Review

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Non-invasive diagnosis and biomarkers in alcohol-related liver disease

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Summary

Even though alcohol-related liver disease (ALD) is a major cause of severe liver disease worldwide, most patients with ALD are diagnosed at the decompensation stage. Liver biopsy is still considered the gold standard for establishing a definite diagnosis and assessing the fibrosis stage of ALD, but it is an invasive procedure, associated with significant morbidity. During the last decade, non-invasive tests have been developed to estimate the severity of liver fibrosis and steatosis. Measurement of liver stiffness by transient elastography has become the most commonly used non-invasive parameter to evaluate fibrosis. In ALD, transient elastography has been demonstrated to have an excellent performance to detect advanced fibrosis and cirrhosis. However, aspartate aminotransferase levels must be considered when interpreting liver stiffness cut-offs. Non-invasive biological tests have also been evaluated to assess liver fibrosis in ALD. The commercially available Enhanced Liver Fibrosis test and FibroTest have comparable performance for the diagnosis of advanced fibrosis in ALD, with studies suggesting that they are better than other biological tests (*i.e.* FIB-4 and APRI). Although ultrasound is still accepted as an initial screen for fatty liver diagnosis, new methods have recently been developed to detect steatosis. Magnetic resonance spectroscopy and magnetic resonance imaging techniques are highly accurate and reproducible, with superior sensitivities and specificities for detecting histological steatosis than ultrasound. However, low availability and high cost limit the use of magnetic resonance techniques in routine clinical practice. More recently, controlled attenuation parameter was developed as a novel tool to non-invasively assess liver steatosis; performed in combination with transient elastography, it was suggested to be superior to regular ultrasound for detecting steatosis and was shown to have acceptable diagnostic accuracy. New serum biomarkers are under investigation to non-invasively diagnose more severe forms of ALD and to predict prognosis of patients.

Keywords: Alcohol-related liver disease; Fibrosis; Steatosis; Non-invasive tests; Transient elastography; Biomarker.

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Introduction

Alcohol-related liver disease (ALD) is the most frequent cause of severe liver disease in Europe. Based on the World Health Organization database, more than 40% of the liver deaths are attributable to alcohol.[1] The number of liver transplants performed for patients with ALD-related cirrhosis has increased over the past 2 decades, both in Europe and in the US.[2,3] Despite the high burden of ALD, it is regrettable that the majority of patients with ALD are diagnosed at the decompensation stage. Moreover, a large proportion of patients with newly diagnosed cirrhosis had recent consultations in primary care or emergency units,[4] without any intervention. Since the risk of developing advanced liver disease decreases with abstinence or reduced alcohol consumption, screening for ALD and interventions in at-risk patients should be routinely implemented.

ALD includes a wide spectrum of lesions ranging from steatosis to steatohepatitis, progressive liver fibrosis, cirrhosis and its complications.[1] Although steatosis is present in almost all heavy drinkers, it is estimated that only 10–20% will eventually develop cirrhosis.[5] Since the presence of advanced fibrosis or cirrhosis in compensated patients is the main predictor of long-term survival,[6] it is of clinical importance to diagnose patients with advanced fibrosis before decompensation occurs, in order to promote abstinence and improve survival.

Liver biopsy is still considered the gold standard for establishing a definite diagnosis of ALD, assessing the stage of fibrosis and excluding alternative causes of liver injury. However, liver biopsy is an invasive procedure, with significant morbidity,[7] and is generally not recommended in routine clinical practice for all patients with suspected ALD.

Initially developed in viral hepatitis, non-invasive tests are increasingly used to estimate the severity of liver fibrosis in almost all aetiologies of liver disease. Serologic tests, radiographic modalities and liver stiffness (LS) have excellent predictive value for diagnosis of advanced fibrosis, particularly in chronic hepatitis C and non-alcoholic fatty liver disease (NAFLD).[8,9] In addition to fibrosis evaluation, recent advances have been made for the diagnosis and staging of steatosis. The aim of the present manuscript is to review the non-invasive methods available for the diagnosis and evaluation of liver fibrosis and steatosis in patients with ALD, while proposing a practical algorithm for the clinician and discussing new biomarkers in development.

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Diagnosis and evaluation of liver steatosis in ALD

Liver steatosis is characterised by the excessive accumulation of fat-containing vacuoles within the cytoplasm of hepatocytes. Up to 90% of patients with heavy alcohol intake have steatosis, which is usually asymptomatic and rapidly reversible with abstinence.[10] However, 5–10% of patients with simple alcoholic steatosis have been shown to progress to cirrhosis within 5 years,[11] and steatosis has been identified as an independent predictive factor of fibrosis progression in heavy drinkers.[12] Consequently, reliable non-invasive methods to diagnose and monitor steatosis in patients with ALD are desirable.

Liver ultrasound

Key point

Ultrasound is accepted as a first screen for steatosis, because it is non-invasive, inexpensive and widely available.

