Current Hepatology Reports (2019) 18:492–502
https://doi.org/10.1007/s11901-019-00493-x

FATTY LIVER DISEASE (V AJMERA, SECTION EDITOR)

Quantification of Liver Fat in NAFLD: Available Modalities and Clinical Significance

Kee-Huat Chuah[1] · Wah-Kheong Chan[1]

Published online: 11 November 2019

Abstract

Purpose of Review To review the available modalities for quantification of liver fat in nonalcoholic fatty liver disease (NAFLD) and their clinical significance.

Recent Findings Ultrasonography remains the first line imaging used to diagnose NAFLD as it is widely available and relatively inexpensive. Controlled attenuation parameter can be used as a screening tool for fatty liver as it is reasonably accurate and provides simultaneous estimation of hepatic fibrosis. Magnetic resonance imaging proton density fat fraction and magnetic resonance spectroscopy are the most accurate noninvasive modalities for quantification of hepatic steatosis. Liver biopsy remains the gold standard but is limited by the invasive nature of the procedure, and observer and sampling variability. This may be improved with novel computer-assisted stereological analysis or second harmonic generation microscopy.

Summary Understanding the advantages and disadvantages of each of the modalities will help one choose the most suitable method for quantifying hepatic steatosis in NAFLD.

Keywords Hepatic steatosis · MRI-PDFF · MRS · CAP

Introduction

Nonalcoholic fatty liver disease (NAFLD) has become the leading cause of chronic liver disease in the world [1]. The global prevalence of NAFLD has been reported to be 25% based on a meta-analysis [2•]. NAFLD is closely associated with obesity and is considered the liver manifestation of the metabolic syndrome. Patients with NAFLD have an increased mortality rate when compared with the general population, mainly due to cardiovascular disease [3, 4]. Identifying patients with NAFLD represents an important first step towards providing more focused lifestyle advice to ameliorate the disease as well as further assessment for other components of the metabolic syndrome, which should be addressed accordingly, if present [5]. The ability to quantify liver fat allows monitoring over time, which is important for evaluation of lifestyle intervention or medical therapy, in both clinical trial and real-world settings. This paper aimed to review the currently available modalities for quantifying liver fat in NAFLD and their clinical significance.

This article is part of the Topical Collection on *Fatty Liver Disease*

✉ Wah-Kheong Chan
wahkheong2003@hotmail.com

[1] Gastroenterology and Hepatology Unit, Department of Medicine, Faculty of Medicine, University of Malaya, 50603 Kuala Lumpur, Malaysia

Ultrasonography

Ultrasonography (US) for assessment of hepatic steatosis is commonly qualitative in nature and typically consists of visual assessment of hepatic echogenicity compared with kidney cortex echogenicity, evaluation of echo penetration into the deep portion of the liver, and determination of the clarity of blood vessel structures in the liver. Normal liver echogenicity is graded as grade 0, while slight increase in liver echogenicity is considered mild steatosis (grade 1). Slight impairment in visualization of intrahepatic vessels and diaphragm with increase liver echogenicity is categorized as moderate steatosis (grade 2). In severe steatosis (grade 3), hepatic echogenicity is markedly increased, in addition to poor penetration to the posterior segment of the right lobe of the liver and poor visualization of hepatic vessels and diaphragm [6, 7]. A previous meta-analysis reported that US is reliable and accurate in detecting moderate-to-severe fatty liver with sensitivity of

84.8% and specificity of 93.6% [8•]. However, US has several limitations, including being highly operator-dependent [9], has poor accuracy in diagnosing mild steatosis [7], and may be less accurate to diagnose steatosis in patients with underlying hepatic fibrosis [10]. A recent study revealed that the sensitivity of US was only 65.1% when compared with magnetic resonance imaging proton density fat fraction (MRI-PDFF) in diagnosing mild steatosis [11]. To improve the accuracy, computer-assisted quantitative US techniques have been developed for the assessment of hepatic steatosis [12]. The most widely reported technique is the computerized hepatorenal index with two studies showing sensitivities and specificities of more than 90% in diagnosing hepatic steatosis of ≥ 5% [13•, 14]. In this technique, the ratio between the echogenicity of the liver and kidney cortex is calculated and analyzed using a histogram. However, a more recent study suggested that the hepatorenal ratio was not as good, while acoustic structure quantification (ASQ) from ultrasound echo amplitude was better for the assessment of hepatic steatosis. By using magnetic resonance spectroscopy (MRS) as the reference standard, the area under receiver operating characteristic curve (AUROC) of using the ASQ for diagnosing hepatic steatosis ≥ 10% was 0.959, with sensitivity of 86.2% and specificity of 100%, while the AUROC of using hepatorenal ratio was only 0.772, with sensitivity of 72.4% and specificity of 73.3% [15, 16]. Another quantitative US technique that measures backscatter coefficient was demonstrated to be accurate in quantifying hepatic steatosis ≥ 5% using the MRI-PDFF as the reference. The sensitivity and specificity were reported as 87–93% and 91–97%, respectively [17]. Overall, US remains the first-line imaging used to diagnose NAFLD as it is noninvasive, widely available, and relatively inexpensive.

Computed Tomography

Hepatic steatosis evaluation by computed tomography (CT) scan is based on the measurement of attenuation value of liver parenchyma, using Hounsfield units (HUs). Different tissue composition will have different attenuation value in HUs, for example, it is usually − 100 HU for fat and around 30–40 HU for soft tissue. With hepatic steatosis, the attenuation value of the liver parenchyma is reduced [12]. Absolute liver attenuation value is developed as a quantitative method to measure steatosis [18]. This is generally performed using unenhanced CT scan to avoid the potential changes in attenuation after contrast injection despite some studies reporting similar efficacy with contrast-enhanced CT. However, the accuracy of the absolute attenuation value for the quantification of hepatic steatosis may be limited by intra- and inter-scanner variability [19]. Established quantitative CT techniques of measuring liver-to-spleen attenuation ratio (CT L/S) and liver-to-spleen difference in attenuation (CT L-S) are able to limit the variability. In these techniques, the spleen, which generally has a lower attenuation value, is used as an internal control. CT has been reported to be reasonably accurate in diagnosing moderate to severe steatosis but lack the sensitivity to detect mild steatosis [18, 20]. The sensitivity of CT in detecting hepatic steatosis ≥ 5% has been reported to be 50.5 to 67.7% using the MRI-PDFF as reference standard [21]. More importantly, the ionizing radiation from CT deters clinician in using it as a routine tool for quantification of hepatic steatosis. The assessment of hepatic steatosis by CT is usually limited to patients who have undergone CT scan for other reasons or to potential liver donor who will need a CT scan of the liver for pre-operative assessment.

