring [4].

In recent years, a new strategy for therapy monitoring has gained attention: theranostic imaging. This strategy combines diagnostic imaging and therapy into a single compound. Using this strategy allows for the selection of patients that are most likely to benefit from the treatment, thereby enabling personalized treatment [20].

Despite the shortcomings of non-invasive techniques for the assessment of liver fibrosis, the major advantage compared to the liver biopsy is that these methods can easily be repeated over time, allowing the response to the therapy to be monitored regularly.

In this review, we will investigate the most promising non-invasive imaging techniques for the detection of early stage fibrosis, as well as evaluate the potential of these methods for theranostics.

2.0 Non-invasive liver fibrosis imaging techniques for early-stage liver fibrosis

The degree of liver fibrosis is very important for the proper diagnosis and treatment in chronic liver disease. As previously described, liver biopsy is currently the golden standard for the diagnosis and staging of fibrosis. However, the risks, sampling errors, and intra- and interobserver variability lead to an increased interest in non-invasive fibrosis imaging and staging techniques.

As previously described, there are already some non-invasive imaging techniques for liver fibrosis applied in the clinic. The most routinely used is standard gray-scale US, which is able to distinguish a smooth liver surface from a severe nodular liver surface (figure 1A) [4]. However, this method is limited to the detection of advanced state liver fibrosis as structural changes in the liver become visible at later stages of liver fibrosis [21].

Besides US, MRI is also commonly used in the clinic. However, similar to US, MRI only shows structural changes (figure 1B) [4]. Besides the lack of sensitivity, there is a high interobserver variability leading to different measures of accuracy within studies [21].

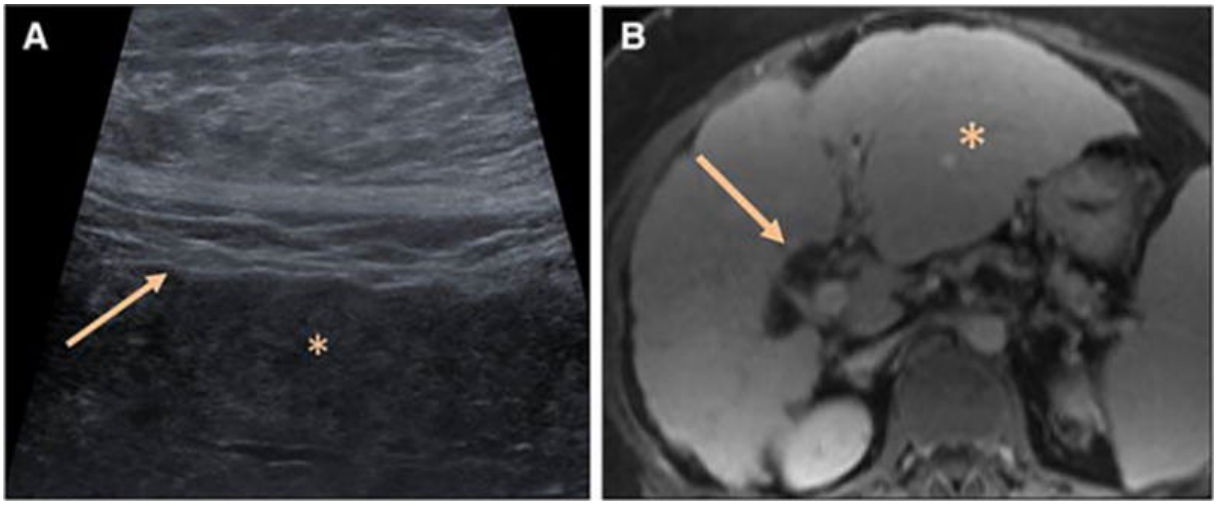

*Figure 1: Morphologic imaging of cirrhosis. A) US shows a nodular surface, indicated with the arrow, and a coarse hepatic echotexture indicated with *. B) MRI image of a cirrhotic liver with a nodular surface (indicated with the arrow) and an enlarged hilar periportal space (indicated with *).*

In summary, although commonly used, standard US and MRI are not sufficient in distinguishing the different stages of liver fibrosis. Therefore, researchers have been looking into techniques than can be used for the diagnosis and staging of liver fibrosis, especially the earlier stages of fibrosis.

2.1 Transient Elastography may be able to detect early-stage fibrosis

Recent research proposes that elastography techniques may be able to detect early-stage fibrosis [19]. Elastography measures liver stiffness, which correlates to liver fibrosis. It was found that clinically useful elastography techniques are either US- or MRI-based.

An example of US-based elastography that is able to differentiate between moderate and severe fibrosis is TE. The first approved TE device is FibroScan®, which measures the shear wave velocity generated on the skin [4,12]. The liver stiffness is expressed as the median value of 10 valid measurements in kiloPascals (kPa) [22]. The cut-off values for the liver fibrosis stages are shown in figure 2 [23].

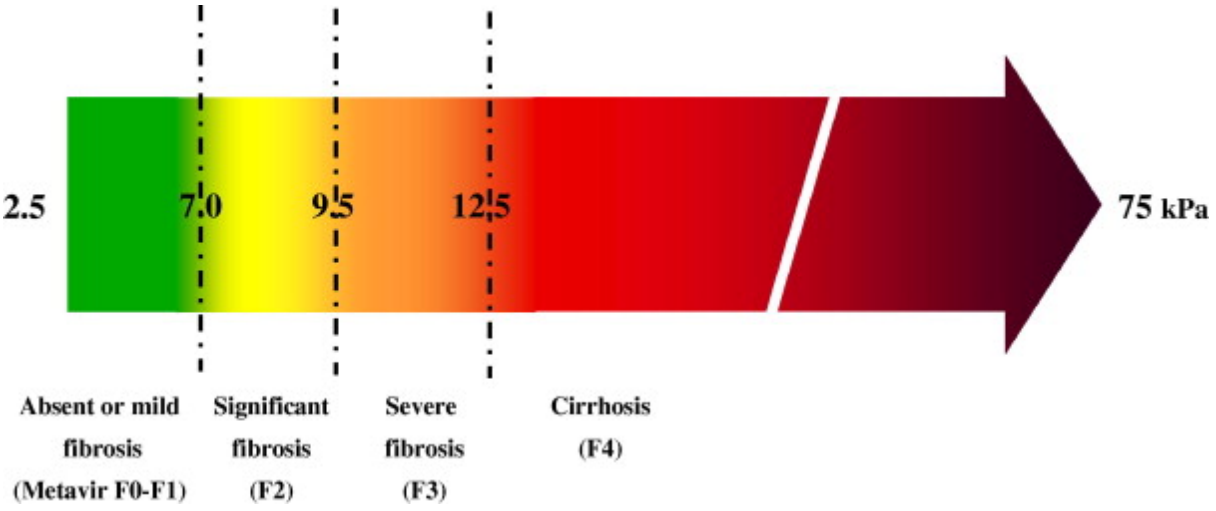

Figure 2: Livers stiffness cut-offs in chronic liver diseases.