Ultrasound (US) is accepted as an initial screen for fatty liver because it is non-invasive, inexpensive and widely available. In US images, steatosis appears as a diffuse hyper echogenicity due to increased parenchymal reflectivity that results from intracellular accumulation of fat inclusions.[13] US has a sensitivity of 60–94% and a specificity of 88–95% in detecting steatosis.[14] US sensitivity significantly varies according to degrees of fatty load, with 80% sensitivity at fat accumulation above 30%, as opposed to 55% when the fat content reaches only 10–20%. The US evaluation of steatosis is mainly qualitative, with a grading conveniently classified as mild, moderate or severe.[13] One major weakness of US is its operator dependency.[15] Another drawback of the technique includes its inaccuracy in differentiating fibrosis from steatosis.

Magnetic resonance imaging

Key point

Controlled attenuation parameter is a simple non-invasive technique for the assessment of steatosis, which has been shown to be superior to ultrasound in ALD, although it requires further validation.

Magnetic resonance spectroscopy (MRS) allows for non-invasive studies into the molecular composition of tissues *in vivo*. MRS quantifies the proton density fat fraction (PDFF), a standardized measure of liver tissue.[16] MRS is highly accurate and reproducible for measuring hepatic fat. However, MRS has limited availability and is not available on routine scanners. Magnetic resonance imaging (MRI)-based methods have been developed that use MRI-PDFF and routinely available clinical MRI scanners to quantify liver fat without needing spectroscopy. MRI-PDFF is not affected by scanner field strength, patient factors, aetiology of liver disease, and concomitant liver abnormalities such as iron overload or liver inflammation.[17,18] MRI sensitivities and specificities in detecting histologic steatosis ≥5% were 76.7–90.0% and 80.2–87.0%, respectively.[19] MRI has several advantages: In addition to its high accuracy and reproducibility for measuring hepatic fat, MRI allows follow-up of response after intervention.[18] The disadvantages of MRI include its high cost and long examination time.

Controlled attenuation parameter

Controlled attenuation parameter (CAP) is a novel tool to non-invasively assess liver steatosis, which measures ultrasound attenuation when travelling through fatty liver tissue, compared to normal liver.[20] The CAP software is incorporated into the Fibroscan® (Echosens, Paris, France) equipment, which enables CAP to be combined with non-invasive liver fibrosis assessment using transient elastography. In an individual data meta-analysis, CAP technology was shown to diagnose moderate and severe steatosis with diagnostic accuracies of between 0.85 and 0.90 in 2,735 patients with mixed liver disease aetiologies (mainly viral hepatitis and NAFLD).[21] In a recent European multicentre prospective study including 562 patients with ALD who underwent CAP, regular US and liver biopsy,[22] CAP diagnosed mild, moderate and severe steatosis with an area under the receiver operating characteristic curve (AUROC) of 0.77, 0.78 and 0.82, respectively. A CAP value above 290 dB/m ruled in any steatosis with 88% specificity. Moreover, CAP was shown to be superior to regular US for diagnosing steatosis in patients with ALD. The procedure is non-invasive, non-ionizing, easy to perform and provides immediate results. In addition, CAP can be performed simultaneously with LS measurement, making the simultaneous evaluation of both fibrosis and steatosis possible.[23] For these reasons, CAP technology is an interesting tool for diagnosing steatosis. However, diagnostic accuracy appears to be poorer at low steatosis stages and seems lower in ALD compared to other liver disease aetiologies. Moreover, optimal cut-offs to rule in, rule out and stage steatosis vary in the different studies performed.

In summary, liver ultrasound remains the first-line screening modality in clinical practice, with acceptable sensitivity and specificity for detection of steatosis. MRI- and MRS-PDFF appear clearly to be the most accurate and reproducible methods for the diagnosis and follow-up of steatosis. However, cost and examination time limit its use in routine clinical practice. CAP is a simple and promising new bedside technique for diagnosing steatosis in patients with ALD, but it requires further validation.

Evaluation of liver fibrosis in ALD

Biological tests

Enhanced Liver Fibrosis (ELF™) and FibroTest (FT) are the most commonly used blood-based assessment tools for liver fibrosis. The ELF test is commercially available and it combines 3 direct serum markers of extracellular matrix remodelling and fibrogenesis including, hyaluronic acid, tissue inhibitor of metalloproteinase-1 and the N-terminal propeptide for collagen type III.[24] The FT score uses an algorithm calculated from 6 serum markers, age and gender of the patient. Numerous studies have evaluated the performance of these tests in liver fibrosis. In a meta-analysis, ELF scores showed good performance for prediction of histological stage of fibrosis.[25]

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Table 1. Comparison of the performance of biological tests for fibrosis.

Biological tests	Sensitivity	Specificity	Ref
Fibrosis			
APRI* threshold 0.5	49%	84%	28
FIB-4* cut-off >3.25	16%	99%	29
FIB-4 cut-off <1.45	42%	83%	29
APRI >1.50	8%	98%	29
APRI <0.5	48%	72%	29
ELF* moderate	83%	73%	25
ELF severe	78%	76%	25
FT* (fibrotest)	61%	80%	30
Cirrhosis			
APRI threshold 1.0–1.5	54%	78%	28
APRI >2.0	0%	1%	29
APRI <1.0	35%	94%	29
ELF	80%	71%	25
FT	62%	91%	30

*APRI = (AST/ULN of AST)/(platelet count × 100). FIB-4 = (age × AST)/(platelet count × (ALT)1/2). Note: ULN of AST: 40 IU/L. ELF = Enhanced Liver Fibrosis score is calculated based on of tissue inhibitor of metalloproteinases 1 (TIMP-1), amino-terminal propeptide of type III procollagen (PIIINP) and hyaluronic acid (HA) levels.
FT = FibroTest consists of an algorithm of 5 fibrosis markers (alfa2-macroglobulin [g/L], apolipoproteinA1 [g/L], total bilirubin [μmol/L], haptoglobin [g/L], Gamma glutamyltransferase [IU/L]. AST, aspartate aminotransferase; ALT, alanine aminotransferase; ULN, upper limit of normal.