Magnetic Resonance Imaging

Evaluation of fat with magnetic resonance imaging (MRI) is common by chemical-shift imaging (CSI) principal, where MRI detect different resonance frequencies between protons bound to water and fat. In a regular MRI sequence, images in "in-phase (IP)" and "opposed phase (OP)" are produced at specific echo times when water and fat signals are added and subtracted. This can produce a qualitative or semi-quantitative assessment of hepatic steatosis. In the presence of fatty liver, the liver will appear dark in opposed phase due to signal loss. Subsequently, dual-echo CSI was developed for fat quantification based on a pair of OP and IP images, but there were biased from T1- and T2*-relaxation effects. To improve accuracy, T1-independent, T2*-corrected proton density fat fraction (PDFF) methods were developed based on multiple-echo CSI that acquire three or more consecutives pairs of OP and IP echoes. Meanwhile, spectral fat modeling was developed to be applied in the T1-independent, T2*-corrected multi-echo CSI technique for more precise fat quantification. This MRI-PDFF technique analyses vector sum of all signals from fat and water of the entire liver and is able to avoid sampling error [10, 12, 22, 23]. This method can assess the fat content of the entire liver in a short breath-hold, in about 20 s. The MRI-PDFF technique has high accuracy in evaluating liver steatosis. The AUROC for diagnosing hepatic steatosis ≥ 5% with histology as reference standard has been reported to be 0.98 in a recent meta-analysis [24].

Meanwhile, the MRS directly measures the chemical composition within tissue, usually based on the acquisition of data from a single voxel (typically 2 cm × 2 cm × 2 cm to 3 cm × 3 cm × 3 cm in size). Spectral tracing of fat and water peaks are attained from the MRS, then PDFF is calculated by signal intensities derived from protons bound to fat divided by amount from all protons in the liver, specifically those bound to fat and water. Despite MRS being regarded as the gold standard MRI technique for hepatic fat quantification, this technique may be limited by sampling error, similar to liver biopsy, especially in

cases with irregular hepatic fat distribution [10, 12, 22, 23]. The MRS is also time-consuming and requires technical expertise for its acquisition and analysis. A summary of meta-analyses on the MRI and MRS for the quantification of hepatic steatosis can be found in Table 1 [24, 25••, 26, 27].

The MRI and MRS have consistently outperformed CT or US in diagnosing and grading of hepatic steatosis [6, 7]. A meta-analysis reported the mean sensitivity and specificity of MRI (82.0–97.4% and 76.1–95.3%) and MRS (72.7–88.5% and 92.0–95.7%) compared with US (73.3–90.5% and 88.1–94.6%) and CT (46.1–72.0% and 76.1–95.3%) for the diagnosis of hepatic steatosis [25••]. On the other hand, the MRI-PDFF and MRS were reported to be similar in accuracy in quantification of hepatic steatosis, when compared with liver histology in NAFLD patients, including in mild hepatic steatosis [28–30]. These techniques were reliable and highly reproducible [31, 32]. There have been studies suggesting that the MRS and MRI-PDFF could serve as a better reference standard to quantify hepatic steatosis compared with current gold standard of liver biopsy. At the moment, the MRI-PDFF has been validated as a modality to be used for early-phase clinical trials to assess hepatic steatosis [33–35]. In addition, to comprehensively evaluate the liver, it is feasible to assess fibrosis by colocalization of MRI-PDFF-derived fat maps and MR elastography-derived stiffness maps at the same settings [36], besides the ability to add a standard liver MRI protocol to assess for structural abnormalities of the liver, if indicated. In a longitudinal study, the MRI-PDFF and MRS correlated well and the MRI-PDFF was more sensitive than histology in detecting small increases or decreases in the liver fat content [37••]. A relative reduction of about 30% in liver fat based on the MRI-PDFF was associated with 2 points or more reduction in nonalcoholic fatty liver disease (NAFLD) activity score (NAS) and had been suggested as a marker for significant improvement in clinical trials [38].

The disadvantage of using the MRI-PDFF or MRS is that the presence of hepatic fibrosis may reduce the correlation with hepatic steatosis [28]. Moreover, MRS has limited clinical applicability as not every MRI scanner is equipped with MRS capabilities and sophisticated post-processing methods are required for MRS. On the other hand, the MRI-PDFF can be used on any clinical MRI platform and is therefore more widely available. Moreover, it allows mapping of fat of the entire liver while MRS measures fat in only a small region of interest [39]. Overall, the high cost and limited availability of MRI scanner limit its use for quantification of hepatic steatosis in day-to-day clinical practice.

Controlled Attenuation Parameter

Controlled attenuation parameter (CAP) is the decrease in amplitude of ultrasound propagating through liver tissue that can be estimated using the same radiofrequency data used for estimation of liver stiffness using Fibroscan (Echosens, France, Paris), an ultrasound-based vibration-controlled transient elastography device [1, 40•]. CAP has been shown to correlate well with hepatic steatosis in several initial studies

Table 1 Summary of meta-analyses on MRI for quantification of hepatic steatosis

Author (year)	Number of studies and patients	MRI technique	Reference standard	Results
Gu J et al. 2019 [27]	6 studies 635 patients	MRI-PDFF	Histology	Diagnosis of steatosis grade ≥ S1: AUROC 0.98, sensitivity 0.93, specificity 0.94 Diagnosis of steatosis grade ≥ S2: AUROC 0.91, sensitivity 0.74, specificity 0.90 Diagnosis of steatosis grade ≥ S3: AUROC 0.90, sensitivity 0.74, specificity 0.87
Qu Y et al. 2019 [24]	13 studies 1100 patients	MRI-PDFF	Histology	Diagnosis of steatosis grade ≥ S1: AUROC 0.98 Diagnosis of steatosis grade ≥ S2: AUROC 0.91, sensitivity 0.83, specificity 0.89 Diagnosis of steatosis grade ≥ S3: AUROC 0.92, sensitivity 0.79, specificity 0.89
Zheng D et al. 2017 [26]	8 studies 934 living liver donors	MRI and/or MRS	Histology	For detection of hepatic steatosis: AUROC 0.92, sensitivity 0.89, specificity 0.84 For detection of substantial hepatic steatosis (> 10 to > 30%): AUROC 0.96, sensitivity 0.91, specificity 0.89
Bohte et al. 2011 [25••]	46 studies 4715 patients	MRI or MRS	Histology	MRI for detection of hepatic steatosis: sensitivity 82.0–97.4%, specificity 76.1–95.3% MRS for detection of hepatic steatosis: sensitivity 72.7–88.5%, specificity 92.0–95.7%

that consisted of patients with chronic liver disease of various aetiologies [41••, 42–46]. A subsequent study on 101 NAFLD patients and 60 non-NAFLD controls found that it was excellent for the diagnosis of significant hepatic steatosis, but its accuracy was impaired by increased body mass index and it was less accurate to distinguish between the different grades of significant hepatic steatosis. In the overall population, the AUROC of CAP for diagnosis of steatosis grade ≥ S1, ≥ S2, and S3 was 0.97, 0.86, and 0.75, respectively. Among non-obese patients, the AUROC for diagnosis of steatosis grade ≥ S1 and ≥ S2 was 0.99 and 0.99, respectively. However, among obese patients, the AUROC for diagnosis of steatosis grade ≥ S1, ≥ S2, and S3 was 0.92, 0.64, and 0.58, respectively. The lower diagnostic accuracy of CAP in obese patients was thought to be due to the thicker subcutaneous tissue in these patients [47•]. This was confirmed by a subsequent study that found the diagnostic accuracy of CAP to be lower in patients with skin capsular distance (SCD) > 25 mm compared with patients with SCD < 25 mm [48•]. In general, studies with larger proportion of NAFLD patients, greater body mass index, and higher grades of hepatic steatosis tended to produce less satisfactory diagnostic accuracy [40•, 41••, 42–46, 47•, 48•, 49–57]. A summary of the studies from which this observation was based on can be found elsewhere [58•]. Subsequent studies that evaluated CAP for the diagnosis of steatosis grades using liver biopsy as reference standard are summarized in Table 2 and a similar trend was observed [58•, 59–63, 64•, 65, 66, 67•, 68–75].