The shear wave propagation velocity is directly related to liver stiffness: the stiffer the tissue, the faster the shear wave propagates [23].

A study from Castera et al. in 2008 discussed the potential of TE in the staging of liver fibrosis. Their findings, shown in figure 3, showed that TE was able to distinguish the different stages of fibrosis. The reproducibility of TE was found to be excellent, in terms of intra- and interobserver agreement, for patients with moderate to severe stages of liver fibrosis. However, in patients with lower degrees of fibrosis (stage F2 or lower), with hepatic steatosis, or with an increased body mass index (BMI) (>25 kg/m^2), the interobserver agreement was significantly reduced.

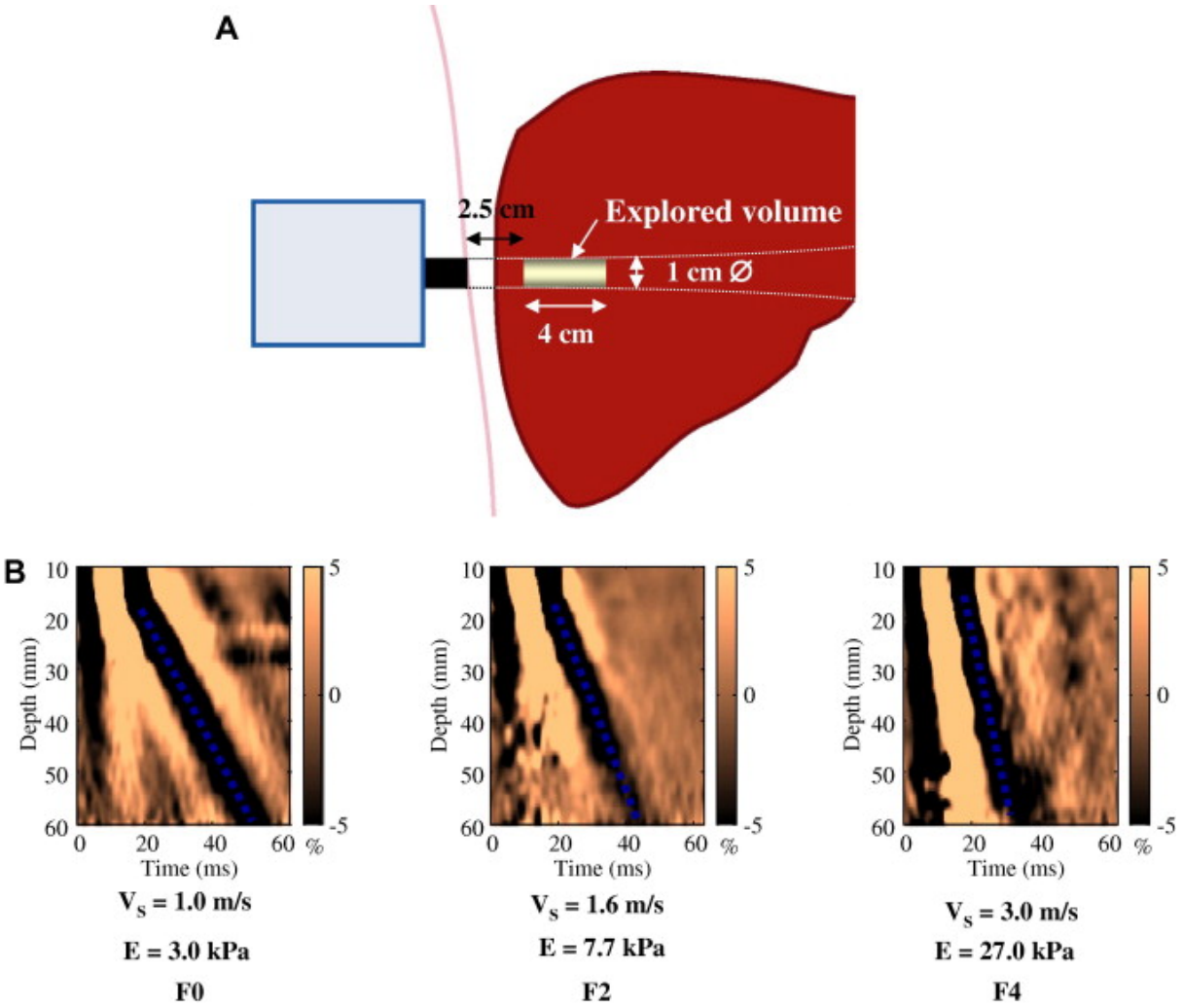

Figure 3: Evaluation of liver fibrosis with TE. A) Position of probe and explored volume. B) Shear wave propagation velocity in different stages of fibrosis (METAVIR). The elastic modulus E expressed as E = 3ρV2, where V is the shear velocity and ρ is the mass density (constant for tissues).

As previously described, the main downside of TE is that it does not directly measure fibrosis, which can result in over-estimation of the fibrotic state, likely due to liver inflammation, cholestasis, lesions in the liver, or liver congestion [12]. Also, TE does not display the location of where the stiffness is measured, thus confirmation that the measurements are from the liver is difficult. Interobserver agreement is low in patients with early-stage fibrosis, making TE less accurate in the diagnosis of early stage fibrosis.

In summary, although TE can be used for diagnosing intermediate liver fibrosis, it may not able to accurately detect early-stage fibrosis [21].

2.2 Magnetic Resonance Elastography can accurately assess early-stage liver fibrosis

The most accurate non-invasive imaging technique for liver fibrosis is MRE. Several studies proved that MRE is superior to TE for the detection and staging of liver fibrosis [19][21]. MRE relies on standard MRI protocols for imaging of the liver.