In a Spanish study, ELF was also found to be cost effective for liver fibrosis assessment in patients with ALD.[26] A study by Thiele *et al.* found that ELF and FT had comparable diagnostic accuracy in patients with ALD with an AUROC of 0.92 for ELF and an AUROC of 0.9 for FT.[27] This prospective study concluded that advanced fibrosis can be ruled out in patients with ALD based on an ELF <10.5 or an FT value below 0.58.[27] Comparison of the performance of the different biological tests suggests that ELF and FT are better than APRI (aspartate aminotransferase [AST] to platelet ratio index) or FIB-4 (Table 1).[25,28–30] A combination of serum-based fibrosis tests with non-invasive imaging or new biomarkers will likely emerge in future clinical studies to aid patient care.

Transient elastography

Diagnosis of fibrosis by transient elastography: general findings

Since the introduction of transient elastography (TE, Fibroscan®) in 2003,[31] LS measurement has become the best non-invasive parameter to screen for liver cirrhosis.[32,33] This success has largely been driven by the fact that a) no extensive training is required, b) TE is non-invasive and rapid and, c) TE is reproducible with a lower sampling error than liver biopsy.[34] Due to these promising findings other competing technologies are now available on the market such as acoustic radiation force impulse imaging (ARFI),[35] shear wave elastography (SWE)[36] and magnetic resonance elastography (MRE).[37] In particular, the latter holds great promise for three-dimensional assessment of stiffness in various organs not restricted to the liver. So far, most published LS studies have been performed with TE.

In general, LS is an excellent surrogate marker of advanced fibrosis (F3) and cirrhosis (F4) in ALD and superior to all serum markers.[38] LS strongly correlates with histological fibrosis stage, independently of the underlying liver disease (r >0.8).[34] During fibrosis progression, LS increases continuously from ca. 2 kPa up to 75 kPa (upper detection limit of the Fibroscan device). A threshold of 12.5 kPa is widely considered a cut-off value of histological cirrhosis, F4 stage, although cut-off values are aetiology dependent.[39] A normal LS measurement (<6 kPa) is thought to exclude liver pathology and liver fibrosis.[40] Finally, LS values strongly correlate with portal pressure and complications such as oesophageal varices and hepatocellular carcinoma and are likely at LS values >20 kPa.[34,41]

In addition, LS has been shown to be an excellent short- and long-term predictor of survival in various settings ranging from the emergency room up to treatment response in viral hepatitis[42–45] although data on LS as a long-term survival predictor in ALD are still not available. Interestingly, a decrease of LS either during a pharmacological intervention or transjugular intrahepatic portosystemic shunt seems to predict a favourable outcome.[43,46] Based on the relation of LS with portal hypertension, LS has also been evaluated as a prognostic tool to predict adverse events and clinical outcome including survival. Thus, patients with higher baseline LS values have worse clinical outcomes including decreased 5-year survival[47] and increased risk of developing hepatocellular carcinoma.[48,49] Longitudinal studies in patients undergoing treatment for chronic hepatitis B and C infection showed that patients were less likely to experience liver-related events if LS had decreased over time.[50,51] In fact, patients whose LS increased by more than 1 kPa/year presented a worse overall survival compared to patients whose LS remained unchanged or even decreased after hepatitis C treatment.[51]

Key point

Liver stiffness measurement, as measured by transient elastography, is an excellent surrogate marker of fibrosis and can act as a prognostic marker of clinical outcomes, including survival.

Confounders of LS other than cirrhosis

LS is affected by many (patho)physiological conditions that should be considered when assessing fibrosis stage (see Fig. 1).[34] Major confounders of LS elevation encompass hepatic necroinflammation,[52,53] congestion,[54] mechanic cholestasis,[55] alcohol[40] and food intake.[56] In addition, arterial[57] and portal pressure are independent factors of LS elevation.[58]

Based on these observations, an inflow/outflow model (see Fig. 1) has been developed recently that includes all known conditions that affect LS at the molecular, cellular and vascular level.[59] Accordingly, the hepatic outflow (bile flow, hepatic veins) and inflow (hepatic artery and portal vein) are important determinants of the sinusoidal