An individual patient data meta-analysis that included 2735 patients with chronic liver disease of various aetiologies defined the cutoffs for CAP for the diagnosis of steatosis grades ≥ S1, ≥ S2, and S3, which were 248 dB/m, 268 dB/m, and 280 dB/m, respectively. However, adjustments were recommended for several factors which were found to impact on CAP. These included deducting 10 dB/m from the CAP value for NAFLD patients, deducting 10 dB/m for patients with diabetes mellitus, and deducting or adding 4.4 dB/m for each unit of BMI above or below 25 kg/m^2 over the range of 20–30 kg/m^2. Moreover, the results of the individual patient data meta-analysis may only be valid for patients with BMI values ≤ 35 kg/m^2 as the study only included examinations with the standard M probe which has limited applicability in more obese patients [76••]. The XL probe has a greater depth of measurement below the skin surface and was introduced to reduce unsuccessful and unreliable liver stiffness measurements in more obese patients [77]. Although CAP measured using the XL probe tended to be higher than CAP measured using the M probe in the same patient, both probes appeared to have similar accuracy for the diagnosis of steatosis grades ≥ S1, ≥ S2, and S3 [58•]. Another study has shown that the 248-dB/m, 268-dB/m, and 280-dB/m recommended cutoffs for CAP for the diagnosis of steatosis grades ≥ S1, ≥ S2, and S3 may be used for the XL probe [67•]. On the other hand, a study by Caussy et al. that used either the M probe or the XL probe based on the recommendation by the device suggested that the cutoff for CAP in detecting hepatic steatosis ≥ 5% based on MRI-PDFF to be 288 dB/m [78].

The reliability criteria for CAP have not been defined. In a study using liver biopsy as the reference standard, the accuracy of CAP for the diagnosis of hepatic steatosis was significantly lower when the IQR was ≥ 40 dB/m [63]. In another study using the MRI-PDFF as reference standard, CAP was found to be more accurate for the diagnosis of hepatic steatosis when the IQR was < 30 dB/m [78]. Currently, CAP is considered reliable if the corresponding liver stiffness measurement is reliable. Liver stiffness measurement is considered reliable if the IQR/median is ≤ 30%, or if the liver stiffness measurement is < 7.1 kPa when the IQR/median is > 30% [79••]. A study found that CAP declined after meal but returned to baseline after 150 min [80], while another study found no difference in CAP after meal [81]. As liver stiffness measurement has been shown to be affected by meal, transient elastography is usually performed after at least 2 h of fasting [82].

As transient elastography is noninvasive and easy to use, CAP has the potential to be used as a screening tool for fatty liver. Patients identified to have fatty liver should be given lifestyle advice and should undergo evaluation for other components of the metabolic syndrome, which should be treated accordingly if present, to reduce the risk of cardiovascular disease [83•]. Moreover, liver stiffness measurement that is available simultaneously with CAP allows identification of patients with more severe liver fibrosis who can then be referred to specialist doctors for further management. On the other hand, patients with chronic liver disease of various aetiologies undergoing liver stiffness measurement with FibroScan to evaluate for liver fibrosis can have evaluation for hepatic steatosis at the same time, which may be helpful in the overall management of the patient. For example, the presence of significant hepatic steatosis has been shown to be associated with more severe liver disease in patients with chronic hepatitis B infection [84], and CAP can prompt the attending doctor to manage the fatty liver and metabolic syndrome in these patients, when present. One of the limitations of CAP is that it does not have the ability for anatomical visualization, which is often required, for example in the investigation of abnormal liver tests or for hepatocellular carcinoma surveillance in patients with chronic hepatitis B. As mentioned before, CAP is impaired by increased body mass index and is less accurate to distinguish between the different grades of significant hepatic steatosis in obese patients. The MRI-PDFF has been shown to perform significantly better than CAP for distinguishing the different grades of significant hepatic steatosis in NAFLD patients [85•, 86•]. Another limitation of CAP is the lack of accuracy in following change in the degree of hepatic steatosis. A study that evaluated CAP for following change in hepatic steatosis determined by the MRI-

Table 2 Summary of studies that evaluated CAP for the diagnosis of steatosis grades using liver biopsy as reference standard since the summary by Chan et al. [58•]

Authors (year)	Study population	Mean BMI (kg per m^2)	Distribution of steatosis grades S0, S1, S2, and S3	AUROC for diagnosis of steatosis grades ≥ S1, ≥ S2, and S3
Cardosa et al. 2015 [59]	136 patients with chronic hepatitis B	25	78%, 10%, 9%, and 3%	0.82, 0.82, and 0.97
de Ledinghen et al. 2016 [60]	261 NAFLD patients	30.2	0%, 29.9%, 38.3%, and 31.8%	The AUROC for diagnosis of steatosis grades ≥ S2 and S3 was 0.80 and 0.66, respectively.
Chen et al. 2016 [61]	189 patients with chronic hepatitis B	22.5	51%, 27%, 16%, and 6%	0.88, 0.92, and 0.94
Hong et al. 2017 [62]	55 potential liver donors	23.6	34.5%, 54.5%, 11.0%, and 0%	The AUROC for diagnosis of steatosis grades ≥ S1 and ≥ S2 was 0.77 and 0.88, respectively.
Chan et al. 2017 [58•]	57 NAFLD patients; transient elastography was performed with both M and X probes.	30.2	29%, 17%, 35%, and 19%	The AUROC for diagnosis of steatosis grades ≥ S1, ≥ S2, and S3 was 0.94, 0.80, and 0.69, respectively, using the M probe, and 0.97, 0.81, and 0.67, respectively, using the XL probe.
Xu et al. 2017 [75]	366 patients with chronic hepatitis B	23.4	62.6%, 33.9%, 2.2%, and 1.4%	0.78, 0.93, and 0.99
Wong et al. 2017 [63]	754 patients with chronic liver disease of various aetiologies; 46% had NAFLD.	27.2	29%, 33%, 19%, and 19%	The AUROC for diagnosis of steatosis grades ≥ S1 was 0.88. In the derivation cohort, the AUROC for diagnosis of steatosis grades ≥ S1 was 0.86, 0.89, and 0.76 in patients with IQR < 20 dB/m, 20–39 dB/m, and ≥ 40 dB/m. In the validation cohort, the AUROC for diagnosis of steatosis grades ≥ S1 was 0.90 and 0.77 in patients with IQR < 40 dB/m and ≥ 40 dB/m
de Ledinghen et al. 2017 [64•]	236 patients with chronic liver disease of various aetiologies; 20.8% had NAFLD; transient elastography was performed with both M and XL probes.	24.4	51.7%, 22.5%, 10.2%, and 15.7%	The AUROC for diagnosis of steatosis grades ≥ S1, ≥ S2, and S3 was 0.82, 0.83, and 0.89, respectively, using the M probe, and 0.88, 0.92, and 0.93, respectively, using the XL probe.
Andrade et al. 2017 [65]	159 patients with chronic liver disease of various aetiologies; 42.1% had NAFLD.	1.9% had BMI < 18.5 kg per m^2, 50.3% had BMI 18.5–24.9 kg per m^2, 30.8% had BMI 25.0–29.9 kg per m^2, and 17.0% had BMI > 30 kg per m^2	18.9%, 32.7%, 22.6%, and 25.8%	0.82, 0.96, and 0.98
Thiele et al. 2018 [66]	269 patients with alcohol overuse	26	28%, 35%, 24%, and 13%	0.77, 0.78, and 0.88
Chan et al. 2018 [67•]	180 patients with chronic liver disease of various aetiologies;	29.5	9.4%, 28.3%, 43.9%, and 18.3%	The AUROC for diagnosis of steatosis grades ≥ S1, ≥ S2, and

