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REVIEW

Transient elastography (FibroScan®) with controlled attenuation parameter in the assessment of liver steatosis and fibrosis in patients with nonalcoholic fatty liver disease - Where do we stand?

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Abstract

Non-alcoholic fatty liver disease (NAFLD) is the most common cause of chronic liver disease worldwide. Currently, the routinely used modalities are unable to adequately determine the levels of steatosis and fibrosis (laboratory tests and ultrasonography) or cannot be applied as a screening procedure (liver biopsy). Among the non-invasive tests, transient elastography (FibroScan®, TE) with controlled attenuation parameter (CAP) has demonstrated good accuracy in quantifying the levels of liver steatosis and fibrosis in patients with NAFLD, the factors associated with the diagnosis and NAFLD progression. The method is fast, reliable and reproducible, with good intra- and interobserver levels of agreement, thus allowing for population-wide screening and disease follow-up. The initial inability of the procedure to accurately determine fibrosis and steatosis in obese patients has been addressed with the development of the obese-specific XL probe. TE with CAP is a viable alternative to ultrasonography, both as an initial assessment and during follow-up of patients with NAFLD. Its ability to exclude patients with advanced fibrosis may be used to identify low-risk NAFLD patients in whom liver biopsy is not needed, therefore reducing the risk of complications and the financial costs.

Key words: Non-alcoholic fatty liver disease; Transient elastography; Controlled attenuation parameter

Core tip: Non-alcoholic fatty liver disease (NAFLD) patients are at risk of NAFLD-related cirrhosis and

hepatocellular carcinoma, particularly in the setting of liver fibrosis with concurrent metabolic syndrome. Transient elastography (TE) with controlled attenuation parameter (CAP) is a fast, reliable, repeatable non-invasive method for the assessment of liver steatosis and fibrosis. TE with CAP may be used to diagnose and monitor patients with NAFLD. TE with CAP is a favorable means of excluding advanced fibrosis.

INTRODUCTION

An increasingly common cause of chronic liver disease in adults and children is nonalcoholic fatty liver disease (NAFLD). In adults, the prevalence of NAFLD ranges from 17% to 33%[1], whereas in children, it ranges from 2.6% to 9.6%, and from 22.5% to 44% in children with obesity[2]. Because of the ongoing epidemics of metabolic syndrome (MetS) and its individual components, the incidence of NAFLD is increasing in both adults and children. The individual components of MetS include diabetes mellitus type 2 (T2DM), obesity, arterial hypertension and dyslipidemia. The presentation of NAFLD may vary from simple steatosis to nonalcoholic steatohepatitis (NASH), liver cirrhosis and hepatocellular carcinoma (HCC)[1-4]. It is expected that NASH related cirrhosis and NASH related HCC may soon become the most frequent indications for liver transplantation[1]. Interestingly, in patients with NAFLD, liver-related mortality is the third cause of death and malignancy, whereas cardiovascular diseases are the main cause[1]; thus, cumulative evidence indicates that NAFLD is correlated with many extrahepatic diseases[1-3]. Many authors have suggested that NAFLD is not only a marker of MetS but also a risk factor for cardiovascular diseases (CVD), chronic kidney disease (CKD) and T2DM. Moreover, it is a risk factor for malignancy (colorectal carcinoma) and other conditions (sleep apnea, osteoporosis, psoriasis, and polycystic ovary syndrome)[1,3]. The clinical implication of these findings is that NAFLD patients may benefit from more intensive monitoring and early therapeutic interventions to lower the risk of cardiovascular and kidney diseases, as well as the risk for malignancies, HCC and colorectal carcinoma[3].

NAFLD AND METS

The association of NAFLD with MetS has been described with respect to the relation of both conditions with insulin resistance (IR), the basic pathogenetic factor underlying NAFLD and MetS. The main problem in assessing MetS risk is that it is not a disease but a clinical syndrome. MetS is manifested by the clustering of acquired metabolic factors that primarily increase the likelihood of cardiovascular events (myocardial infarction, stroke, and peripheral arterial disease). Thus, it has been suggested that NAFLD should be included as a fifth risk factor in the definition of MetS (the other four factors include obesity, dyslipidemia, arterial hypertension and glucose intolerance/diabetes mellitus); however, others consider NAFLD not as a hepatic manifestation of MetS but a separate condition only associated with MetS[3,4]. Whether the former or latter is true, NAFLD is strongly associated with all components of MetS. The prevalence of NAFLD in the general population is 20%-30%, whereas the prevalence rates are approximately 50%, 50%-90%, 30%-50%, and 80%-90% in patients with hypertension, hyperlipidemia, T2DM, and obesity, respectively. Compared with MetS, one-third of patients with NAFLD have MetS, and 90% of NAFLD patients have at least one positive MetS criterion[5-7].

However, cross-sectional studies cannot define the time relationship between NAFLD and MetS components. The general belief that MetS precedes NAFLD has been questioned after a longitudinal study demonstrated an increased risk for the development of MetS in NAFLD patients (HR = 1.70, 95%CI: 1.55-1.87)[8]. The similar, yet opposing effect of MetS increasing the risk for NAFLD development has also been confirmed, with a slightly increased hazard ratio (1.94, 95%CI: 1.78-2.13) compared with that reported in the previous study[9]. This bidirectionality is not limited to the occurrence of NAFLD and MetS; it also affects MetS components and disease severity. The presence of NAFLD increases the risk of developing arterial hypertension (HR = 1.07, 95%CI: 1.00-1.15 for mild steatosis; HR = 1.14, 95%CI: 1.00-1.30 for severe steatosis) and type 2 diabetes mellitus (two-fold risk increase)[10,11]. However, the presence of MetS increases the risk of developing NAFLD, depending on the number of MetS components present, with the combination of dyslipidemia and central obesity carrying a 3.01 hazard ratio (95%CI: 2.68-3.37) of NAFLD development. NAFLD severity is also affected by the presence of MetS, which increases the risk of inflammation - NASH (OR = 3.2, 95%CI: 1.2-8.9) and severe fibrosis (OR = 3.5, 95%CI: 1.1-11.2)[12]. Of the individual components, the strongest correlation appears to occur with abdominal obesity. A 1% increase in visceral fat carries an OR of 2.4 (95%CI: 1.3-4.2) for increasing liver inflammation and an OR of 3.5 (95%CI: 1.7-7.1) for increasing fibrosis. The predictive value regarding advanced steatohepatitis and fibrosis remains after correcting for IR and hepatic steatosis, with an OR of 2.1 (95%CI: 1.1-4.2) for advanced steatohepatitis and an OR of 2.9 (95%CI: 1.1-4.2) for fibrosis[13]. From a clinical viewpoint, the presence of NAFLD increases the risk of cardiovascular

event-associated deaths by approximately two-fold, independently of other cardiovascular risk factors[14,15].