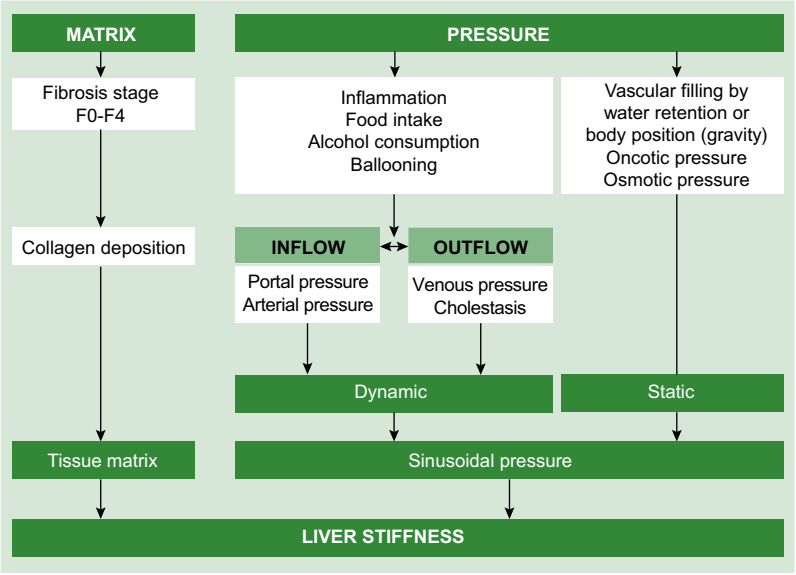

Fig. 1. Factors influencing liver stiffness measurement.

pressure ultimately underlying LS. Pharmacological modulation in rodent models of cirrhosis demonstrated a sophisticated effect of the inflow/outflow components on LS depending not only on central venous, arterial and portal pressure but also on heart rate.[46] While cardiac circulation is tightly connected to these components by dynamic pressure, static pressure also strongly affects LS through water retention resulting from hormonal, osmotic or albumin-related conditions.[59] Finally, although not all subcellular factors associated with LS have been clarified yet, it is well established that histological features such as fibrosis, ballooning and inflammation are associated with LS elevation.[60,61] The role of fat, which has been shown to lower LS in the absence or presence of inflammation, remains controversial.[60]

Elastographic assessment of fibrosis in ALD

In contrast to other common liver diseases such as viral hepatitis, the performance of LS in ALD was assessed rather late. An actual meta-analysis[62] has carefully analysed biopsy-proven studies on LS in patients with ALD based on various quality measures (Table 2). Early direct comparison with serum fibrosis markers showed a better performance of TE in patients with ALD[38] and AUROCs are typically >0.9 to detect F4 cirrhosis. Although an excellent performance was shown in all studies, the cut-off values used differed drastically, ranging from 11.5–25.8 kPa. Mueller *et al.* could then demonstrate that this was primarily related to the presence of inflammation as assessed by aminotransferase levels.[34] In this study, it was shown that LS decreases in patients with ALD during alcohol withdrawal.[34] The decrease of LS was best estimated based on AST levels. When only considering patients with low or normal aminotransferase levels, cut-off values were comparable to those observed in patients with viral hepatitis.[34] In addition, the diagnostic accuracy of LS could be improved when considering the AST levels. These data have also been confirmed by others[63] and resuming alcohol drinking was associated with an increased LS value.[64] In our present cohort of 365 patients undergoing alcohol withdrawal (Centre for Alcohol Research Heidelberg), the overall mean decrease of LS was 10%, which led to an underestimation of fibrosis stage in 27% of patients. In some patients, fibrosis stage changed by up to 3 degrees after alcohol withdrawal (unpublished). For these reasons, we require actual laboratory testing to correctly interpret LS values (see Fig. 2).

It is not yet completely clear why AST is best correlated with LS, however a recent multicentre study on both ALD and HCV confirmed the impact of AST on LS elevation.[65] Based on 677 patients with ALD and 1,391 with HCV, AST correlated best with LS in both diseases (HCV: r = 0.54, p <0.0001 and ALD: r = 0.34, p <0.0001) (Fig. 3). In the absence of elevated aminotransferases, cut-off values were almost identical between HCV and ALD for F1-2, F3 and F4 (HCV 5.1, 9.0 and 11.9 kPa *vs.* ALD 4.9, 8.1 and 10.5 kPa). These cut-off values increased exponentially as a function of median AST level. The impact of AST on LS was higher in lobular-pronounced ALD compared to portal tract-localised HCV. Most notably, Cohen's weighted Kappa displayed an improved agreement of the novel AST-dependent cut-off values with histological fibrosis stage both for HCV (0.68 *vs.* 0.65) and ALD (0.80 *vs.* 0.76).

In ALD, AST levels are typically higher than alanine aminotransferase (ALT) and in ca. 70% of patients the AST/ALT ratio is higher than 2.[66] However, AST levels higher than 300 IU/L are rarely detected. In cirrhotic stages, aminotransferases may normalise while AST levels may be continuously increased despite the absence of alcohol consumption.[65] Novel markers such as caspase-cleaved cytokeratin 18 fragments (M30) and M65 are more sensitive than aminotransferases and more specifically detect apoptotic cell death.[61] Notably, and in contrast to M65 and AST levels, M30 levels significantly increase during alcohol withdrawal, which highlights the specific role of apoptosis in ALD.[61]

Table 2. Liver stiffness and fibrosis stages in ALD (biopsy-proven studies).