MRE measures the mechanical property of the tissue by using propagating shear waves, in a similar fashion as TE. Shear waves propagate faster in stiffer tissue, and the velocity of the waves is reflected in the wavelength [24]. Hence, the wavelength becomes longer in stiffer tissues. Mechanical shear waves with a low frequency (around 60 Hz) are generated by a special acoustic driver system, along with a modified phase-contrast pulse sequence to image the displacements associated with the wave propagation. The wave images are processed with specialized software to generate quantitative cross-sectional images showing the tissue stiffness, shown in figure 4 [24]. The low frequency vibrations are well tolerated by patients, and do not cause any discomfort.

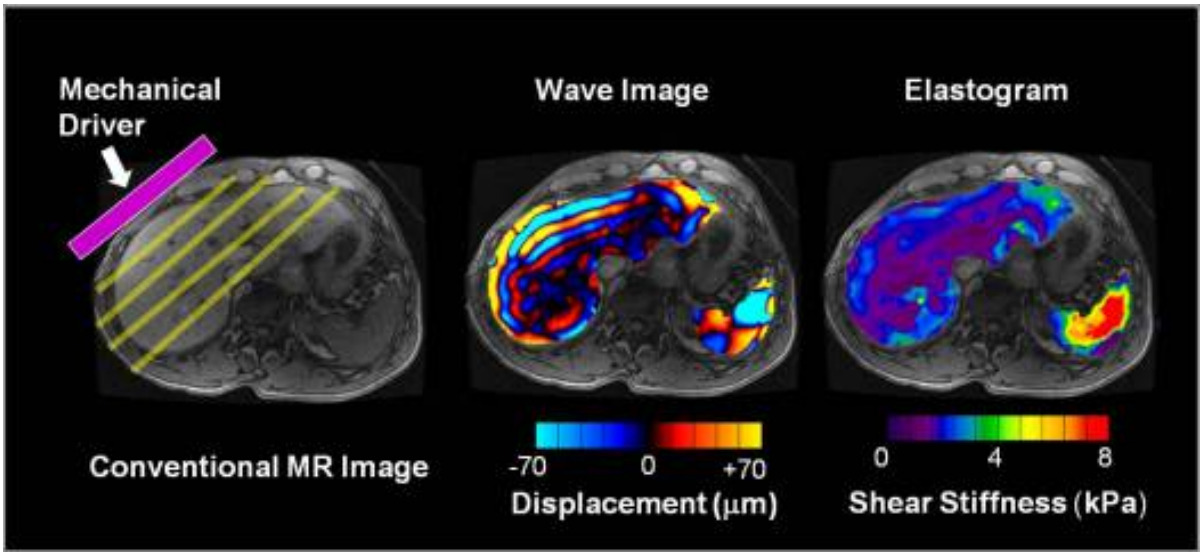

Figure 4: Process of MRE imaging. The figure on the left shows the placement of the driver, the middle shows the wave image, and the right figure shows the image after being processed, referred to as the elastogram.

The liver stiffness is assessed by drawing regions of interest (ROI), which can be geographic or oval in shape [24]. Normal hepatic parenchyma has a shear stiffness value less than 3 kPa, liver fibrosis can be diagnosed with high specificity and sensitivity is the stiffness is above this value (figure 5).

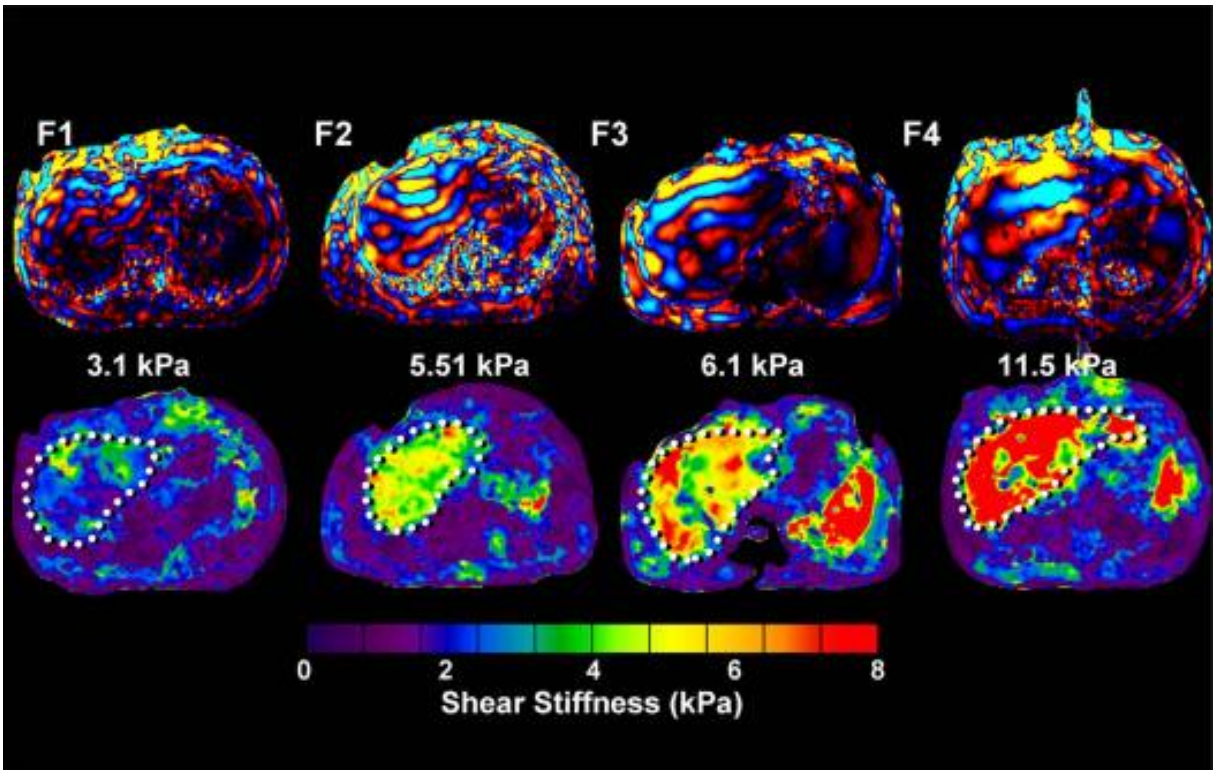

Figure 5: Elastograms from patients with different stages of liver fibrosis. The top row shows wave images from patients with biopsy-confirmed liver fibrosis ranging from METAVIR stage 1 to 4. The bottom row shows elastograms from these patients.