PATHOGENESIS OF NAFLD

The pathogenesis of NAFLD remains an unsolved problem with a plethora of implications and potential solutions for clinical practice. Many people are affected by NAFLD; however, most (approximately 60%-70%) remain asymptomatic, with simple liver steatosis. The main challenge faced by researchers is the identification of patients in whom the disease will progress and why. Elucidating the details of pathogenesis will provide the answer to this question, thereby allowing researchers to focus on the 30%-40% of NAFLD patients who require intensive observation, follow-up and prevention (treatment) to halt the development of cirrhosis and HCC[16,17].

The central role in the development of NAFLD is reserved for IR. IR disrupts lipid metabolism by increasing peripheral fatty acid release. The increased free fatty acid levels combined with hyperinsulinemia result in the development of hepatic IR and increased hepatic triglyceride synthesis. Hepatic triglycerides accumulate as fat droplets in hepatocytes, thus resulting in what is referred to as a fatty liver[18,19]. Whether IR may represent a consequence of NAFLD appears to have been disproved by the discovery of specific genetic mutations (*e.g.*, PNPLA gene), which result in the development of NAFLD without peripheral IR[20]. This simplistic view is mirrored by the lack of an effect of IR-reducing medications on NAFLD and the current lack of an effective, established anti-NAFLD treatment other than lifestyle modifications and increased physical activity. IR affects NAFLD; however, the interplay of multiple factors appears to affect the character of the disease[3,21].

Another important factor underlying NAFLD is oxidative stress. Multiple studies have demonstrated a close relationship among mitochondrial dysfunction, the overproduction of reactive oxygen species (ROS) and NAFLD, namely, NASH[22,23]. Imperfect fatty acid degradation results in increased free radical production, which is manifested by the production of various lipid peroxides. If sufficient mitochondria are affected, the resulting leak of mitochondrial components may induce hepatocyte apoptosis[24,25].

Extracellular signals, such as proinflammatory cytokines, also appear to affect the development of NAFLD. Studies have demonstrated an effect of free hepatocyte lipids on the induction of various, predominately proinflammatory, intracellular signaling pathways (NF-κB, c-Jun, and diacylglycerol), which in turn worsen IR and contribute to the hepatic production of various proinflammatory cytokines (*e.g.*, interleukin-6 and tumor necrosis factor α)[26].

In previous years, studies have implicated gut microbiota dysbiosis as a potential building block in NAFLD pathogenesis. The effect does not appear to be directly mediated by bacteria; instead, it may be mediated by various bacterial products that enter the portal blood stream. Moreover, the mechanisms include the effects of bacteria-produced short-chain fatty acids on the energy balance and intestinal barrier permeability of the host, the effect of bacteria on intestinal motility and the effect of various absorbed toxins (lipopolysaccharides) on the liver[27].

The main challenge with understanding NAFLD pathogenesis is correctly positioning the small pieces (risk factors) in their respective places to "see the big picture". Currently, there are three main theories regarding how the "pieces" should be positioned: the "two-hit", "multi-hit" and "distinct-hit" theories[28].

The two-hit theory was initially suggested in the late 1990s. It is based on the finding that only a portion of patients with a fatty liver develop advanced forms of NAFLD, indicating that the "first hit", IR-induced liver steatosis, is like a barrel of gasoline that requires a "second hit", ignition (*e.g.*, mitochondrial dysfunction, cytokines, or bacterial endotoxins), for activation[29].

The theory is highly contested because of findings that some patients develop hepatic inflammation without pre-existing IR, and in most patients, more than two factors are typically present. Thus, the second "multiple parallel-hits" theory was created. It is based on the premise that the "first hit" is not a single factor, but a sum of multiple distinct factors that wear down liver defenses. Again, the basic mechanism is IR and its associated metabolic disturbances. The result, a fatty liver, is prone to multiple "hits", which wear down liver defenses and eventually lead to inflammation (NASH) and fibrosis[30].

In recent years, a third "distinct-hit" theory has been proposed. It is based on the presumption that NAFLD and NASH are two separate diseases, which are associated with IR but unrelated to each other. This theory is based on epidemiological data indicating that patients with NASH have a 10%-20% chance of disease progression to cirrhosis during a 5-10-year period, whereas individuals with NAFLD typically manifest a stable disease, with a low risk of disease progression. Other data include the previously described genetic NASH predisposition, in which liver inflammation occurs without peripheral IR[31,32].

TRANSIENT ELASTOGRAPHY (FIBROSCAN®) WITH CONTROLLED ATTENUATION PARAMETER

In clinical practice, the initial diagnosis of NAFLD is typically established through laboratory findings (increased levels of aminotransferases and gamma-glutamil transferases) and radiological imaging techniques in the absence of other recognized causes of fatty liver (*e.g.*, alcohol, virus, drugs, or autoimmunity). Because of space limitations, this review will

not discuss the use of the various NAFLD diagnostic techniques[3]. In everyday clinical practice, biomarkers are needed to determine excess fat in the liver, as well as inflammation and fibrosis of the liver. However, it is less likely that specific proteins/biomarkers will be identified for the detection of liver steatosis/fibrosis. Whether currently available biomarkers for NAFLD severity are useful in monitoring NAFLD progression (or regression) in people with MetS is uncertain. In recent years, substantial attention has been focused on one dimensional transient elastography (TE). TE is a non-invasive ultrasound-based method that uses shear wave velocity to assess tissue (*e.g.*, liver) stiffness. Shear (secondary or S-) waves were initially discovered in seismology as slow waves that follow the primary compressional wave, hence their name. They are the manifestation of elastic waves that travel through the body of an object, as opposed to the surface waves, which, as the name implies, travel on the surface. In contrast to sound waves, which are longitudinal, shear waves are transverse, thus the motion of the affected tissue is perpendicular to the direction of wave propagation. As a result, shear waves move slowly (< 50 m/s) and are rapidly attenuated by liver parenchyma. This effect depends on the elastic properties of the tissue, with the speed of shear waves inversely proportional to the tissue elasticity. The method was designed at the Langevin institute in 1995 and was initially implemented for quality control in the food industry; however, since 2001, it has been applied in medical practice under the name FibroScan®[33].

In practical terms, the TE device consists of a vertically oriented mobile cuboid main body and one or several cylindrical probes. Measurements are performed on patients lying supine with their right rib cage spread (which is accomplished by elevating the right hand and/or crossing the right leg over the left). After gel application, the probe is positioned perpendicular to the skin surface in one of the intercostal spaces adjacent to the right lobe of the liver (typically the 9th to 11th intercostal space, on the midaxillary line). Shear waves are affected by changes in the medium density, particularly in the presence of liquid medium; thus, the operator must avoid large vascular structures. To avoid this problem and ensure better results, the TE device is equipped with a small scale, real-time, ultrasonographic display of the tissue that underlies the probe in both A- and M-modes. After adequate positioning, a low frequency shear wave is induced by a small piston positioned on the tip of the probe that hits the skin surface. On the basis of the physical characteristics (velocity and intensity attenuation) of the shear wave, the acquired data are processed and displayed on the screen as the liver stiffness measurement (LSM) and controlled attenuation parameter (CAP). Unsuccessful measurements are automatically excluded by the device; the numerical results are not displayed, and the message *"invalid measurement"* is displayed[34,35].