Number of patients	Correlation	AUROC, F4	Cut-off, F4	Ref
174	0.70, p <0.0001	0.87	22.6	Nahon *et al.*, 2008
103	0.72, p <0.014	0.92	19.5	Nguyen-Khac *et al.*, 2008
45		0.97	25.8	Kim *et al.*, 2009
217	0.87, p <0.02	0.91	17.3	Boursier *et al.*, 2009
101	0.72, p <0.001	0.92	11.5	Mueller *et al.*, 2010
49		0.86	21.1	Janssens *et al.*, 2010
15		0.93	18.0	Fernandez *et al.*, 2015
199		0.94	16.9	Thiele *et al.*, 2016
217	0.73, p <0.0001	0.93	20.8	Voican *et al.*, 2017

AUROC, area under the receiver operating characteristic curve; ALD, alcohol-related liver disease.

Finally, in the aforementioned meta-analysis[62] of more than 1,000 patients, AST as well as bilirubin concentrations had a significant effect on LS. The presence of histological features of asymptomatic and non-severe alcoholic hepatitis was associated with increased LS (p <0.0001). In a multivariate analysis, AST (p <0.0001) and bilirubin (p = 0.0002) concentrations, and prothrombin activity (p = 0.01), were independently associated with the presence of histological features of asymptomatic and non-severe alcoholic hepatitis. It remains to be confirmed whether bilirubin levels really add to the overall performance of LS, since patients with ALD develop jaundice at end-stage cirrhosis, where LS is normally higher than 30 kPa and the status of cirrhosis is unquestionable. In contrast, patients with clinical alcoholic hepatitis may develop high levels of bilirubin in the absence of drastic LS elevation.

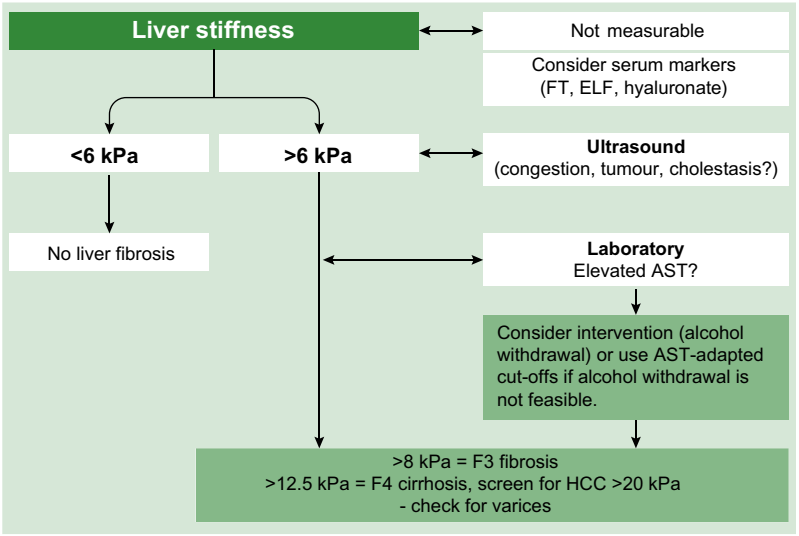

Fig. 2. Practical algorithm in patient with excessive alcohol consumption. AST, aspartate aminotransferase; HCC, hepatocellular carcinoma.

Transfer into clinical practice

Fibrosis assessment with TE in combination with ultrasound and alcohol withdrawal

Fig. 2 shows a typical interpretation of LS if ultrasound and laboratory testing is available. If ALD is suspected based on patient reporting, alongside clinical and/or laboratory signs, then TE is performed directly after the abdominal ultrasound and routine blood tests. Patients should be kept in a horizontal position for a minimum of 5 minutes to stabilise haemodynamics. During the ultrasound, liver size, spleen size, morphology, abnormalities such as congestion, cholestasis, morphological signs of cirrhosis, the presence of ascites and the diameter of the lower caval vein are assessed. TE is then performed either with the M probe or in cases of M probe failure, obvious obesity or ascites with the XL probe.[67,68] Ascites is no contraindication for the XL probe, which performs well in such cases.[67] If LS is elevated and patients have AST >100 U/ml, alcohol withdrawal for at least 2 weeks is recommended followed by a second LS measurement. In patients with LS >30 kPa, the diagnosis of cirrhosis is confirmed, irrespective of steatohepatitis as measured by elevated aminotransferase levels. At these levels, the development of ascites is very likely.

This approach enables definitive non-invasive assessment of fibrosis stage in ca. 95% of patients. Compared to conventional routine ultrasound, TE identifies twice as many patients with advanced fibrosis/cirrhosis (Mueller S, unpublished) and has a smaller sample error than histology (3–5% *vs.* 20–50%). In a recent French elastography screening study on more than 1,000 apparently healthy people older than 45 years, 7.5% had a pathologically increased liver stiffness >8 kPa, which in 36% of cases was eventually linked to ALD.[69] Therefore, it is anticipated that these novel non-invasive screening tools will improve the early recognition and follow-up of patients with ALD, the most common and unfortunately too often underestimated liver disease. Whether in addition AST-adapted cut-off values should be used *e.g.* for *ad hoc* decisions in patients with no time or option to withdraw from alcohol, remains a matter of debate.