MRE allows for liver fibrosis to be detected well before other imaging signs of fibrosis are seen [24]. Also, fibrosis is not a homogenous process, which in the case of a biopsy this can potentially create sampling errors, but with MRE this pattern of fibrosis can be visualized.

The problem that arose with TE, in which obese patients could not be accurately diagnosed, is eliminated with MRE. In an obese patient with a BMI of 43, the liver wave image is not affected by the thick layer of fat, as shown in figure 6.

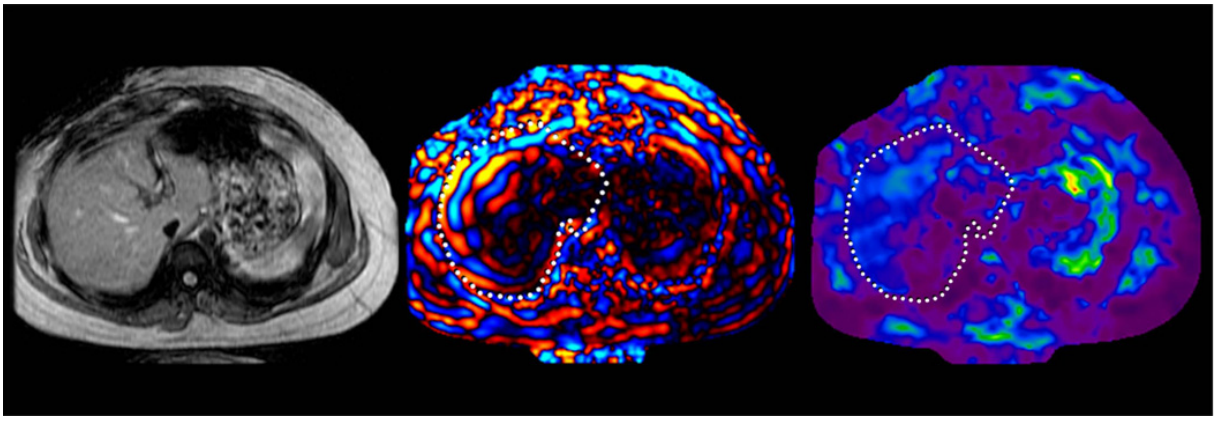

Figure 6: MRE images of a patient with obesity. The middle image shows that the wave image is not affected by a thick layer of fat. The elastogram (right) shows that the patient has normal liver stiffness.

Although the problem with obese patients has been overcome, a new problem has arisen. Steatosis alone does not appear to have an effect of liver stiffness. This affects patients with NALFD. However, research has shown that when the disease progresses to inflammation (NASH) the liver stiffness does increase, and can be accurately measured with MRE.

Besides the many advantages of MRE, the technique also has its shortcomings. During MRE, the patients need to hold their breath consistently. When patients fail to properly do this, the wave image and elastogram will be affected. In patients with moderate to severe cases of hemochromatosis the MRI signal may be too low, affecting the visualization of the waves. Examples of both cases are shown in figure 7. The resulting elastograms from both patients were not valid.

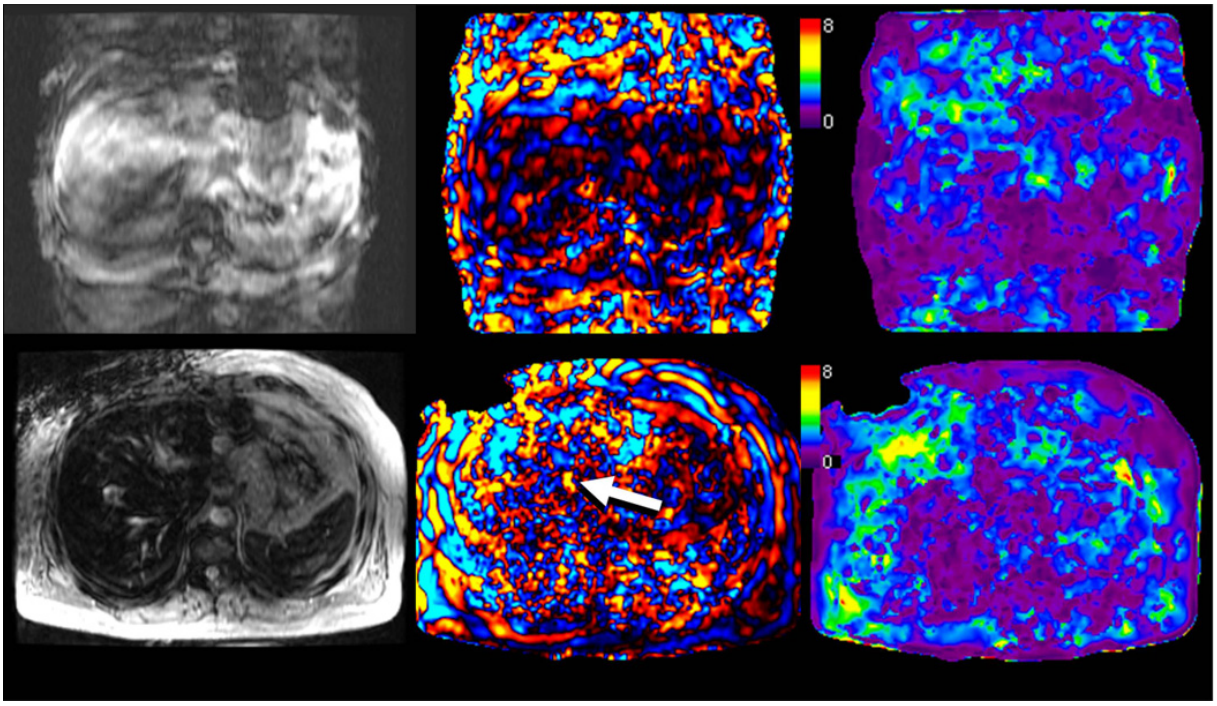

Figure 7: Limitations of MRE. The top row shows images from patients who could not hold their breath properly and consistently. The bottom row shows images from a patients with hemochromatosis and high. The wave pattern (arrow) could not be visualized.