The measurement of liver stiffness is based

on Hook's law, which states that the velocity of shear waves that travel through an elastic object is proportional to the object's stiffness (*i.e.*, inversely proportional to the object's elasticity). The law is mathematically expressed as $E = 3\phi v^2$, where E represents Young's modulus (expressed in kPa), φ represents the tissue density (expressed in kg/m^3, assumed to be the same as water) and v shear represents the wave velocity (expressed in m/s). Young's modulus clinically corresponds to the LSM and is typically referred to as E or *LSM*. The practical application is made possible using a probe that emits two types of waves. The probe (piston) initially causes a slow-spreading low-frequency (50 Hz) shear wave, after which the fast ultrasound waves (emitted from the same probe) in a pulse-echo fashion are used to determine the position of the shear wave front in relation to time; thus, the velocity of the shear wave and hence the LSM are determined. LSM values range from 1.5 to 75 kPa; lower values indicate a more elastic liver. The shear waves spread from the point of skin impact in a spherical manner, whereas the ultrasound waves are released in a straight line along the probe's axis, *i.e.*, in one dimension. To ensure that the measurements are accurate and reproducible in the same patient and are comparable among different patients, the accompanying software modifies the shear wave characteristics by maintaining the shear wave frequency and shape while modifying the shear wave amplitude and energy output. Thus, the full name of the method is vibration-controlled 1D TE. The results are also affected by the amount of pressure applied to the probe, in which a lack of pressure results in incomplete contact with the underlying skin, whereas too much pressure modifies the shear wave by changing the stiffness of the underlying tissue. These errors are prevented by the software, which displays warning signs and disables probe activation when the applied pressure is not adequate for measurement[36,37]. The applied technical solutions have resulted in high intra- and interobserver levels of agreement, 98% in both cases, according to clinical data[38]. The resulting LSM is translated into an estimate of the level of liver fibrosis in a simple and straightforward manner. However, this is estimation is possible only under the assumption that the liver is homogeneous and non-viscous, and its elasticity is predominantly affected by the level of fibrosis. This feature is true for liver parenchyma; however, a problem arises with regard to the capsule of Glisson. The capsule is a sturdy tissue that provides the liver with its form and protects it from mechanical injuries. It adapts over time to changes in the liver size; however, it does not respond well to abrupt changes. Consequently, a rapidly developing mass effect inside the liver will increase the intrahepatic pressure and thereby reduce the liver elasticity. These conditions include right-sided (global) congestive heart failure, acute inflammation and/or edema of the hepatic

tissue, and extrahepatic cholestasis[39-41]. Therefore, in everyday practice, LSM is not an absolute measure of liver fibrosis but is instead a component of liver assessment and cannot be interpreted independently of other clinical results (*e.g.*, anamnesis, physical examination, laboratory tests, and imaging methods). Interestingly, food intake and alcohol consumption have also been demonstrated to affect LSM. Regarding food intake, different studies have reported varying results; however, a minimum two-hour fast is currently recommended prior to the exam[42,43]. Active alcohol consumption appears to lead to an overestimation of the LSM because one study has found that patients who were actively drinking at the first TE exam and subsequently stopped had significantly lower LSM values at the control TE exam several months later[44].

The basis for TE development was the measurement of liver stiffness; thus, LSM has been present in TE devices from its inception. However, conventional ultrasonography has demonstrated that liver steatosis, another important liver parameter, affects ultrasound waves by strongly attenuating their intensity. The changes in signal attenuation are followed by an increased reflection of incoming ultrasound waves, which results in the liver appearing bright (hyperechoic). The main problems with conventional ultrasonography are its subjective operator-dependent nature and multiple uncontrolled variables included in the examination, which decrease the sensitivity of the examination in the detection of liver steatosis. The effect is more pronounced when small amounts of fat are observed, and the sensitivity becomes substantially lower (12% in patients with a 5%-9% fat content in contrast to 80% in patients with a $\geq$ 30% fat content)[45]. The theoretical background consists of the formula for intensity attenuation: $I_z = I_0 e^{-\alpha_f z}$, where I_z represents the ultrasound intensity (expressed in W/m^2) at depth z (expressed in m), I_0 represents the initial intensity (expressed in W/m^2) and α_f represents the ultrasound attenuation coefficient (expressed in dB/m). The α_f coefficient is primarily affected by two parameters, including the frequency of the emitted ultrasound wave and the properties of the conducing object (liver). With a fixed and known frequency (3.5 MHz), α_f is directly affected and proportional to the level of steatosis; thus, it is typically referred to as the CAP. CAP values range from 100 to 400 dB/m, and higher numbers indicate more pronounced steatosis. The advantage of CAP is that it is simultaneously calculated with the LSM and from the same region of interest. The clinical application of CAP began in 2011, ten years after the introduction of LSM[46].

The benefit of TE compared with liver biopsy is that it measures a larger region of interest, namely, a cylindrical liver segment 1 cm wide and 4 cm long at a medium depth of 4.5 cm. This region amounts to a volume of 3 cm^3, which is approximately 100 times larger than the volume of the liver cylinder obtained by liver biopsy. The drawback is that the information (LSM and CAP) cannot be obtained by a single measurement. The final result is obtained as a median of at least 10 measurements. The procedure is deemed a failure if 10 valid measurements cannot be obtained, the percentage of valid measurements compared with the total number of measurements is less than 60% and/or the interquartile range exceeds 30% of the median[47]. Boursier *et al*[48] have investigated a group of 1165 chronic liver disease patients and have determined that an interquartile median ratio $\leq$ 10% is the best predictor of accuracy. In addition to the previously described factors that are controlled by the device, two important factors that increase the measurement failure and may be only partially offset by the device include the body size and intercostal space width. Similarly to conventional ultrasonography, the body mass index (BMI) negatively affects TE measurements, resulting in falsely increased LSM values in obese individuals and rendering the standard probe unreliable in patients with a BMI $\geq$ 28 (30) kg/m^2. A study by Castéra *et al*[49] has reported a 3.1% failure rate for obtaining valid results, which was associated with a BMI $\geq$ 30 kg/m^2 (OR = 7.5) and operator inexperience (defined as having performed fewer than 500 examinations, OR = 2.5). The number of unreliable results was higher and affected 15.8% of the examined patients; again, unreliability was related to a BMI $\geq$ 30 kg/m^2 (OR = 3.3) and operator inexperience (OR = 3.1). Of the obesity measures, LSM failure and LSM unreliability were predominantly related to waist circumference (> 80 cm in women, > 94 cm in men; OR = 3.0). To solve this problem, a new probe was developed with a more sensitive ultrasound transducer using a lower shear wave frequency, increased vibration amplitude, deeper focal length (mean depth 5.5 cm) and a greater depth of measurement. The probes were renamed after clothing sizes, and the standard probe represents the M probe, whereas the new probe represents the XL probe. Similar problems regarding narrow intercostal spaces have been identified in children and asthenic adults, thus necessitating the development of the S probe. Similarly to the M probe, the new probes could initially measure only the LSM; however, this issue has been resolved with the adjustment of CAP measurements for the new probes[50-53]. The advantages and disadvantages of TE are summarized in Tables 1 and 2.