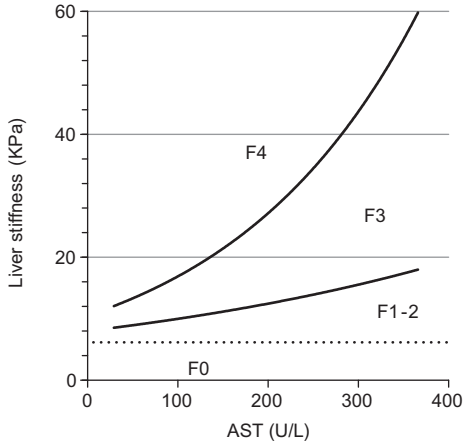

Fig. 3. Influence of AST elevation on liver stiffness measurement in ALD patients. ALD, alcohol-related liver disease; AST, aspartate aminotransferase.

Fibrosis assessment with TE using inflammation adapted cut-off values

We have recently developed an algorithm to avoid repetitive re-assessment of LS in patients with ALD and elevated AST levels (Fig. 3). In this multicentre study of more than 2,000 biopsy-proven patients with ALD and HCV, cut-off values for fibrosis increased exponentially as a function of median AST level.[65] While AST-adapted cut-off values allow an immediate assessment of fibrosis stage,

even in patients with pronounced steatohepatitis, and avoid overestimation of fibrosis stages, it remains unclear why AST is so strongly correlated with LS. Moreover, AST may not only be derived from hepatocytes but also myocytes and erythrocytes. It also remains to be studied whether indeed all patients with elevated AST levels will necessarily develop elevated LS.

LS follow-up in patients with ALD

LS measurement enables drinking activity and ALD progression to be monitored, as LS encompasses the sum of all pathological features from inflammation, ballooning to fibrosis. LS has been shown to improve, shortly after alcohol withdrawal, in more than 80% of patients.[40] Preliminary unpublished mortality data from a 10-year survey in Heidelberg indicate that LS predicts mortality independently of bilirubin and international normalized ratio.

Comparison of various elastographic techniques

Several studies have been performed to directly compare the performance of TE with ARFI or SWE, however, no robust data are available on ALD. Thus, ARFI had a similar predictive value as TE in both chronic hepatitis B and C.[70] In an Asian non-alcoholic steatohepatitis (NASH) population, AUROCs of TE, SWE, and ARFI were 0.757, 0.759, and 0.657 for significant fibrosis and 0.870, 0.809, and 0.873 for advanced fibrosis. Thus, these elastographic methods had similar diagnostic performance for staging fibrosis in patients with NAFLD.[71] Similar data were found in a large study of 349 consecutive patients with various chronic liver diseases who underwent liver biopsy and SWE, ARFI and TE.[72] Although larger trials are required to finally settle advantages and limitations of the various elastographic methods, they seem to perform similarly.

Key point

Larger trials are required to conclusively determine the comparative advantages and limitations of various elastographic methods, although their performances seem similar.

Non-invasive diagnosis of alcoholic hepatitis

Alcoholic hepatitis is a clinical syndrome characterised by the recent onset of jaundice in patients with ongoing alcohol abuse. Other signs of liver decompensation (*i.e.* ascites and/or encephalopathy) can be present, particularly in severe forms of the disease.[1] Underlying this clinical syndrome is steatohepatitis, a disease defined histologically by steatosis, hepatocyte ballooning and infiltrates by polymorphonuclear neutrophils.[73] Laboratory findings in patients with alcoholic hepatitis reveal neutrophilia, hyperbilirubinemia (>3 mg/dl), AST >50 IU/L (but rarely above 300 IU/L), with an AST/ALT ratio typically greater than 1.5–2. Diagnosis of alcoholic hepatitis is based on clinical and laboratory findings, and ideally confirmed by a transjugular liver biopsy. Liver biopsy is also useful to rule out other diagnoses and is of prognostic interest.[74] However, in routine clinical practice, liver biopsy is restricted by the lack of availability in some countries, the risk of the procedure, and the costs. Therefore, it appears reasonable that a liver biopsy should be mandatory when there is diagnostic uncertainty, and that stringent clinical and biological criteria should be applied in the absence of liver biopsy, in order to avoid misdiagnosis. Recently, the NIAAA Alcoholic Hepatitis consortia proposed a classification of alcoholic hepatitis diagnosis into 3 degrees of confidence:[75] *Definite alcoholic hepatitis*, clinically diagnosed and biopsy-proven; *Probable alcoholic hepatitis*, clinically diagnosed, and without confounding factors (including 1 or more of the following: presence of auto-antibodies, sepsis, shock, cocaine use, recent use of a drug with potential hepatotoxicity within 30 days, uncertain alcohol use assessment, atypical laboratory tests); *Possible alcoholic hepatitis*, clinically diagnosed but with 1 of the confounding factors listed before. We strongly recommend performing a liver biopsy in this last case to confirm the diagnosis of alcoholic hepatitis.

Very little attention has been given to patients with a non-severe form of the disease (*i.e.* Maddrey discriminant function [MDF] <32, see below), probably related to the low risk of death in the short term (1 month) in this group of patients. However, our group recently reported (in an abstract form) a 2-year mortality rate of 35% in patients with non-severe alcoholic hepatitis, which suggests that those patients should be identified and could benefit from specific therapies in the future, while alcohol abstinence is a key target to achieve in this population.