However, there seems to be a solution for patients with hemochromatosis. A MRE study with a spin-echo based echo planar imaging (EPI) technique, which is designed to be less affected by hepatic iron, showed an improved wave image and elastogram (figure 8).

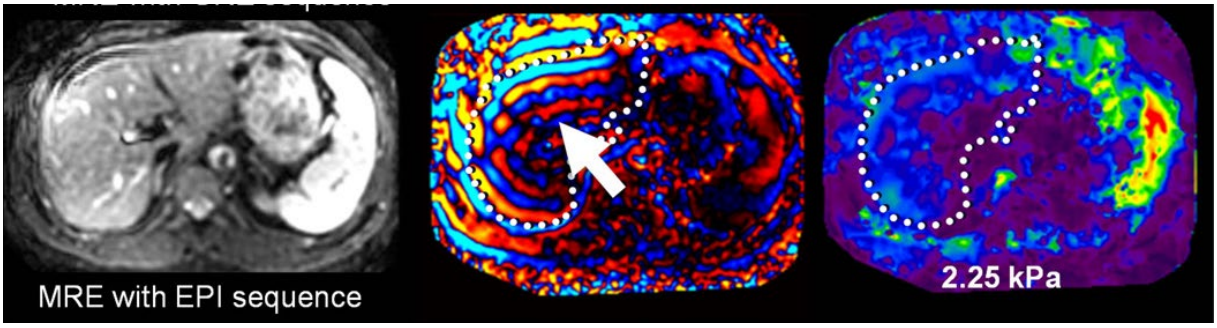

Figure 8: Echo-based EPI MRE of patient with hemochromatosis. The arrow indicates the wave pattern in the liver.

In summary, MRE is a promising non-invasive alternative to biopsies in patients with liver fibrosis. The technique is also relevant for the monitoring of patients that are receiving treatment for liver fibrosis. There is still room for further improvement, as the image resolution is still low.

MRE is not only safer, but also less expensive compared to biopsies. But most importantly, patient compliance is higher as it is painless.

3.0 Can MRE be employed for theranostics?

MRE is a very promising non-invasive imaging technique for early-stage liver fibrosis, making it possible for patients to receive the proper treatment earlier, increasing the chance of reversal of liver fibrosis. As MRE has shown potential for monitoring the fibrotic state, the next question is: can it be applied in theranostics?

As previously described, MRE is performed in sequence with MRI and only images the liver stiffness. Because it is applied in combination with MRI, targeted contrast agents with a therapeutic effect become an interesting possibility. Liver-specific contrast agents are already applied in the clinic, an example being Primovist®. Primovist® mainly consist of gadolinium (Gd), which was shown to not have an significant effect on liver stiffness [24]. Therefore, MRE can be performed either before or after intravenous Primovist® injection.

Besides Primovist® there are other liver-specific contrast agents used in the clinic, such as MultiHance and OptiMARK. Both MultiHance and OptiMARK are based on gadolinium. Recent research has shown that pullulan-conjugated Gd-DTPA is also hepatocyte specific, and may have an extended plasma half-life [24][25]. Coupling of these contrast agents with a therapeutic may enable MRI/MRE to be used in theranostics.

Magnetic Nanoparticles (MNP) have already been developed to enhance diagnostics, and are used as MR contrast agents [24]. MNPs can be synthesized of particles with various sizes and properties, allowing therapeutic molecules to be carried to and released in a specific environment. Besides controlling the size of the particle, poly ethylene glycol (PEG), amongst other polymers, can be used to stabilize the particle in solution. Albumin is commonly used to avoid immunogenicity and increase cellular uptake [24].

Maeng et al. have shown that MR sensitivity and the anticancer efficacy of MNPs loaded with doxorubicin is increased in rat and rabbit liver cancer models [27]. A novel polymeric nanoparticle (YCC-DOX) composed of poly(ethylene oxide) trimellitic anhydride chloride-folate (PEO-TMA-FA), doxorubicin, Fe_3O_4, and folate, was synthesized and tested for its anticancer effect. The animals were divided in four groups: a saline control, free doxorubicin (FD), DOXIL® (nanoparticle containing doxorubicin), and YCC-DOX. Each formula was injected, and the signal intensity compared to Resovist® (conventional contrast agent) was measured in all animals using MRI (figure 9A). Before and after the treatment the size of the tumor was also analyzed with MRI (figure 9B)