Table 2 (continued)

Authors (year)	Study population	Mean BMI (kg per m^2)	Distribution of steatosis grades S0, S1, S2, and S3	AUROC for diagnosis of steatosis grades ≥ S1, ≥ S2, and S3
	86.7% had NAFLD; transient elastography was performed with both M and XL probes.			S3 was 0.84, 0.76, and 0.61, respectively, using the M probe, and 0.91, 0.78, and 0.65, respectively, using the XL probe.
Mendes et al. 2018 [68]	312 patients with chronic hepatitis C infection; steatosis grade was based on digital morphometric quantification of liver biopsy slides.	27.2	19.2%, 28.5%, 31.1%, and 21.2%	The AUROC for diagnosis of steatosis grades ≥ S1 was 0.94.
Yen et al. 2018 [69]	54 liver donors	24.0	47 subjects had no hepatic steatosis while the remaining 7 subjects had hepatic steatosis ranging from 10 to 30%.	The AUROC for diagnosis of steatosis grade ≥ S1 was 0.96.
Eddowes et al. 2019 [70]	404 consecutive adults who underwent liver biopsy for suspected NAFLD	33.8	12%, 23%, 28%, and 36%.	0.87, 0.77, and 0.70
Rout G et al. 2019 [71]	462 patients with chronic liver disease of various aetiologies; 19.3% had NAFLD	23.4	71.6%, 16.0%, 8.4%, and 3.9%	0.88, 0.89, and 0.88
Baumeler et al. 2019 [72]	224 patients with chronic liver disease of various aetiologies; 23.2% had NAFLD.	26.8	37.9%, 36.6%, 14.7%, and 10.7%	0.78, 0.83, and 0.82
Somda et al. 2019 [73]	249 morbidly obese patients who underwent sleeve gastrectomy; in cases with probe-to-capsule distance > 35 mm, CAP was adapted for a deeper measurement.	43.8	15.7%, 26.1%, 20.5%, and 37.7%	CAP was not performed at a sufficient depth in 130 patients, and the adapted CAP was significantly lower than the standard CAP. After adapting CAP in patients with probe-to-capsule distance > 35 mm, the AUROC for diagnosis of steatosis grades ≥ S1, ≥ S2, and S3 was 0.86, 0.83, and 0.79, respectively.
Semmler et al. 2019 [74]	88 patients with advanced chronic liver disease and/or portal hypertension defined by liver stiffness measurement ≥ 10 kPa and/or hepatic venous pressure gradient (HVPG) ≥ 6 mmHg who underwent liver biopsy, CAP, and HVPG.	25.4	53.4%, 31.8%, 12.5%, and 2.3%	AUROC for diagnosis of steatosis grades ≥ S1 was 0.69 in the overall study population, 0.83 in patients with HVPG < 10 mmHg, and 0.63 in patients with HVPG ≥ 10 mmHg.

PDFF found that the accuracy was 87.5% when the change in CAP was > 38 dB/m, but the accuracy was only 29.3% when the change in CAP was < 38 dB/m. The prognostic value of CAP is currently unclear. One study found CAP to be predictive of clinical decompensation and bacterial infections independent of liver stiffness measurement in patients with compensated advanced chronic liver disease [87], while another study found CAP to be not predictive of first and further hepatic decompensation among patients with compensated advanced chronic liver disease [88]. In a prospective study on 4282 patients with reliable baseline transient elastography, CAP did not predict liver-related event, cancer, or cardiovascular event [89]. Further studies on the prognostic value of CAP is awaited.

Histopathological Examination of Liver Biopsy Specimen

Nonalcoholic steatohepatitis (NASH) is defined histologically by the presence of significant hepatic steatosis, lobular inflammation, and hepatocyte ballooning with or without fibrosis. Currently, the diagnosis of NASH can only be reliably made by histopathological examination of a liver biopsy specimen [90•]. The NASH Clinical Research Network has introduced a scoring system for a standardized approach towards the reporting of histopathological findings of liver biopsy of NAFLD patients [91••]. The grading for hepatic steatosis is S0 for < 5%, S1 for 5–33%, S2 for 33–66%, and S3 for > 66%. However, the grading is subjective to the observer's estimation and may suffer from intra- and inter-observer variability. Moreover, the semi-quantitative nature of the grading means that a large degree of change in hepatic steatosis is required for a change in grade. This means that it will not be able to detect small changes in hepatic steatosis following intervention. Liver biopsy may also be limited by sampling variability, although a study on patients with two liver biopsies taken at different angles did show substantial agreement for grading of steatosis [92]. These factors challenge the role of liver biopsy in the assessment of hepatic steatosis as the procedure is also invasive and associated with a small risk of serious complications. Moreover, several other noninvasive methods are available for the diagnosis of hepatic steatosis, from the simple and widely available ultrasonography to the more sophisticated transient elastography that come with simultaneous assessment of steatosis and fibrosis, and the highly accurate MRI-based methods. Nevertheless, a liver biopsy is still essential for the diagnosis of NASH, which has important prognostic value, is recommended prior to the use of certain medications such as vitamin E and pioglitazone, and is often required prior to enrolment in clinical trial, although this may change when safer and more effective drugs addressing the many faces of metabolic syndrome become available in the future.

Enhancement of Histopathological Grading of Hepatic Steatosis

Stereological analysis using grid-point counting method combined with the Delesse principle has been shown to have superior conformity and repeatability for the measurement of hepatic steatosis on liver biopsy specimens compared with histopathologist grading. For example, the intraclass coefficient for assessment of hepatic steatosis by three experienced liver pathologists in a study on 59 liver biopsy specimens was 0.79 with a repeatability coefficient of 38%, whereas the intraclass coefficient for stereological measurement of hepatic steatosis by three analysts was 0.86 with a repeatability coefficient of 8% [93•]. However, stereological analysis is time-consuming when performed manually and is not practical outside of research setting. The development of computer-assisted stereology system may improve the uptake of this method for measurement of hepatic steatosis in the future. Second harmonic generation microscopy is an automated novel optical imaging modality that can potentially identify and assess clinically pertinent aspects of liver histology [94]. A laser is passed through a pulse compressor and an acoustic-optic modulator, before being routed by a dichroic mirror through an objective lens to the liver biopsy sample, where two photon-excited fluorescence emissions and second harmonic generation signal are collected and processed for detection. Specific aspects of liver histology can be measured using dedicated algorithms. The technology has been shown to provide reliable and reproducible measurements of fibrosis in liver biopsy specimen previously [95], and steatosis more recently [96•, 97•]. In a study on 86 liver biopsy specimens, very strong correlation was observed in the assessment of hepatic steatosis by histopathologists and second harmonic generation microscopy with Pearson correlation of 0.93. In a subsequent study on 329 liver biopsy specimens incorporating second harmonic generation–based tool for quantification of fibrosis, inflammation, hepatocyte ballooning, and steatosis, a very strong correlation was similarly observed for steatosis with Pearson correlation of 0.88. The correlation was strong for lobular inflammation and fibrosis but only moderate for hepatocyte ballooning [94]. This technology appears promising in overcoming observer variability and providing more precise quantification of the different histological components of NAFLD but requires further refining and validation. However, a dedicated machine is required for image acquisition and the analysis is performed using proprietary algorithms, which may hamper its uptake for wider use.