Prognosis and new biomarkers in ALD

Currently there are few prognostic markers or biomarkers in ALD.[76] However, there is a major need for biomarkers in ALD and in alcoholic hepatitis, particularly of diagnostic markers, indicators of response to therapy, prognostic markers, early indicators of inflammation and markers of liver regeneration.[77] Recent studies attempted to address these questions, however, larger studies validating emerging biomarkers have not yet been performed. Biomarker discovery focusses on easily accessible specimens such as circulating blood markers (serum, plasma, blood cell populations), urine, hair, saliva or stool.

Originally described prognostic calculators include the MDF, model for end-stage liver disease (MELD), ABIC (age, serum bilirubin, international normalized ratio, serum creatinine) and other less frequently used scoring models in alcoholic hepatitis.[78] An MDF >32 and MELD >20 are defined as severe alcoholic hepatitis.[75] However, a recent study suggests that combining data from scoring systems is better for predicting outcomes in alcoholic hepatitis.[79]

A recent study suggested that MELD performs better than MDF in alcoholic hepatitis.[79] However, new biomarkers are needed to better evaluate individual responses to steroids and other emerging therapies under investigation.

In severe alcoholic hepatitis, infection, sepsis and multi-organ dysfunction syndrome have been shown to correlate with poor survival.[80,81] Consistent with this, the Glasgow coma scale and Glasgow alcoholic hepatitis score (GAHS) may help to stratify patients and identify those who will benefit from corticosteroids in alcoholic hepatitis.[82] Close monitoring and supportive care in the intensive care setting are the most important factors in improving survival in acute alcoholic hepatitis. Current treatment in acute alcoholic hepatitis is prednisolone for 28 days. The Lille score is a commonly used and validated prognostic marker of response or lack of thereof to corticosteroid therapy.[83]

In the setting of chronic, otherwise stable, ALD or cirrhosis, acute alcohol binge has been identified as a major trigger of acute-on-chronic liver failure (ACLF).[84] ACLF is characterised by a rapid increase in bilirubin.[85,86] Acute alcoholic binge can also trigger ACLF in advanced liver disease of other aetiologies such as viral hepatitis.[87] It has been proposed that alcohol-related increased gut permeability, increased circulating endotoxin and induction of pro-inflammatory cytokines mediate the ACLF event.[88] Clinical prognosis of ACLF is poor with development of infection, sepsis and multi-organ failure. The CLIF-C ACLF score has been proposed as the best currently available predictor of mortality in patients with ACLF.[89]

The characteristic AST >ALT ratio greater than 1.5 is considered as a classic diagnostic biomarker in ALD and alcoholic hepatitis. However, increased aminotransferases do not distinguish between key components of the disease pathology such as hepatocyte damage and inflammation. Recent investigations focussed on circulating small non-coding RNA (miRNA) and long-non-coding RNAs (lncRNAs). Increased levels of serum miR-122 have been found in mouse models of ALD and in human patients.[90] Because miR-122 is a miRNA primarily found in hepatocytes, its increase in the serum is believed to represent hepatocyte damage. Indeed, circulating miR-122 is increased in many different forms of liver disease, including NASH, viral hepatitis and drug-induced liver injury. Therefore, it cannot serve as a disease-specific marker in ALD.[91] Another miRNA, miR-155 is a master regulator of inflammation. Increased levels of circulating miR-155 were found in a mouse model of ALD and both miR-155 and miR-122 were enriched in circulating exosomes.[92] Further analysis of exosome miRNA content revealed that miR-192 and miR-30a are also highly abundant in exosomes in mice with ALD and more importantly, in patients with alcoholic hepatitis compared to normal controls.[93] (Fig. 4). Unique lncRNAs were shown to be expressed in the serum and liver of patients with alcoholic cirrhosis.[94] Among those, AK128652 and AK054921 were the most abundantly increased lncRNAs in patients with alcoholic cirrhosis. Furthermore, in 480 prospectively followed patients, AK128652 and AK054921 inversely correlated with survival in patients with alcoholic cirrhosis.[94] Prospective analysis of these emerging diagnostic and prognostic biomarkers in larger patient populations is awaited.

Because of the presence of systemic increase in inflammation in alcoholic hepatitis, circulating cytokine levels have been evaluated as biomarkers.[95] Significantly increased circulating levels of IL-1β, TNF and IL-8 were found in severe alcoholic hepatitis in several independent studies.[96] However, it remains to be evaluated whether the dynamics of increases/decreases in specific cytokines have potential as disease-specific or prognostic biomarkers. Cytokeratins have emerged as markers of liver, particularly hepatocyte, damage. Mallory bodies, the hallmark of alcoholic hepatitis, contain cytokeratin-18 (CK-18) and cytokeratin-19 (CK-19).[97] Serum levels of CK-18 and CK-19 were increased in patients with alcoholic hepatitis compared to fatty liver or controls.[97] In a recent study, Bissionette *et al.* reported higher levels of total and microvesicle-bound M65 and M30, circulating fragments of CK-18 in the circulation in biopsy-proven alcoholic hepatitis.[98] A cut-off of 2,000 IU/L for M65 has a positive predictive value of 91% and a cut-off of 642 IU/L has a negative predictive value of 88%.[98] It should be noted that CK-18 fragments are markers of hepatocyte apoptosis, thus, are not specific for alcoholic hepatitis.[99] Furthermore, high levels of CK-18 were reported in non-alcoholic hepatitis and toxin-related steatohepatitis.[100,101]