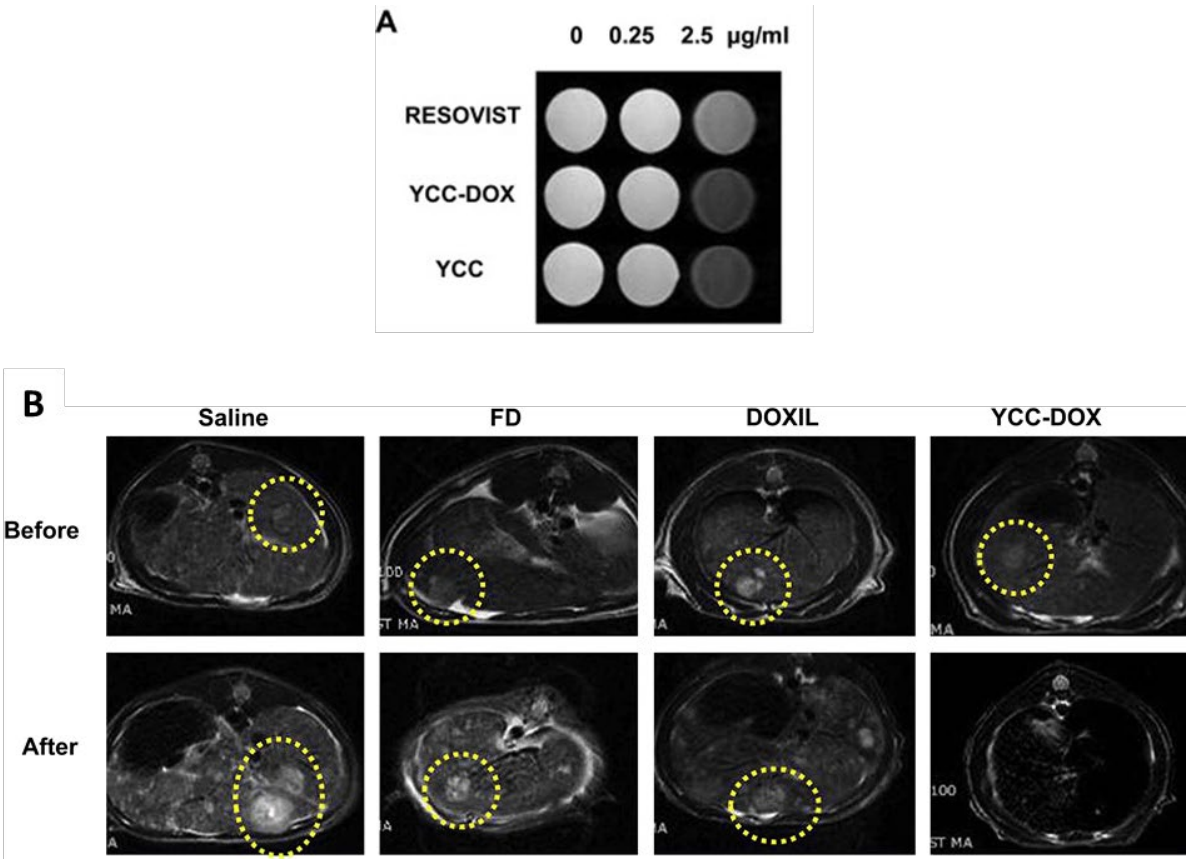

Figure 9: Anticancer effect and MRI sensitivity. A) Signal intensities of Resovist®, YCC-DOX and YCC. B) MR images before and after treatment.

The analysis revealed that the MRI signal intensity of YCC and YCC-DOX was comparable to Resovist®, even though YCC-DOX has a lower iron concentration than Resovist®. The MRI signal enhancement of YCC and YCC-DOX were increased compared with Resovist®, and the tumor size was significantly decreased in YCC-DOX treated animals.

The results proved that YCC-DOX is not only a proper contrast agent, but also an excellent anti-cancer therapeutic. Thus, YCC-DOX is a great example of a theranostic agent. These results may also open up the possibility that a similar compound could be designed for the staging, treatment, and monitoring of liver fibrosis.

In conclusion, in combination with MRI, MRE can provide accurate diagnosis of early-stage liver fibrosis. Using a targeted compound that is not only a contrast agent but also a therapeutic opens up the possibility for MRI/MRE to be used in theranostics. MRE is not only easier and more comfortable than liver biopsies, it is also safer. Fatality is a problem with liver biopsies, as 1 in every 10,000 patients dies of biopsy complications [30]. According to a 2010 article in Clinical Anatomy, it was reported that MRE has a 99% accurate diagnosis rate [29].

4.0 Discussion

Liver disease is a worldwide problem, where cirrhosis in particular is responsible for over 1 million deaths per year [2]. As many stages of fibrosis are reversible, early diagnosis of liver fibrosis is very important. The current golden standard are liver biopsies, however, this method has many limitations. Besides the risks involved in taking a liver biopsy, there is low representativeness and patient compliance. Also, it is not a suitable technique for monitoring a patient over the course of their therapy. Therefore, non-invasive fibrosis staging and imaging techniques are of great interest.

Liquid-based biopsies, such as serum biomarkers, are easy to obtain and measure. However, serum biomarkers are usually not direct markers of liver fibrosis, and cannot detect fibrosis with high accuracy rates. Methods like the FibroTest® do not exceed accuracy rates beyond 80-85% [8].

Although frequently applied in the clinic, US and MRI cannot detect early stage liver fibrosis and therefore do not have the potential to replace liver biopsies. Elastography on the other hand, may be able to detect early stage fibrosis. TE, an US-based elastography method, has shown great potential. TE measures the shear wave propagation velocity, which is directly related to liver stiffness. Although TE was able to distinguish the different stages of liver fibrosis, the main limitation is that it does not show the liver. This makes it difficult to confirm that the measured stiffness is actually from liver tissue. The low observer agreement in earlier stages of liver fibrosis also do not make it a reliable technique for the diagnosis of early-stage liver fibrosis.

The MRI-based elastography method, MRE, showed a greater potential. Contrary to TE, MRE does display the position of the liver, and intra- and interobserver agreement is higher. Because the technique is MRI-based, this opens up the opportunity of using the technique for theranostics. A liver specific contrast agent with a therapeutic effect, YCC-DOX, showed a greater antitumor effect than free doxorubicin or the targeted form of doxorubicin, DOXIL®. It also showed comparable MRI signal intensity to Resovist®, a commercial contrast agent. These findings confirmed that YCC-DOX is not only a great anticancer drug, but also a good contrast agent.

The success of YCC-DOX in animal studies proved that MRI is a suitable technique for theranostics. In liver fibrosis, the combination of using a theranostic agent for MRI and the measurement of the liver stiffness with MRE could give an accurate analysis of liver stiffness, while at the same time allowing for monitoring of the treatment. As MRE is not limited to measuring stiffness in the liver, the method can also be applied to other tissues. Therefore, targeted organ-specific contrast agents with a therapeutic effect may allow for treatment and monitoring of many other diseases. As MRI is already widely used in the clinic, and MRE is used increasingly more, investigating these possibilities becomes very appealing.

Measuring stiffness does not only give insight in the fibrotic state, it may also help in characterizing tumors. A study by Venkatesh et al. showed that the stiffness of malignant tumors was significantly higher than that of benign tumors [31]. This further broadens the spectrum of diseases in which MRE can be applied.

As of today, the MRE scan still has its shortcomings. Failure to conduct shear waves can occur, especially in patients with extreme obesity it can be difficult to position the patient into the scanner and apply the passive driver. This will result in poor contact between the driver and the patient. Chest wall deformities can also result in poor contact, but this problem can be resolved by changing the position of the passive driver to obtain good contact to the largest part of the liver. Recently, new passive drivers have been designed which, contrary to the conventional disc driver, are flexible [32]. This allows for better contact between the driver and the patient.