The main initial clinical focus of TE was to assess the level of liver fibrosis (LSM) in patients with chronic viral hepatitis and to reduce the need for invasive procedures (liver biopsy). To date, the studies performed have demonstrated a good correlation of LSM with liver biopsy in the identification of significant liver fibrosis (F $\geq$ 2) and cirrhosis (F4). The AUROC for the identification of significant fibrosis in hepatitis B patients (cut-off values from 5.2 to 8.0 kPa) ranges from 0.86 to 0.97, with a sensitivity range of 70%-94% and a specificity range of 38%-99%. The AUROC for the identification

Table 1 Limitations of transient elastography with controlled attenuation parameter

Limitations	Explanation
Ascites	Elastic waves do not travel through liquids
Obesity	BMI > 30 kg/m^2 is associated with TE failure. With the development of the XL probe, the failure rate in obese patients has decreased
Acute hepatitis	Tissue changes in acute hepatitis may increase LSM
Chronic hepatitis with transaminases flare	At ALT levels greater than 5 × the upper normal limit, there is a risk of overestimating the fibrosis stage. LSM interpretations in patients with high ALT levels must be made with caution
Extrahepatic cholestasis	Increases LSM independently of fibrosis stage
Congestive heart failure	May lead to increased LSM because of an increased blood volume in the liver
Narrow intercostal spaces	Associated with a lower success rate or failed acquisition of LSM. Reduced failure rate with the development of the S probe

BMI: Body mass index; LSM: Liver stiffness measurement; ALT: Alanine-aminotransferase.

Table 2 Advantages of transient elastography with controlled attenuation parameter

Most widely used and validated non-invasive technique
High range of values
Well defined quality criteria
Good reproducibility
Detects liver stiffness and steatosis from the same region of interest
Excellent for the exclusion of cirrhosis
Prognostic value in cirrhosis
User-friendly
Short duration, painless
Applicable as a screening method in large populations

of significant fibrosis in hepatitis C patients (cut-off values from 5.2 to 9.5 kPa) ranges from 0.73 to 0.91, with a sensitivity range of 56%-97% and a specificity range of 32%-91%. Regarding cirrhosis, the AUROC for identification in hepatitis B patients (cut-off values from 9.7 to 14.0 kPa) ranges from 0.80 to 0.97, with a sensitivity range of 52%-98% and a specificity range of 59%-99%. The AUROC for the identification of cirrhosis in hepatitis C patients (cut-off values from 11.9 to 14.8 kPa) ranges from 0.87 to 0.98, with a sensitivity range of 72%-94% and a specificity range of 85%-98%[39,54-69]. In summary, TE is better at the identification of liver cirrhosis compared with significant fibrosis (mean AUROC 94% *vs* 84%, respectively), and among hepatitis patients, it is better at excluding than confirming liver cirrhosis (negative predictive value 96%, positive predictive value 74%)[70,71]. The main drawback is the lack of clear-cut cut-off values for different stages of liver fibrosis because the ranges for different fibrosis levels often overlap, particularly with lower levels of liver fibrosis. One recently published meta-analysis including 4386 chronic hepatitis B patients has confirmed these statements. The meta-analysis has indicated cut-off values for significant fibrosis (F ⩾ 2), a fibrosis level ⩾ 3 and cirrhosis in the following ranges: 5.85-8.8 kPa, 7.0-13.5 kPa and 9.0-16.9 kPa, respectively. The respective mean AUROCs for the cut-off values are 0.88, 0.91 and 0.93, respectively. Thus, the increasing accuracy of TE in the diagnosis of higher levels of fibrosis should be noted, as well as the substantial range in the cut-off values used in different studies. The latter finding may be explained by the differences in the cirrhosis prevalence in the studies, which affects the interpretation of the results, as well as the significance of the cut-off

values[72].

Despite the shortcomings, the role of TE in the assessment of the level of fibrosis in viral hepatitis patients has been recognized by the most recent EASL guidelines[54,73,74]. TE is currently considered to be the non-invasive standard for the measurement of liver stiffness, and it is the most accurate non-invasive method for the identification of liver cirrhosis in patients with chronic viral hepatitis[55]. Consequently, initial hepatitis C (HCV) staging includes the performance of TE to exclude liver cirrhosis. The gold standard for the non-invasive assessment of the degree of fibrosis includes performing TE with serum biomarkers because of the superior accuracy in comparison with that of either test alone. However, the use of two tests also results in increased costs, as well as the need to perform a liver biopsy when the tests are not in agreement[59,75,76]. In the case of hepatitis B (HBV), values less than 5-6 kPa indicate absent or minimal liver fibrosis, whereas values greater than 12-14 kPa are highly suggestive of cirrhosis. TE is also recommended in the initial assessment for HBV.

The use of TE in HCV patients to monitor the therapeutic response (reversal of cirrhosis) is discouraged because of a lack of clinical data. Even more disappointing, a single study has demonstrated a low sensitivity of 61% with 95% specificity in determining the reversal of cirrhosis[77]. Regarding HBV, the disease activity is a primary concern because inflammation and increased ALT levels are correlated with the overestimation of liver stiffness. The recommendations prompt the use of TE at least several months after ALT normalization to reduce the number of false positive results and to obtain a realistic value of the liver stiffness. However, the use of TE has been demonstrated to have a good prognostic value regarding the development of HCC. This association was initially identified in cross-sectional studies; however, because of the study design, the prognostic value could not be established[78,79]. Proof has come from subsequent prospective longitudinal studies that have demonstrated a progressive increase in the risk of HCC development with increased initial LSM values (Table 3)[80,81].

Table 3 Hazard ratio of hepatocellular carcinoma development in relation to liver stiffness measurement (according to Masuzaki *et al*[80] and Jung *et al*[81])

HCV		HBV	
LSM (kPa)	HR	LSM (kPa)	HR
10.1-15	16.7	13.1-18	4.68
15.1-20	20.9	18.1-23	5.55
20.1-25	25.6	> 23	6.60
> 25	45.5		

HCV: Hepatitis C virus infection; HBV: Hepatitis B virus infection; LSM: Liver stiffness measurement.