Conclusion

Quantification of hepatic steatosis is important, especially in clinical trials and for follow-up of NAFLD patients on lifestyle intervention or medical therapy. Although liver biopsy is regarded as the gold standard, its use is limited by the invasive nature of the procedure and the availability of noninvasive tests such as US, MRI-PDFF, and CAP. A good understanding of the advantages and disadvantages of each of these modalities will help one choose the most suitable method for quantifying hepatic steatosis in NAFLD patients in clinical trials as well as in day-to-day clinical practice.

Compliance with Ethical Standards

Conflict of Interest Kee-Huat Chuah declares no potential conflicts of interest.

Wah-Kheong Chan reports speaking for Echosens and a grant from Resonance Health for an investigator-initiated study.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

References

Papers of particular interest, published recently, have been highlighted as:
- • Of importance
- •• Of major importance

1. Wah-Kheong C, Khean-Lee G. Epidemiology of a fast emerging disease in the Asia-Pacific region: non-alcoholic fatty liver disease. Hepatol Int. 2013;7:65–71.
2.• Younossi ZM, Koenig AB, Abdelatif D, Fazel Y, Henry L, Wymer M. Global epidemiology of nonalcoholic fatty liver disease-meta-analytic assessment of prevalence, incidence, and outcomes. Hepatology. 2016;64:73–84 **The meta-analysis reporting on the latest global prevalence and clinical and economic burden of NAFLD**.
3. Musso G, Gambino R, Cassader M, Pagano G. Meta-analysis: natural history of non-alcoholic fatty liver disease (NAFLD) and diagnostic accuracy of non-invasive tests for liver disease severity. Ann Med. 2011;43:617–49.
4. Sayiner M, Otgonsuren M, Cable R, Younossi I, Afendy M, Golabi P, et al. Variables associated with inpatient and outpatient resource utilization among Medicare beneficiaries with nonalcoholic fatty liver disease with or without cirrhosis. J Clin Gastroenterol. 2017;51:254–60.
5. Wong VW, Chan WK, Chitturi S, Chawla Y, Dan YY, Duseja A, et al. Asia-Pacific Working Party on Non-alcoholic Fatty Liver Disease guidelines 2017-Part 1: definition, risk factors and assessment. J Gastroenterol Hepatol. 2018;33:70–85.
6. Saadeh S, Younossi ZM, Remer EM, Gramlich T, Ong JP, Hurley M, et al. The utility of radiological imaging in nonalcoholic fatty liver disease. Gastroenterology. 2002;123:745–50.
7. Lee SS, Park SH, Kim HJ, Kim SY, Kim MY, Kim DY, et al. Non-invasive assessment of hepatic steatosis: prospective comparison of the accuracy of imaging examinations. J Hepatol. 2010;52:579–85.
8.• Hernaez R, Lazo M, Bonekamp S, Kamel I, Brancati FL, Guallar E, et al. Diagnostic accuracy and reliability of ultrasonography for the detection of fatty liver: a meta-analysis. Hepatology. 2011;54:1082–90 **Important major meta-analysis reporting on the reliabilty and accuracy in detecting moderate-to-severe fatty liver, compared with histology**.
9. Strauss S, Gavish E, Gottlieb P, Katsnelson L. Interobserver and intraobserver variability in the sonographic assessment of fatty liver. AJR Am J Roentgenol. 2007;189:W320–3.
10. Lee SS, Park SH. Radiologic evaluation of nonalcoholic fatty liver disease. World J Gastroenterol. 2014;20:7392–402.
11. Kromrey ML, Ittermann T, Berning M, Kolb C, Hoffmann RT, Lerch MM, et al. Accuracy of ultrasonography in the assessment of liver fat compared with MRI. Clin Radiol. 2019;74:539–46.
12. Lee DH. Imaging evaluation of non-alcoholic fatty liver disease: focused on quantification. Clinical and molecular hepatology. 2017;23:290–301.
13.• Webb M, Yeshua H, Zelber-Sagi S, Santo E, Brazowski E, Halpern Z, et al. Diagnostic value of a computerized hepatorenal index for sonographic quantification of liver steatosis. AJR Am J Roentgenol. 2009;192:909–14 **The original article describing hepatorenal sonograhic index as a sensitive noninvasive method for steatosis quantification**.
14. de Almeidae Borges VF, ALD D, Cotrim HP, HLOG R, Andrade NB. Sonographic hepatorenal ratio: a noninvasive method to diagnose nonalcoholic steatosis. J Clin Ultrasound. 2013;41:18–25.
15. Son JY, Lee JY, Yi NJ, Lee KW, Suh KS, Kim KG, et al. Hepatic steatosis: assessment with acoustic structure quantification of US imaging. Radiology. 2016;278:257–64.
16. Kuroda H, Kakisaka K, Kamiyama N, Oikawa T, Onodera M, Sawara K, et al. Non-invasive determination of hepatic steatosis by acoustic structure quantification from ultrasound echo amplitude. World J Gastroenterol. 2012;18:3889–95.
17. Lin SC, Heba E, Wolfson T, Ang B, Gamst A, Han A, et al. Noninvasive diagnosis of nonalcoholic fatty liver disease and quantification of liver fat using a new quantitative ultrasound technique. Clin Gastroenterol Hepatol. 2015;13:1337–1345.e1336.
18. Pickhardt PJ, Park SH, Hahn L, Lee SG, Bae KT, Yu ES. Specificity of unenhanced CT for non-invasive diagnosis of hepatic steatosis: implications for the investigation of the natural history of incidental steatosis. Eur Radiol. 2012;22:1075–82.
19. Birnbaum BA, Hindman N, Lee J, Babb JS. Multi-detector row CT attenuation measurements: assessment of intra- and interscanner variability with an anthropomorphic body CT phantom. Radiology. 2007;242:109–19.
20. Park YS, Park SH, Lee SS, Kim DY, Shin YM, Lee W, et al. Biopsy-proven nonsteatotic liver in adults: estimation of reference range for difference in attenuation between the liver and the spleen at nonenhanced CT. Radiology. 2011;258:760–6.
21. Zhang Y, Wang C, Duanmu Y, Zhang C, Zhao W, Wang L, et al. Comparison of CT and magnetic resonance mDIXON-Quant sequence in the diagnosis of mild hepatic steatosis. Br J Radiol. 2018;91:20170587.
22. Reeder SB, Cruite I, Hamilton G, Sirlin CB. Quantitative assessment of liver fat with magnetic resonance imaging and spectroscopy. J Magn Reson Imaging. 2011;34:729–49.
23. Chartampilas E. Imaging of nonalcoholic fatty liver disease and its clinical utility. Hormones (Athens). 2018;17:69–81.
24. Qu Y, Li M, Hamilton G, Zhang YN, Song B. Diagnostic accuracy of hepatic proton density fat fraction measured by magnetic resonance imaging for the evaluation of liver steatosis with histology as reference standard: a meta-analysis. Eur Radiol 2019.