Also, MRE is motion sensitive. Therefore, a poor breath-hold technique can disrupt the signal. Recently it has become possible to obtain a single slice of MRE in a short time (11-12 seconds), minimizing the chance of motion during the breath hold [29]. Another limitation of MRE and MRI is that it cannot be used for patients with implants, as the techniques use a magnetic field [40].

As described in chapter 2.2 (figure 7), high iron can cause failure of the propagation of the shear waves, resulting in a poor signal. To improve the liver signal, alternative pulse sequences such as the spin-echo EPI sequence can be used. However, in some rare cases, the iron overload is so severe that the patient should first receive treatment to reduce the iron content in the liver, before receiving the MRE scan [29].

The current MRE sequence that is applied in the clinic is a 2D sequence, which assumes that the shear waves propagate in two dimensions. However, this can lead to over-estimation of liver stiffness in some cases [29][34]. Recently, 3D MRE has been developed to further improve the accuracy and reproducibility of MRE [34]. 3D MRE is better in evaluating the spatial patterns of liver fibrosis, and also allows for the analysis of additional parameters, like volumetric strain [29]. The technique also provides substantially more images (32 slices), which is useful in the characterization of liver tumors [34].

Besides stiffness, inflammation also plays a role in the development and progression of liver fibrosis [35]. Therefore, assessment of inflammation in the liver may give an indication of the stage of liver fibrosis. Results from a study on mice and pigs by Yin et al. showed that liver stiffness increased in NASH, inflammation, fibrosis, and portal hypertension [36]. In patients with cirrhosis, liver stiffness positively correlates with the hepatic venous pressure gradient (HVPG), a marker for liver cirrhosis an hepatocellular carcinoma [37]. This implies that, although indirectly, MRE can also give an indication of the venous pressure gradient. Results from a study on rats by Salameh et al. showed that MRE could detect inflammation before the onset of fibrosis [33]. Chen et al. performed a retrospective study and found that stiffness measurements with MRE could help identify patients with steatohepatitis even before the first signs of fibrosis [39].

Although promising results have been published, MRE may have a new-found limitation. Yin et al. suggested that a high level of steatosis could possibly lead to unchanged or even decreased liver stiffness in the early stages of NAFLD [36]. Not only in NAFLD, but also in borderline NASH the inflammation and fibrosis are mild, making MRE less accurate in the assessment of these diseases. However, the effects of fibrosis and steatosis could be frequency dependent, with higher frequencies possibly being more useful in detecting the early phases of NAFLD and borderline NASH [36].

The damping ratio can also be assessed with MRE. In physics, damping is described a resistive force that reduces the amplitude of oscillations. The damping ratio reflects how rapidly the oscillations decay. In tissues, damping is related to tissue resistance. The damping ratio can distinguish inflammation from fibrosis in early stages of liver disease [36]. In a study by Yin et al., results showed that the damping ratio increased in case of edema, and decreased when early fibrosis developed [36].

In conclusion, MRE can accurately indicate the stage of hepatic fibrosis, and the combination of a targeted therapeutic contrast agent for MRI allows for the non-invasive monitoring of the treatment. MRE can not only assess liver fibrosis, but the technique can also be applied for other tissues. Most importantly, MRE can detect early stages of liver fibrosis with high accuracy, which allows for the patient to receive the treatment sooner, thus increasing the chance of recovery. Because the technique is MRI based, a theranostic contrast agent could potentially be used for therapy and monitoring of liver fibrosis.

MRE has been approved by the Food and Drug Administration (FDA) for clinical use, and is currently being investigated for its potential use in other pathologies [38][40].

5.0 Bibliography

[1] Johns Hopkins Medicine (2017, December 21) After the Cure: What comes next for patients with post-HCV liver fibrosis? Retrieved from: https://clinicalconnection.hopkinsmedicine.org

[2] Asrani, S. K., Devarbhavi, H., Eaton, J., & Kamath, P. S. (2018). Burden of liver diseases in the world. Journal of hepatology.

[3] Schuppan, D., & Afdhal, N. H. (2008). Liver cirrhosis. The Lancet, 371(9615), 838-851.

[4] Baues, M., Dasgupta, A., Ehling, J., Prakash, J., Boor, P., Tacke, F., ... & Lammers, T. (2017). Fibrosis imaging: Current concepts and future directions. Advanced drug delivery reviews, 121, 9-26.

[5] Bataller, R., & Brenner, D. A. (2005). Liver fibrosis. The Journal of clinical investigation, 115(2), 209-218.

[6] Bio Predictive, FibroTest-ActiTest is a non-invasive diagnostic tool for the liver. Retrieved from: https://www.biopredictive.com/

[7] Snyder, N., Gajula, L., Xiao, S. Y., Grady, J., Luxon, B., Lau, D. T., ... & Petersen, J. (2006). APRI: an easy and validated predictor of hepatic fibrosis in chronic hepatitis C. Journal of clinical gastroenterology, 40(6), 535-542.

[8] Padoan, A., Bernardi, D., & Plebani, M. (2015). Biomarker Panels and Multiple Readouts. In Principles of Translational Science in Medicine (pp. 159-166). Academic Press.

[9] Siemens Healthcare GmbH. Enhanced Liver Fibrosis (ELF™) Test. Retrieved from: https://www.siemens-healthineers.com/

[10] Gungoren, M. S., Efe, C., Kav, T., & Akbiyik, F. (2018). Diagnostic accuracy of enhanced liver fibrosis (ELF) test for significant fibrosis in patients with autoimmune hepatitis. Journal of Laboratory and Precision Medicine, 3(3).

[11] Pavlov, C. S., Casazza, G., Nikolova, D., Tsochatzis, E., Burroughs, A. K., Ivashkin, V. T., & Gluud, C. (2015). Transient elastography for diagnosis of stages of hepatic fibrosis and cirrhosis in people with alcoholic liver disease. Cochrane Database of Systematic Reviews, (1).

[12] Kemp, W., & Roberts, S. (2013). FibroScan and transient elastography. Australian family physician, 42(7), 468.

[13] Castera, L. (2012). Noninvasive methods to assess liver disease in patients with hepatitis B or C. Gastroenterology, 142(6), 1293-1302.

[14] Bruno, C., Minniti, S., Bucci, A., & Mucelli, R. P. (2016). ARFI: from basic principles to clinical applications in diffuse chronic disease—a review. Insights into imaging, 7(5), 735-746.