LSMS FOR THE PREDICTION OF FIBROSIS STAGE IN NAFLD

In daily clinical practice, specific biomarkers are needed that will demonstrate the amount of excess fat present in the liver, the level of fibrosis and the level of inflammation. In patients with NAFLD, the most important factor is the assessment of fibrosis severity and monitoring fibrosis progression. Most patients remain asymptomatic until their liver function is compromised; thus, the identification of the presence and severity of liver fibrosis remains a clinical challenge. This issue is important because efficient treatment for NAFLD has not yet been established. Therefore, the identification of risk factors for HCC and liver cirrhosis, such as liver fibrosis, should facilitate the implementation of risk-reduction mechanisms in NAFLD patients[82,83]. For the evaluation of fibrosis severity, a liver biopsy represents the "gold standard" in various liver diseases. Nevertheless, it is restricted by its complications and costs[84]. It is unrealistic to perform a liver biopsy for the diagnosis or monitoring of disease progression on all patients because 15%-40% of adults have NAFLD.

Despite the potential presence of high risk factors for fibrosis in NAFLD patients, such as diabetes, the population remains too large to implement an invasive method to exclude fibrosis[84,85]. Thus, noninvasive methods have been intensively investigated[84]. The various approaches include standard biochemical and hematological tests, surrogate fibrosis markers in the blood and their algorithms and, the most recently investigated approach, TE[85]. To date, TE assessment of liver fibrosis has predominantly been implemented in patients with chronic viral hepatitis, as well as patients with other chronic liver diseases of different etiologies[86]. Recent studies have examined the usefulness of LSM compared with liver biopsy to identify fibrosis in NAFLD patients. Table 4 shows details of eight studies that have examined the usefulness of LSMs in the identification of different stages of liver fibrosis in NAFLD patients compared with liver biopsy[83,84,87-92]. In these studies, for F ≥ 2, the LSM cut-off values range from 6.2 to 11 kPa, with 62%-90% sensitivity and 74%-100% specificity. For

Table 4 Usefulness of liver stiffness measurement compared with liver biopsy in the detection of fibrosis in nonalcoholic fatty liver disease patients

Study	Probe	Cut-off (kPa)	Sensitivity	Specificity	Number of patients with liver biopsy
Fibrosis stage ≥ F2					
Imajo *et al*[83] (2016)	M	11.0	61.7	100	142
Pathik *et al*[84] (2015)	M	9.1	Not reported	Not reported	110
Yoneda *et al*[87] (2007)	M	6.65	81.8	91.2	67
Cassinotto *et al*[88] (2015)	M	6.2	≥ 90	Not available	291
Wong *et al*[89] (2010)	M	7.0	88	74	246
Lupsor *et al*[90] (2010)	M	6.8	67	84	72
Yoneda *et al*[91] (2008)	M	6.65	88	74	97
Kumar *et al*[92] (2013)	M	7.0	78	79	205
Fibrosis stage ≥ F3					
Imajo *et al*[83] (2016)	M	11.4	85.7	83.8	142
Pathik *et al*[84] (2015)	M	12.0	90	80	110
Yoneda *et al*[87] (2007)	M	8.0	87.5	84.3	67
Cassinotto *et al*[88] (2015)	M	8.2	≥ 90	Not available	291
Wong *et al*[89] (2010)	M	8.7	84	83	246
Lupsor *et al*[90] (2010)	M	10.4	100	97	72
Yoneda *et al*[91] (2008)	M	9.8	85	81	97
Kumar *et al*[92] (2013)	M	9.0	85	88	205
Fibrosis stage F4					
Imajo *et al*[83] (2016)	M	14.0	100	75.9	142
Pathik *et al*[84] (2015)	M	20.0	90	80	110
Yoneda *et al*[87] (2007)	M	17.0	100	98.4	67
Cassinotto *et al*[88] (2015)	M	9.5	≥ 90	Not available	291
Wong *et al*[89] (2010)	M	10.3	92	97	246
Yoneda *et al*[91] (2008)	M	17.5	100	97	97
Kumar *et al*[92] (2013)	M	11.8	90	88	205

F ≥ 3, the LSM cut-off values range from 8 to 12 kPa, with 84%-100% sensitivity and 83%-97% specificity. For F4, the LSM cut-off values range from 9.5 to 20 kPa, with 90%-100% sensitivity and 75.9%-98.4% specificity.

A meta-analysis[93] in 2014 has indicated that TE is excellent in diagnosing F ≥ 3 (85% sensitivity, 82% specificity) and F4 (92% sensitivity, 92% specificity), and it has a moderate accuracy for F ≥ 2 in NAFLD

patients. LSMs were performed with an M probe, and obesity was the major reason for unsuccessful LSM. This problem may be avoided with the use of the novel XL probe. In a study by Wong *et al*[94], the XL probe was used to identify fibrosis in 193 NAFLD patients with a BMI $\geq$ 30 kg/m^2 compared with liver biopsy, "the gold standard". Ten valid measurements were obtained in 93% of the patients, with AUROCs of 0.80, 0.85 and 0.91 for F $\geq$ 2, F $\geq$ 3 and F4, respectively. In a study conducted by Friedrich-Rust *et al*[85], the AUROC for significant fibrosis diagnosis (F $\geq$ 2) for the XL probe was 0.82 compared with 0.84 for a severe fibrosis diagnosis (F $\geq$ 3) and 0.95 for an F4 diagnosis. This study demonstrates that when measured with the XL probe, the median LSM is significantly lower than that measured with the M probe (6.9 kPa *vs* 8.4 kPa, respectively). According to these two studies, the LSM cut-off values should be approximately 1.5-2 kPa lower when the XL probe is used rather than the M probe for the same stage of fibrosis. This issue justifies the need for more studies on this topic because the existing cut-off values, which are defined for the M probe, cannot be used for the XL probe. Available data indicate that in patients with NAFLD, TE is a highly accurate, non-invasive method for advanced fibrosis exclusion and a moderately accurate method for significant fibrosis exclusion.

The use of paired biopsies for monitoring the progression of the disease in NAFLD patients has been reported. A prospective four-year study has been conducted by Suzuki *et al*[95], in which the disease progression in NAFLD patients was evaluated using TE. Ninety-seven NAFLD patients (demonstrated by liver biopsy) had their LSM obtained at the beginning of the study; of the 97 patients, 36 patients were available for reevaluation after four years, in which their stage of fibrosis was compared with that from their initial assessment. The authors concluded that LSM may be used to monitor hepatic fibrosis severity in patients with NAFLD. Nevertheless, additional prospective studies regarding the monitoring of LSM progression in patients with NAFLD are necessary.