25.•• Bohte AE, van Werven JR, Bipat S, Stoker J. The diagnostic accuracy of US, CT, MRI and 1H-MRS for the evaluation of hepatic steatosis compared with liver biopsy: a meta-analysis. Eur Radiol. 2011;21:87–97 **The meta-analysis describing diagnostic accuracy of US, CT, MRI, and MRS, compared with histology in evaluating hepatic steatosis.**

26. Zheng D, Guo Z, Schroder PM, Zheng Z, Lu Y, Gu J, et al. Accuracy of MR imaging and MR spectroscopy for detection and quantification of hepatic steatosis in living liver donors: a meta-analysis. Radiology. 2017;282:92–102.

27. Gu J, Liu S, Du S, Zhang Q, Xiao J, Dong Q, et al. Diagnostic value of MRI-PDFF for hepatic steatosis in patients with non-alcoholic fatty liver disease: a meta-analysis. Eur Radiol. 2019;29:3564–73.

28. Idilman IS, Keskin O, Celik A, Savas B, Elhan AH, Idilman R, et al. A comparison of liver fat content as determined by magnetic resonance imaging-proton density fat fraction and MRS versus liver histology in non-alcoholic fatty liver disease. Acta Radiol. 2016;57:271–8.

29. Heba ER, Desai A, Zand KA, Hamilton G, Wolfson T, Schlein AN, et al. Accuracy and the effect of possible subject-based confounders of magnitude-based MRI for estimating hepatic proton density fat fraction in adults, using MR spectroscopy as reference. J Magn Reson Imaging. 2016;43:398–406.

30. Yokoo T, Serai SD, Pirasteh A, Bashir MR, Hamilton G, Hernando D, et al. Linearity, bias, and precision of hepatic proton density fat fraction measurements by using MR imaging: a meta-analysis. Radiology. 2018;286:486–98.

31. Kang GH, Cruite I, Shiehmorteza M, Wolfson T, Gamst AC, Hamilton G, et al. Reproducibility of MRI-determined proton density fat fraction across two different MR scanner platforms. J Magn Reson Imaging. 2011;34:928–34.

32. Bachtiar V, Kelly MD, Wilman HR, Jacobs J, Newbould R, Kelly CJ, et al. Repeatability and reproducibility of multiparametric magnetic resonance imaging of the liver. PLoS One. 2019;14:e0214921.

33. Adams LA. End-points for drug treatment in NASH. Hepatol Int. 2019;13:253–8.

34. Caussy C, Reeder SB, Sirlin CB, Loomba R. Noninvasive, quantitative assessment of liver fat by MRI-PDFF as an endpoint in NASH trials. Hepatology. 2018;68:763–72.

35. Siddiqui MS, Harrison SA, Abdelmalek MF, Anstee QM, Bedossa P, Castera L, et al. Case definitions for inclusion and analysis of endpoints in clinical trials for nonalcoholic steatohepatitis through the lens of regulatory science. Hepatology. 2018;67:2001–12.

36. Loomba R, Sirlin CB, Ang B, Bettencourt R, Jain R, Salotti J, et al. Ezetimibe for the treatment of nonalcoholic steatohepatitis: assessment by novel magnetic resonance imaging and magnetic resonance elastography in a randomized trial (MOZART trial). Hepatology. 2015;61:1239–50.

37.•• Noureddin M, Lam J, Peterson MR, Middleton M, Hamilton G, Le TA, et al. Utility of magnetic resonance imaging versus histology for quantifying changes in liver fat in nonalcoholic fatty liver disease trials. Hepatology. 2013;58:1930–40 **The original paper describing MRI-PDFF and MRS correlated well and more sensitive than histology in quantifying steatosis changes**.

38. Patel J, Bettencourt R, Cui J, Salotti J, Hooker J, Bhatt A, et al. Association of noninvasive quantitative decline in liver fat content on MRI with histologic response in nonalcoholic steatohepatitis. Ther Adv Gastroenterol. 2016;9:692–701.

39. Di Martino M, Pacifico L, Bezzi M, Di Miscio R, Sacconi B, Chiesa C, et al. Comparison of magnetic resonance spectroscopy, proton density fat fraction and histological analysis in the quantification of liver steatosis in children and adolescents. World J Gastroenterol. 2016;22:8812–9.

40.• Sasso M, Beaugrand M, de Ledinghen V, Douvin C, Marcellin P, Poupon R, et al. Controlled attenuation parameter (CAP): a novel VCTE guided ultrasonic attenuation measurement for the evaluation of hepatic steatosis: preliminary study and validation in a cohort of patients with chronic liver disease from various causes. Ultrasound Med Biol. 2010;36:1825–35 **The original paper describing controlled attenuation parameter for evaluation of hepatic steatosis in patients with chronic liver disease**.

41.•• Sasso M, Tengher-Barna I, Ziol M, Miette V, Fournier C, Sandrin L, et al. Novel controlled attenuation parameter for noninvasive assessment of steatosis using Fibroscan((R)): validation in chronic hepatitis C. J Viral Hepat. 2012;19:244–53 **The original paper providing validation of controlled attenuation parameter for evaluation of hepatic steatosis**.

42. Myers RP, Pollett A, Kirsch R, Pomier-Layrargues G, Beaton M, Levstik M, et al. Controlled attenuation parameter (CAP): a noninvasive method for the detection of hepatic steatosis based on transient elastography. Liver Int. 2012;32:902–10.

43. de Ledinghen V, Vergniol J, Foucher J, Merrouche W, le Bail B. Non-invasive diagnosis of liver steatosis using controlled attenuation parameter (CAP) and transient elastography. Liver Int. 2012;32:911–8.

44. Kumar M, Rastogi A, Singh T, Behari C, Gupta E, Garg H, et al. Controlled attenuation parameter for non-invasive assessment of hepatic steatosis: does etiology affect performance? J Gastroenterol Hepatol. 2013;28:1194–201.

45. Chon YE, Jung KS, Kim SU, Park JY, Park YN, Kim DY, et al. Controlled attenuation parameter (CAP) for detection of hepatic steatosis in patients with chronic liver diseases: a prospective study of a native Korean population. Liver Int. 2014;34:102–9.

46. Masaki K, Takaki S, Hyogo H, Kobayashi T, Fukuhara T, Naeshiro N, et al. Utility of controlled attenuation parameter measurement for assessing liver steatosis in Japanese patients with chronic liver diseases. Hepatol Res. 2013;43:1182–9.

47.• Chan WK, Nik Mustapha NR, Mahadeva S. Controlled attenuation parameter for the detection and quantification of hepatic steatosis in nonalcoholic fatty liver disease. J Gastroenterol Hepatol. 2014;29:1470–6 **The original paper on controlled attenuation parameter for evaluation of hepatic steatosis in NAFLD patients that described reduced accuracy with increased body mass index**.

48.• Shen F, Zheng RD, Shi JP, Mi YQ, Chen GF, Hu X, et al. Impact of skin capsular distance on the performance of controlled attenuation parameter in patients with chronic liver disease. Liver Int. 2015;35:2392–400 **The original paper on controlled attenuation parameter for evaluation of hepatic steatosis that showed reduced accuracy with increased skin capsular distance**.