[15] Drehfal, L. (2018). Elasticity Imaging in Obstetrics. In Obstetric Imaging: Fetal Diagnosis and Care (pp. 696-700). Elsevier.

[16] Park, C. C., Nguyen, P., Hernandez, C., Bettencourt, R., Ramirez, K., Fortney, L., ... & Valasek, M. A. (2017). Magnetic resonance elastography vs transient elastography in detection of fibrosis and noninvasive measurement of steatosis in patients with biopsy-proven nonalcoholic fatty liver disease. Gastroenterology, 152(3), 598-607.

[17] Zhao, X., Kwan, J. Y. Y., Yip, K., Liu, P. P., & Liu, F. F. (2019). Targeting metabolic dysregulation for fibrosis therapy. Nature Reviews Drug Discovery, 1-19.

[18] Kendall, R. T., & Feghali-Bostwick, C. A. (2014). Fibroblasts in fibrosis: novel roles and mediators. Frontiers in pharmacology, 5, 123.

[19] Venkatesh, S. K., Yin, M., Takahashi, N., Glockner, J. F., Talwalkar, J. A., & Ehman, R. L. (2015). Non-invasive detection of liver fibrosis: MR imaging features vs. MR elastography. Abdominal imaging, 40(4), 766-775.

[20] Drude, N., Tienken, L., & Mottaghy, F. M. (2017). Theranostic and nanotheranostic probes in nuclear medicine. Methods, 130, 14-22.

[21] Horowitz, J. M., Venkatesh, S. K., Ehman, R. L., Jhaveri, K., Kamath, P., Ohliger, M. A., ... & Wells, M. L. (2017). Evaluation of hepatic fibrosis: a review from the society of abdominal radiology disease focus panel. Abdominal Radiology, 42(8), 2037-2053.

[22] Pavlov, C. S., Casazza, G., Nikolova, D., Tsochatzis, E., Burroughs, A. K., Ivashkin, V. T., & Gluud, C. (2015). Transient elastography for diagnosis of stages of hepatic fibrosis and cirrhosis in people with alcoholic liver disease. Cochrane Database of Systematic Reviews, (1).

[23] Castera, L., Forns, X., & Alberti, A. (2008). Non-invasive evaluation of liver fibrosis using transient elastography. Journal of hepatology, 48(5), 835-847.

[24] Venkatesh, S. K., Yin, M., & Ehman, R. L. (2013). Magnetic resonance elastography of liver: technique, analysis, and clinical applications. Journal of magnetic resonance imaging, 37(3), 544-555.

[25] Jeong, Y., Hwang, H. S., & Na, K. (2018). Theranostics and contrast agents for magnetic resonance imaging. Biomaterials research, 22(1), 20.

[26] Jenjob, R., Kun, N., Ghee, J. Y., Shen, Z., Wu, X., Cho, S. K., ... & Yang, S. G. (2016). Enhanced conjugation stability and blood circulation time of macromolecular gadolinium-DTPA contrast agent. Materials Science and Engineering: C, 61, 659-664.

[27] Maeng, J. H., Lee, D. H., Jung, K. H., Bae, Y. H., Park, I. S., Jeong, S., ... & Lee, J. (2010). Multifunctional doxorubicin loaded superparamagnetic iron oxide nanoparticles for chemotherapy and magnetic resonance imaging in liver cancer. Biomaterials, 31(18), 4995-5006.

[29] Mariappan, Y. K., Glaser, K. J., & Ehman, R. L. (2010). Magnetic resonance elastography: a review. Clinical anatomy, 23(5), 497-511.

[30] Diagnostic Imaging (2013, January 24) MR Elastography Growing as Preferred Modality for Liver Diagnosis. Retrieved from: https://www.diagnosticimaging.com/

[31] Venkatesh, S. K., Yin, M., Glockner, J. F., Takahashi, N., Araoz, P. A., Talwalkar, J. A., & Ehman, R. L. (2008). MR elastography of liver tumors: preliminary results. American Journal of Roentgenology, 190(6), 1534-1540.

[32] Huang, X., Chafi, H., Matthews II, K. L., Carmichael, O., Li, T., Miao, Q., ... & Jia, G. (2019). Magnetic resonance elastography of the brain: A study of feasibility and reproducibility using an ergonomic pillow-like passive driver. Magnetic resonance imaging, 59, 68-76.

[33] Salameh, N., Larrat, B., Abarca-Quinones, J., Pallu, S., Dorvillius, M., Leclercq, I., ... & Van Beers, B. E. (2009). Early detection of steatohepatitis in fatty rat liver by using MR elastography. Radiology, 253(1), 90-97.

[34] Dzyubak, B., Glaser, K. J., Manduca, A., & Ehman, R. L. (2017, March). Automated liver elasticity calculation for 3D MRE. In Medical Imaging 2017: Computer-Aided Diagnosis (Vol. 10134, p. 101340Y). International Society for Optics and Photonics.

[35] Koyama, Y., & Brenner, D. A. (2017). Liver inflammation and fibrosis. The Journal of clinical investigation, 127(1), 55-64.

[36] Yin, M., Glaser, K. J., Manduca, A., Mounajjed, T., Malhi, H., Simonetto, D. A., ... & Elgilani, F. M. (2017). Distinguishing between hepatic inflammation and fibrosis with MR elastography. Radiology, 284(3), 694-705.

[37] Suk, K. T. (2014). Hepatic venous pressure gradient: clinical use in chronic liver disease. Clinical and molecular hepatology, 20(1), 6.

[38] Serai, S. D., Yin, M., Wang, H., Ehman, R. L., & Podberesky, D. J. (2015). Cross-vendor validation of liver magnetic resonance elastography. Abdominal imaging, 40(4), 789-794.

[39] Chen, J., Talwalkar, J. A., Yin, M., Glaser, K. J., Sanderson, S. O., & Ehman, R. L. (2011). Early detection of nonalcoholic steatohepatitis in patients with nonalcoholic fatty liver disease by using MR elastography. Radiology, 259(3), 749-756.

[40] Ortiz, J. A. A., Konik, E., Eckert, E. C., Pepin, K. M., & Greenberg-Worisek, A. (2018). Premarket Approval Through the 510 (k) Process: Lessons from the Translation Process of Magnetic Resonance Elastography. Clinical and Translational Science, 11(5), 447.