CAP FOR THE PREDICTION OF STEATOSIS GRADES IN NAFLD

Liver steatosis may be defined radiologically as a fat mass comprising $\geq$ 5% of the wet weight of the liver or histologically as a fatty deposit presence in $\geq$ 5% of hepatocytes. Metabolic dysfunction of the liver may develop over time as a result of liver steatosis, which may consequently progress into irreversible damage to the liver, with the development of fibrosis, cirrhosis and HCC[96]. Steatosis of the liver is a key parameter in liver transplantation. Because any liver with a fat content > 30% is automatically ineligible for donation, determining the liver steatosis level is of substantial importance for the evaluation and clinical

prognosis of patients with NAFLD[83]. Efforts regarding the development of reliable non-invasive methods for liver steatosis detection and quantification have been made over the past 10 years[96,97]. A strong correlation of CAP with fat accumulation in the liver (demonstrated by liver biopsy) has been identified in clinical studies investigating TE with CAP; moreover, CAP has been reported to be useful in the diagnosis of steatosis of the liver in numerous chronic liver diseases[83,96-104]. Table 5 shows that (similarly to the LSM cut-off values) the different CAP cut-off values presented by different studies for distinct grades of liver steatosis defined by biopsy (range from S0, which indicates no steatosis, to S3, which indicates the highest level of steatosis); for S $\geq$ 1 ($\geq$ 10% of hepatocytes with fat), the CAP cut-off values range from 214 to 289 dB/m, with a 64%-91% sensitivity range and a 64%-94% specificity range; for S $\geq$ 2 ($\geq$ 33% hepatocytes with fat), the CAP cut-off values range from 255 to 311 dB/m, with a 57%-96% sensitivity range and a 62%-94% specificity range; finally, for S3 ($\geq$ 66% hepatocytes with fat), the CAP cut-off values range from 281 to 310 dB/m, with a 64%-100% sensitivity range and a 53%-92% specificity range. According to these studies, CAP is useful in the detection of S $\geq$ 1, S $\geq$ 2, and S3 steatosis as a result of its good sensitivity and specificity; however, the exact cut-off values remain to be defined[83,98-103,105].

De Lédinghen *et al*[104] conducted a study in 2014 regarding the diagnosis of S1, S2 and S3. The cumulative AUROCs of CAP were 0.79 (95%CI: 0.75-0.84), 0.84 (95%CI: 0.80-0.88) and 0.84 (95%CI: 0.80-0.88), respectively. The study included 440 patients who had undergone a liver biopsy. Compellingly, obesity (defined as a BMI >30 kg/m^2) was determined to be the main cause of CAP measurement failure. It must be taken into account that both the 2014 de Lédinghen study and all studies described in Table 5 excluded the benefits of the CAP-enabled XL probe by using only the M probe. Furthermore, these studies demonstrate that the CAP cut-off values are not affected by the cause of the chronic liver disease, in contrast to LSM, in which the cut-off values depend on the type of liver disease[101].

WHAT IS THE POSITION OF TE WITH CAP IN THE ASSESSMENT OF NAFLD?

The significance of metabolic factors in the pathogenesis of NAFLD has been emphasized by numerous studies. As previously discussed, at least one component of MetS is present in approximately 90% of patients with NAFLD, whereas all diagnostic criteria for MetS are met in 35%-75% of patients. Furthermore, the risk for NAFLD is increased 4-11 times by the presence of MetS[3,106-109]. Mena *et al*[110] have identified an association between different MetS components and fibrosis in chronic hepatitis B patients. The pre-

Table 5 Performance of controlled attenuation parameter compared with liver biopsy for the detection of various steatosis grades

Study	Etiology of CLD	Probe	Cut-off (dB/m)	AUC	Sensitivity (%)	Specificity (%)	Number of patients with liver biopsy
Steatosis grade ≥ 1							
Sasso et al[98] (2010)	CLD, ALD, NAFLD	M	238	0.91	91	81	115
de Lédinghen et al[100] (2012)	NAFLD, HCV, ALD, other	M	266	0.84	69	85	112
Shen et al[102] (2014)	NAFLD, HBV	M	253	0.92	88	83	189
Kumar et al[101] (2015)	HBV, HCV, NAFLD	M	214	0.68	64	64	317
Myers et al[99] (2012)	Hepatitis, NAFLD, other	M	289	0.79	68	88	153
Chan et al[103] (2014)	NAFLD, control	M	263	0.97	91	94	101
Imajo et al[83] (2016)	NAFLD, control	M	236	0.88	82.3	91	127
Lupşor-Platon et al[105]	HCV, HBV, NAFLD, other CLD	M	260	0.81	64.8	82.3	201
Steatosis grade ≥ 2							
Sasso et al[98] (2010)	CLD, ALD, NAFLD	M	259	0.95	89	86	115
de Lédinghen et al[100] (2012)	NAFLD, HCV, ALD, other	M	311	0.86	57	94	112
Shen et al[102] (2014)	NAFLD, HBV	M	285	0.92	93	83	189
Kumar et al[101] (2015)	HBV, HCV, NAFLD	M	255	0.79	77	80	317
Myers et al[99] (2012)	Hepatitis, NAFLD, other	M	288	0.76	85	62	153
Chan et al[103] (2014)	NAFLD, control	M	263	0.86	96	67	101
Imajo et al[83] (2016)	NAFLD, control	M	270	0.73	64.3	73.6	127
Lupşor-Platon et al[105]	HCV, HBV, NAFLD, other CLD	M	285	0.82	69.7	85.1	201
Steatosis grade 3							
Sasso et al[98] (2010)	CLD, ALD, NAFLD	M	292	0.89	100	78	115
de Lédinghen et al[100] (2012)	NAFLD, HCV, ALD, other	M	318	0.93	87	91	112
Shen et al[102] (2014)	NAFLD, HBV	M	310	0.88	92	79	189
Kumar et al[101] (2015)	HBV, HCV, NAFLD	M	305	0.91	71	92	317
Myers et al[99] (2012)	Hepatitis, NAFLD, other	M	283	0.70	94	47	153
Chan et al[103] (2014)	NAFLD, control	M	281	0.75	100	53	101
Imajo et al[83] (2016)	NAFLD, control	M	302	0.70	64.3	73.6	127
Lupşor-Platon et al[105] (2015)	HCV, HBV, NAFLD, other CLD	M	294	0.83	83.3	82.5	201

CLD: Chronic liver disease; ALD: Alcoholic liver disease; NAFLD: Nonalcoholic fatty liver disease; HBV: Hepatitis B virus; HCV: Hepatitis C virus.

sence of multiple MetS components is associated with fibrosis development, whereas significant fibrosis is uncommon in the absence of MetS.

The occurrence of NAFLD has increased as a result of the rapid increase in the prevalence of metabolic risk factors. Patients with NAFLD are at risk for liver-related morbidity and mortality. Numerous recent studies have indicated a significantly increased incidence of HCC in obese patients and patients with T2DM, and moreover, increasing evidence suggests an increased risk of HCC in NAFLD patients. As a result of the increasing incidence of NAFLD, an increase in NAFLD-related HCC is expected in the future[111].