Preclinical studies in mouse models of ALD applied proteomic (MALDI-TOF) analysis of livers and identified a cluster of differentially expressed proteins associated with ALD.[102] Interestingly, most of these proteins were also increased in NASH livers and only ornithine aminotransferase, vitamin D binding protein and phosphatidylethanolamine-binding protein were higher in ALD compared to NASH and normal controls.[102] In a mouse model of ALD, proteomic analysis of circulating extracellular vesicles (EVs) revealed a unique cluster or EV-associated proteins that were increased compared to control EVs.[103] Among these was heat-shock protein-90 (hsp90) that was also associated with a biological effect in macrophages after exosome transfer both *in vitro* and *in vivo,* indicating both the biomarker potential of EV-associated proteins as well as the potential role of EVs in cell-to-cell communication.[103] In addition to serum-based biomarkers, new approaches include identification of urine-based biomarkers by targeting urinary metabolomics.[104]

The clinical prognosis in alcoholic cirrhosis is affected by ongoing alcohol use.[105] While early

Key point

A number of miRNAs have been found to associated with ALD and could prove to be useful diagnostic or prognostic markers in the future.

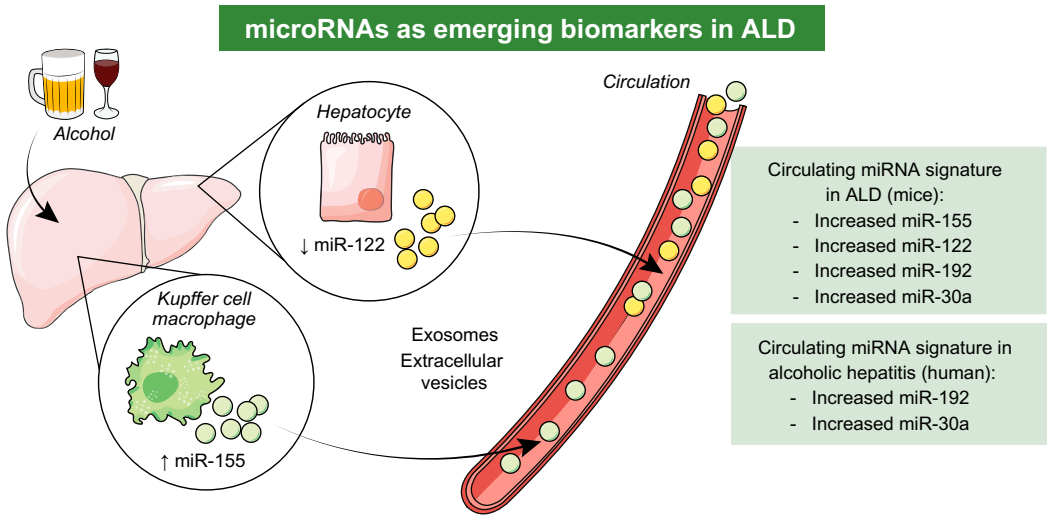

Fig. 4. MicroRNAs as emerging biomarkers in ALD. In ALD, miR-155 expression is increased in the liver in hepatocytes and particularly in Kupffer cells and macrophages. Hepatocyte expression of miR-122 is reduced in ALD in mice. These changes in the liver occur while circulating levels of several miRNAs increase including miR-122, miR-155, miR-192 and miR-30a. Most of these miRNAs a found in the circulation packaged into exosomes and/or extracellular vesicles. ALD, alcohol-related liver disease; miRNAs, small non-coding RNAs.

survival in alcoholic hepatitis at 28 and 90 days is largely determined by liver-related causes, infection and organ failure, longer-term survival is related to non-hepatic causes including a return to alcohol use.[105] Cessation of alcohol use can improve long-term survival while continued alcohol use accelerates decompensation in alcoholic cirrhosis and increases other causes of death related to trauma and alcohol use. In the alcoholic patient, alcohol cessation is a key therapeutic goal and also a determinant of long-term survival. Thus, biomarkers of alcohol use are useful in the management of alcoholic hepatitis and alcoholic cirrhosis, particularly in the pre- and post-transplant settings.[106]

Conclusions and future directions

While the topics of non-invasive diagnosis and biomarkers in ALD have been the focus of many clinical and translational research studies, reliable and validated approaches that are practical in general practice are still awaited. Many issues still need to be addressed in the application of elastographic techniques to patients with ALD. These include better comparison of various elastographic techniques, clarification of inflammation-induced LS and its molecular basis, defining inflammation markers other than AST to prevent overestimation of LS in the presence of steatohepatitis, data on LS as a long-term survival predictor in patients with ALD, and clarification of the role of fat on LS in patients with ALD.

There is a continued need for studies with large and clinically well-characterised patient populations to address the above list of highly clinically relevant questions. Consortium-based projects within the EU and United States, as well as international collaborations will help to assess larger sets of data and diverse patient populations.

Conflict of interest

Christophe Moreno and Gyongyi Szabo have no conflict of interest, Sebastian Mueller is adviser for Echosens.

Please refer to the accompanying ICMJE disclosure forms for further details.

Authors' contributions

All authors contributed equally to the production of this manuscript.

Supplementary data

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