49. Yilmaz Y, Yesil A, Gerin F, Ergelen R, Akin H, Celikel CA, et al. Detection of hepatic steatosis using the controlled attenuation parameter: a comparative study with liver biopsy. Scand J Gastroenterol. 2014;49:611–6.

50. Shen F, Zheng RD, Mi YQ, Wang XY, Pan Q, Chen GY, et al. Controlled attenuation parameter for non-invasive assessment of hepatic steatosis in Chinese patients. World J Gastroenterol. 2014;20:4702–11.

51. Karlas T, Petroff D, Garnov N, Bohm S, Tenckhoff H, Wittekind C, et al. Non-invasive assessment of hepatic steatosis in patients with NAFLD using controlled attenuation parameter and 1H-MR spectroscopy. PLoS One. 2014;9:e91987.

52. de Ledinghen V, Vergniol J, Capdepont M, Chermak F, Hiriart JB, Cassinotto C, et al. Controlled attenuation parameter (CAP) for the diagnosis of steatosis: a prospective study of 5323 examinations. J Hepatol. 2014;60:1026–31.

53. Ferraioli G, Tinelli C, Lissandrin R, Zicchetti M, Dal Bello B, Filice G, et al. Controlled attenuation parameter for evaluating liver steatosis in chronic viral hepatitis. World J Gastroenterol. 2014;20:6626–31.

54. Jung KS, Kim BK, Kim SU, Chon YE, Chun KH, Kim SB, et al. Factors affecting the accuracy of controlled attenuation parameter

(CAP) in assessing hepatic steatosis in patients with chronic liver disease. PLoS One. 2014;9:e98689.
55. Wang CY, Lu W, Hu DS, Wang GD, Cheng XJ. Diagnostic value of controlled attenuation parameter for liver steatosis in patients with chronic hepatitis B. World J Gastroenterol. 2014;20:10585–90.
56. Lupsor-Platon M, Feier D, Stefanescu H, Tamas A, Botan E, Sparchez Z, et al. Diagnostic accuracy of controlled attenuation parameter measured by transient elastography for the non-invasive assessment of liver steatosis: a prospective study. J Gastrointestin Liver Dis. 2015;24:35–42.
57. Mi YQ, Shi QY, Xu L, Shi RF, Liu YG, Li P, et al. Controlled attenuation parameter for noninvasive assessment of hepatic steatosis using Fibroscan(R): validation in chronic hepatitis B. Dig Dis Sci. 2015;60:243–51.
58.• Chan WK, Nik Mustapha NR, Wong GL, Wong VW, Mahadeva S. Controlled attenuation parameter using the FibroScan(R) XL probe for quantification of hepatic steatosis for non-alcoholic fatty liver disease in an Asian population. United European Gastroenterol J. 2017;5:76–85 **The original paper on controlled attenuation parameter using XL probe in NAFLD patients, showing similar accuracy for evaluation of hepatic steatosis as the conventional M probe**.
59. Cardoso AC, Beaugrand M, de Ledinghen V, Douvin C, Poupon R, Trinchet JC, et al. Diagnostic performance of controlled attenuation parameter for predicting steatosis grade in chronic hepatitis B. Ann Hepatol. 2015;14:826–36.
60. de Ledinghen V, Wong GL, Vergniol J, Chan HL, Hiriart JB, Chan AW, et al. Controlled attenuation parameter for the diagnosis of steatosis in non-alcoholic fatty liver disease. J Gastroenterol Hepatol. 2016;31:848–55.
61. Chen J, Wu D, Wang M, Chen E, Bai L, Liu C, et al. Controlled attenuation parameter for the detection of hepatic steatosis in patients with chronic hepatitis B. Infect Dis (Lond). 2016;48:670–5.
62. Hong YM, Yoon KT, Cho M, Chu CW, Rhu JH, Yang KH, et al. Clinical usefulness of controlled attenuation parameter to screen hepatic steatosis for potential donor of living donor liver transplant. Eur J Gastroenterol Hepatol. 2017;29:805–10.
63. Wong VW, Petta S, Hiriart JB, Camma C, Wong GL, Marra F, et al. Validity criteria for the diagnosis of fatty liver by M probe-based controlled attenuation parameter. J Hepatol. 2017;67:577–84.
64.• de Ledinghen V, Hiriart JB, Vergniol J, Merrouche W, Bedossa P, Paradis V. Controlled attenuation parameter (CAP) with the XL probe of the fibroscan((R)): a comparative study with the M probe and liver biopsy. Dig Dis Sci. 2017;62:2569–77 **The original paper on controlled attenuation parameter using XL probe in patients with chronic liver disease of various aetiologies, showing similar accuracy for evaluation of hepatic steatosis as the conventional M probe**.
65. Andrade P, Rodrigues S, Rodrigues-Pinto E, Gaspar R, Lopes J, Lopes S, et al. Diagnostic accuracy of controlled attenuation parameter for detecting hepatic steatosis in patients with chronic liver disease. GE Port J Gastroenterol. 2017;24:161–8.
66. Thiele M, Rausch V, Fluhr G, Kjaergaard M, Piecha F, Mueller J, et al. Controlled attenuation parameter and alcoholic hepatic steatosis: diagnostic accuracy and role of alcohol detoxification. J Hepatol. 2018;68:1025–32.
67.• Chan WK, Nik Mustapha NR, Mahadeva S, Wong VW, Cheng JY, Wong GL. Can the same controlled attenuation parameter cut-offs be used for M and XL probes for diagnosing hepatic steatosis? J Gastroenterol Hepatol. 2018;33:1787–94 **The original paper on controlled attenuation parameter using XL probe in patients with chronic liver disease of various aetiologies, suggesting that the same cutoffs may be used as for the conventional M probe for the diagnosis of the different grades of hepatic steatosis**.
68. Mendes LC, Ferreira PA, Miotto N, Zanaga L, Lazarini MS, Goncales ESL, et al. Controlled attenuation parameter for steatosis grading in chronic hepatitis C compared with digital morphometric analysis of liver biopsy: impact of individual elastography measurement quality. Eur J Gastroenterol Hepatol. 2018;30:959–66.
69. Yen YH, Kuo FY, Lin CC, Chen CL, Chang KC, Tsai MC, et al. Predicting hepatic steatosis in living liver donors via controlled attenuation parameter. Transplant Proc. 2018;50:3533–8.
70. Eddowes PJ, Sasso M, Allison M, Tsochatzis E, Anstee QM, Sheridan D, et al. Accuracy of FibroScan controlled attenuation parameter and liver stiffness measurement in assessing steatosis and fibrosis in patients with nonalcoholic fatty liver disease. Gastroenterology. 2019;156:1717–30.
71. Rout G, Kedia S, Nayak B, Yadav R, Das P, Acharya SK, et al. Controlled attenuation parameter for assessment of hepatic steatosis in Indian patients. J Clin Exp Hepatol. 2019;9:13–21.
72. Baumeler S, Jochum W, Neuweiler J, Bergamin I, Semela D. Controlled attenuation parameter for the assessment of liver steatosis in comparison with liver histology: a single-centre real life experience. Swiss Med Wkly. 2019;149:w20077.
73. Somda S, Lebrun A, Tranchart H, Lamouri K, Prevot S, Njike-Nakseu M, et al. Adaptation of controlled attenuation parameter (CAP) measurement depth in morbidly obese patients addressed for bariatric surgery. PLoS One. 2019;14:e0217093.
74. Semmler G, Stift J, Scheiner B, Woran K, Schwabl P, Paternostro R, Bucsics T, et al. Performance of controlled attenuation parameter in patients with advanced chronic liver disease and portal hypertension. Dig Dis Sci 2019.
75. Xu L, Lu W, Li P, Shen F, Mi YQ, Fan JG. A comparison of hepatic steatosis index, controlled attenuation parameter and ultrasound as noninvasive diagnostic tools for steatosis in chronic hepatitis B. Dig Liver Dis. 2017;49:910–7.
76.•• Karlas T, Petroff D, Sasso M, Fan JG, Mi YQ, de Ledinghen V, et al. Individual patient data meta-analysis of controlled attenuation parameter (CAP) technology for assessing steatosis. J Hepatol. 2017;66:1022–30 **The individual patient data meta-analysis that defined the optimal cutoffs for the diagnosis of the different grades of hepatic steatosis**.
77. Myers RP, Pomier-Layrargues G, Kirsch R, Pollett A, Duarte-Rojo A, Wong D, et al. Feasibility and diagnostic performance of the FibroScan XL probe for liver stiffness measurement in overweight and obese patients. Hepatology. 2012;55:199–208.
78. Caussy C, Alquiraish MH, Nguyen P, Hernandez C, Cepin S, Fortney LE, et al. Optimal threshold of controlled attenuation parameter with MRI-PDFF as the gold standard for the detection of hepatic steatosis. Hepatology. 2018;67:1348–59.
79.•• Boursier J, Zarski JP, de Ledinghen V, Rousselet MC, Sturm N, Lebail B, et al. Determination of reliability criteria for liver stiffness evaluation by transient elastography. Hepatology. 2013;57:1182–91 **The original article that defined the reliability criteria for transient elastography**.
80. Ratchatasettakul K, Rattanasiri S, Promson K, Sringam P, Sobhonslidsuk A. The inverse effect of meal intake on controlled attenuation parameter and liver stiffness as assessed by transient elastography. BMC Gastroenterol. 2017;17:50.
81. Silva M, Costa Moreira P, Peixoto A, Santos AL, Lopes S, Goncalves R, et al. Effect of meal ingestion on liver stiffness and controlled attenuation parameter. GE Port J Gastroenterol. 2019;26:99–104.
82. Arena U, Lupsor Platon M, Stasi C, Moscarella S, Assarat A, Bedogni G, et al. Liver stiffness is influenced by a standardized meal in patients with chronic hepatitis C virus at different stages of fibrotic evolution. Hepatology. 2013;58:65–72.
83.• Kwak MS, Chung GE, Yang JI, Yim JY, Chung SJ, Jung SY, et al. Clinical implications of controlled attenuation parameter in a health check-up cohort. Liver Int. 2018;38:915–23 **The original article evaluating the use of CAP in a health check-up population**.