The risk of developing CVD, CKD and T2DM in long-term follow-up is increased by the presence of NAFLD. The course of these diseases is also affected by NAFLD, because it increases the CVD and CKD risk. Moreover, NAFLD and MetS are more tightly associated because the presence of one condition increases the risk of developing the other condition. From a therapeutic standpoint, the prevention and treatment of hepatic IR, MetS and related complications represent a rational approach in reversing NAFLD, which is why various specialists, such as hepatologists, nephrologists, endocrinologists, cardiologists, general internists, and primary care physicians, should be involved in the care of NAFLD patients[108,112]. The strongest predictor of the progression of liver disease in NAFLD patients is the presence of NASH at the initial liver biopsy. In addition,

the main determinant of all-cause and cause-specific mortality in patients with NAFLD is the severity of liver fibrosis[105-107,113,114]. Complicating matters, a recent study conducted by McPherson et al[114] has reported that 44% of the patients with simple steatosis at the index liver biopsy progressed to NASH, whereas in 37% of the patients, fibrosis progression was present at the follow-up biopsy. Furthermore, at the follow-up biopsy, 22% of the patients with baseline simple steatosis reached stage three fibrosis. The progression potential from simple steatosis to fibrosis and NASH has also been confirmed by other small studies[115,116]. An association between non-cirrhotic NAFLD and HCC risk has been demonstrated in recent studies[117,118]. According to a study in the United States, conducted between 2002 and 2008, the most common underlying risk factor for HCC is NAFLD, followed by T2DM and HCV infection[119]. Thus, contrary to current opinion, simple steatosis may progress to NASH and significant fibrosis, which, in turn, would indicate that most patients with NAFLD are at long-term risk of progressive liver disease[114-116]. Liver biopsy is the gold standard for liver fibrosis assessment; however, its limitations, complications and cost, given that approximately one-third of the population has NAFLD, make it unreasonable to perform it on all patients. A substantial number of physicians consider liver biopsy to be a diagnostic tool in patients who persistently exhibit increased liver function tests because of the substantial

number of patients at risk (T2DM, obesity, arterial hypertension, dyslipidemia and/or MetS). However, it must be considered that in more than half of NAFLD patients, aminotransferases are within the normal limits; therefore, deciding which patients with NAFLD are candidates for liver biopsy and how to monitor their liver disease progression leaves gastroenterologists with more questions than answers[120].

According to the most recent guidelines by the American Association for the Study of Liver Diseases (AASLD) in 2012[121], liver biopsy should be considered in high-risk patients with NAFLD, patients with an increased risk of NASH and advanced fibrosis. The presence of T2DM, MetS and/or an age > 50 years are risk factors. Systematic screening, at least for higher-risk patients (diabetic and obese patients), has been argued for by many authors. According to the AASLD guidelines, "screening for NAFLD in adults attending primary care clinics or high-risk groups attending diabetes or obesity clinics is not advised at this time because of the uncertainties surrounding diagnostic tests and the cost-effectiveness of screening"[121]. That guidelines were published in 2012 must be considered, and given the new data accumulating, updated guidelines are urgently needed.

MetS may be used to identify patients as candidates for liver biopsy, specifically when it is present with a noninvasive marker of liver steatosis/fibrosis because it predicts the presence of steatohepatitis in patients with NAFLD. TE with CAP may find its place in this approach, according to recent investigations. Interestingly, a study conducted by de Lédinghen *et al*[104] has demonstrated that the CAP value significantly increases with the number of parameters of MetS, BMI, waist circumference, presence of arterial hypertension and T2DM. Our recent analysis, including 648 patients with one or more components of MetS, has provided similar results; specifically, the CAP measurements progressively increase with the number of MetS components. In addition, the presence of MetS (or its individual components), IR, increased uric acid levels and an LSM > 7 kPa are factors independently associated with increased CAP (unpublished data). Kwok *et al*[113] have analyzed 1900 patients with T2DM for NAFLD using TE with CAP and have determined prevalence rates of increased CAP and LSM of 72.8% and 17.7%, respectively. Furthermore, that study included 94 T2DM patients with suspected advanced fibrosis or cirrhosis who had undergone liver biopsy, and of these 94 patients, 56% had NASH, 21% had advanced fibrosis and 29% had cirrhosis. Naveau *et al*[122] have analyzed 100 patients who were candidates for bariatric surgery. TE was performed 15 d prior to liver biopsy. The AUROC generated by TE was 0.81 ± 0.05 for the prediction of fibrosis stage F ≥ 2 and 0.85 ± 0.04 for the prediction of fibrosis stage F3. The authors have concluded that TE may be used for the early diagnosis of fibrosis in severely obese patients. In a similar study of hospitalized diabetic patients, de

Ledinghen *et al*[123] have demonstrated an increased prevalence of severe fibrosis (defined by LSM), which was the highest in the > 50 year-old group of T2DM patients. Cho *et al*[2] have tested the feasibility of TE with CAP in 201 children in a comparison of CAP and LSM values in obese children, non-obese healthy controls and non-obese patients with liver disease. The authors found that the CAP values were increased in the obese group compared with the other two groups. Furthermore, they identified significantly higher LSM values in the obese group compared with the healthy control; however, no statistically significant differences in the LSM values were identified between the group with liver disease and the other two groups. In the obese group, the LSM values were highly correlated with the CAP values, whereas there was no correlation in the healthy control group or the group with liver disease[2].

On the basis of the findings in the previously described studies, CAP and LSM have a good correlation with the presence of MetS and its individual components.

The potential effect of NASH on the course and prognosis of NAFLD is a significant issue. The gold standard for the diagnosis and follow-up of NASH is liver biopsy. According to the study by Friedrich-Rust *et al*[85], the AUROC of TE for steatohepatitis diagnosis (according to the NAFLD activity score) was 0.79 for the M probe and 0.74 for the XL probe. In the study by Cho *et al*[2], LSM was mildly increased in patients with steatohepatitis, which may be attributed to inflammation, whereas similar results have been obtained in patients with alcoholic liver disease[124]. Thus, there is a possibility that LSM values in obese patients may be affected by steatohepatitis[2]. The obtained results indicate the urgency to conduct additional research to further clarify the position of TE with CAP in steatohepatitis management.