84. Seto WK, Hui RWH, Mak LY, Fung J, Cheung KS, Liu KSH, et al. Association between hepatic steatosis, measured by controlled attenuation parameter, and fibrosis burden in chronic hepatitis B. Clin Gastroenterol Hepatol. 2018;16:575–83 **e572**.

85.• Imajo K, Kessoku T, Honda Y, Tomeno W, Ogawa Y, Mawatari H, et al. Magnetic resonance imaging more accurately classifies steatosis and fibrosis in patients with nonalcoholic fatty liver disease than transient Elastography. Gastroenterology. 2016;150:626–637 e627 **The original article that compared the diagnostic accuracy of MRI-PDFF and CAP for the diagnosis of the different grades of hepatic steatosis in NAFLD patients**.

86. Runge JH, Smits LP, Verheij J, Depla A, Kuiken SD, Baak BC, et al. MR spectroscopy-derived proton density fat fraction is superior to controlled attenuation parameter for detecting and grading hepatic steatosis. Radiology. 2018;286:547–56.

87. Margini C, Murgia G, Stirnimann G, De Gottardi A, Semmo N, Casu S, et al. Prognostic significance of controlled attenuation parameter in patients with compensated advanced chronic liver disease. Hepatol Commun. 2018;2:929–40.

88. Scheiner B, Steininger L, Semmler G, Unger LW, Schwabl P, Bucsics T, et al. Controlled attenuation parameter does not predict hepatic decompensation in patients with advanced chronic liver disease. Liver Int. 2019;39:127–35.

89. Liu K, Wong VW, Lau K, Liu SD, Tse YK, Yip TC, et al. Prognostic value of controlled attenuation parameter by transient elastography. Am J Gastroenterol. 2017;112:1812–23.

90.• Chalasani N, Younossi Z, Lavine JE, Charlton M, Cusi K, Rinella M, et al. The diagnosis and management of nonalcoholic fatty liver disease: practice guidance from the American Association for the Study of Liver Diseases. Hepatology. 2018;67:328–57 **The latest guidelines from the AASLD on diagnosis and management of NAFLD**.

91.•• Kleiner DE, Brunt EM, Van Natta M, Behling C, Contos MJ, Cummings OW, et al. Design and validation of a histological scoring system for nonalcoholic fatty liver disease. Hepatology. 2005;41:1313–21 **The original article on the histological scoring system for NAFLD, including the grading of hepatic steatosis, which has been used as the reference standard for most studies on diagnostic tests for hepatic steatosis**.

92. Ratziu V, Charlotte F, Heurtier A, Gombert S, Giral P, Bruckert E, et al. Sampling variability of liver biopsy in nonalcoholic fatty liver disease. Gastroenterology. 2005;128:1898–906.

93.• St Pierre TG, House MJ, Bangma SJ, Pang W, Bathgate A, Gan EK, et al. Stereological analysis of liver biopsy histology sections as a reference standard for validating non-invasive liver fat fraction measurements by MRI. PLoS One. 2016;11:e0160789 **The original article describing the use of stereological anlaysis as reference standard for measuring hepatic steatosis**.

94. Sun W, Chang S, Tai DC, Tan N, Xiao G, Tang H, et al. Nonlinear optical microscopy: use of second harmonic generation and two-photon microscopy for automated quantitative liver fibrosis studies. J Biomed Opt. 2008;13:064010.

95. Xu S, Wang Y, Tai DCS, Wang S, Cheng CL, Peng Q, et al. qFibrosis: a fully-quantitative innovative method incorporating histological features to facilitate accurate fibrosis scoring in animal model and chronic hepatitis B patients. J Hepatol. 2014;61:260–9.

96.• Goh GB, Leow WQ, Liang S, Wan WK, Lim TKH, Tan CK, et al. Quantification of hepatic steatosis in chronic liver disease using novel automated method of second harmonic generation and two-photon excited fluorescence. Sci Rep. 2019;9:2975 **The original article describing the use of second harmonic generation microscopy for measurement of hepatic steatosis**.

97. Pilichiewicz AN, Feltrin KL, Horowitz M, Holtmann G, Wishart JM, Jones KL, et al. Functional dyspepsia is associated with a greater symptomatic response to fat but not carbohydrate, increased fasting and postprandial CCK, and diminished PYY. Am J Gastroenterol. 2008;103:2613–23.