A subgroup of NAFLD patients (in the population of patients with one or more MetS components) who are at high risk of developing progressive liver disease may be identified by using TE with CAP because CAP and LSM show good correlations among MetS and its individual components and liver biopsy findings. The presence of MetS, which predicts the presence of steatohepatitis in NAFLD patients, in combination with a non-invasive method for liver fibrosis and steatosis detection may be used to identify candidate patients for liver biopsy. The identification of patients who are at risk for the development of NASH and advanced fibrosis and who require a liver biopsy may be performed through using MetS with high CAP values and particularly with increased LSM values. Available data suggest that to exclude advanced fibrosis in patients with NAFLD, TE is a highly accurate, non-invasive method, whereas it is a moderately accurate method for excluding significant fibrosis in patients with NAFLD, which is why TE with CAP may eventually replace liver ultrasound in the initial evaluation of patients with NAFLD. Taking into account the early observations that MetS and its individual components

(T2DM and obesity) are risk factors for the progression of liver disease in NAFLD patients, the identification of patients in need of a liver biopsy may be accomplished when MetS and its components are present together with increased CAP and particularly increased LSM. As a result of the parallel increasing incidence of both NAFLD and obesity, T2DM and MetS, including the consequences of MetS and NAFLD, *i.e.*, the associated morbidity and mortality, the consideration of screening for NAFLD in all patients with one or more MetS components by a non-invasive method, such as TE with CAP, appears reasonable[125,126]. Per our analysis, increased CAP values have been found in patients with only one MetS component[126]. There is a disproportionately small number of studies conducted that have investigated TE in the setting of NAFLD and MetS, given that NAFLD is a common disease, and TE is becoming an increasingly used non-invasive method. Moreover, NAFLD is the most common cause of increased liver enzymes; however, it is critical to consider that AST and ALT may be within their normal ranges even in advanced NAFLD. Thus, the earlier opinion that NAFLD patients with persistently increased liver enzymes should be the only patients who undergo liver biopsy should be revised[125,126].

In parallel to the increasing need for a noninvasive assessment of liver fibrosis and steatosis, several imaging methods have emerged. Two other methods, in addition to TE, have shown promising results. The first method, acoustic radiation force impulse imaging (ARFI), is based on shear wave propagation, similarly to TE. Compared with TE, the inspected liver volume is smaller (1 cm in length); however, ARFI can be used on modified commercial ultrasound machines. Thus, the point of interest can be pinpointed using an ultrasound's B-mode. The downside of this method includes a narrow range of results (0.5-4.4 m/s) with unclear cut-off values for different fibrosis stage levels. Bota *et al*[127] have summarized the studies comparing the two methods in a meta-analysis, indicating comparable sensitivity (0.87 with 95%CI: 0.79-0.92 for ARFI *vs* 0.89 with 95%CI: 0.80-0.94 for TE) and specificity values (0.87 with 95%CI: 0.81-0.91 for ARFI *vs* 0.87 with 95%CI: 0.82-0.91 for TE) of both methods in the detection of liver cirrhosis. The reliability of the measurements is the principal difference between the two methods. ARFI fares better, with 2.1% unreliable results, compared with 6.6% for TE. The main reason for the unreliable results is obesity, and the studies included in the meta-analysis were based on TE measurements performed by the M probe; thus, these percentages should be interpreted with caution. The unreliability highlighted in that study is why the actual reliability difference between TE and ARFI must be re-assessed, including studies using the XL probe. Compared with TE, the inability to determine the level of steatosis is the clear disadvantage of AFRI. The other significant field of noninvasive liver fibrosis and steatosis assessment involves magnetic resonance imaging (MRI)-based methods. These approaches assess the liver in its entirety. The main advantage of MRI methods is that they are not affected by obesity or the presence of ascites. However, distinct methods are required to properly and independently assess liver fibrosis (*e.g.*, MRI elastography) and steatosis (*e.g.*, proton density fat fraction MRI). The high performance of MRI-based methods in assessment of advanced fibrosis (AUROC 0.957 with 95%CI: 0.918-0.996 for 2D-MRI elastography), as well as steatosis levels (correlation coefficient for the quantification of liver steatosis of 0.82 for proton density fat fraction MRI) in NAFLD patients has been demonstrated in recent studies[128,129]. Although MRI-based methods have demonstrated better diagnostic performances in non-invasive liver fibrosis and steatosis detection in patients with NAFLD compared with TE with CAP, there are major factors that limit this method, particularly in monitoring of the progression of the disease; these factors include cost, patient claustrophobia and duration of the examination[83,125].

Several questions should be addressed with additional studies. First, the question arises as to whether TE with CAP may be used to monitor NAFLD progression and whether the progression of LSM values may be used as a parameter of liver fibrosis severity. Because the only treatment option for NAFLD includes lifestyle changes and individual MetS component treatment, the question arises as to whether monitoring the changes in the CAP and LSM values could be used to assess the treatment of individual MetS components and the effect of treatment on NAFLD.

Second, taking into account portal hypertension, TE may potentially be useful in the identification of patients who are at risk of developing varices, as several studies have demonstrated. Furthermore, some studies have highlighted the potential utility of spleen stiffness measurements in the prediction of esophageal varices and portal hypertension level assessment in liver cirrhosis[124]. Thus, additional studies are required regarding this topic in patients with NAFLD.

The use of TE has been demonstrated to have a good prognostic value regarding the development of HCC in patients who suffer from viral hepatitis[78,79]; however, interestingly, there are no studies regarding the prediction of HCC development in patients with NAFLD *via* an assessment of the value of high LSM measurements. Thus, additional prospective studies are urgently required to answer this question. If the predictive value of TE with CAP were verified, clinicians would be able to assess and monitor the risk of HCC development and to establish optimal and personalized monitoring and treatment strategies in patients with NAFLD. Additional studies should also focus on investigating the accuracy of TE with CAP for all clinically significant events (*i.e.*, liver cirrhosis and HCC) in patients with one or more MetS components.

Third, what is the place of TE with CAP in steatohepatitis detection? Yoneda *et al*[87] have demonstrated

a substantial increase of LSM in NASH patients, as confirmed by liver biopsy results; however, additional studies must be conducted. According to Yoneda *et al*[87], the LSM values are not affected by the degree of steatosis; however, additional studies must clarify this issue, and the influence of high grade steatosis on LSM values remains controversial.

Fourth, an investigation regarding whether the increased CAP and LSM values could predict the development of MetS in patients with one or two MetS components is needed. In addition, a question arises as to whether it is possible to monitor the changes in MetS and its individual components by monitoring the changes in CAP and LSM.

Given the associations between NAFLD and CVD and CKD risks, additional studies should determine whether patients with NAFLD with both increased CAP and particularly an increased LSM might benefit from early CKD and CVD screening. Finally, large studies are required for the development of new cut-off values for liver fibrosis staging using the XL probe and to investigate the differences between the CAP cut-off values used for the M and XL probes, respectively[84].

In conclusion, an easy, quick and non-invasive mass screening for NAFLD in patients with one or more MetS components may be reasonably achieved with TE with CAP. Once NAFLD is diagnosed, particularly liver fibrosis using LSM values, these patients should be directed to hepatologists, diabetologists and nephrologists. If TE with CAP is used as a screening method, liver biopsy may consequently be avoided in a substantial number of patients. This approach may also be useful in the early diagnosis of associated metabolic abnormalities and may enable the appropriate treatment of MetS, which is highlighted by its being the only available treatment option for patients with NAFLD to date. The accuracy of TE with CAP in the prediction of clinical events (*i.e.*, liver cirrhosis and HCC) in patients with one or more MetS components should be investigated in additional studies.

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