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TOPIC HIGHLIGHT

WJG 20th Anniversary Special Issues (10): Alcoholic liver disease

Non-invasive diagnosis of alcoholic liver disease

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Abstract

Alcoholic liver disease (ALD) is the most common liver disease in the Western world. For many reasons, it is underestimated and underdiagnosed. An early diagnosis is absolutely essential since it (1) helps to identify patients at genetic risk for ALD; (2) can trigger efficient abstinence namely in non-addicted patients; and (3) initiate screening programs to prevent life-threatening complications such as bleeding from varices, spontaneous bacterial peritonitis or hepatocellular cancer. The two major end points of ALD are alcoholic liver cirrhosis and the rare and clinically-defined alcoholic hepatitis (AH). The prediction and early diagnosis of both entities is still insufficiently solved and usually relies on a combination of laboratory, clinical and imaging findings. It is not widely conceived that conventional screening tools for ALD such as ultrasound imaging or routine laboratory testing can easily overlook ca. 40% of manifest alcoholic liver cirrhosis. Non-invasive methods such as transient elastography (Fibroscan), acoustic radiation force impulse imaging or shear wave elastography have significantly improved the early diagnosis of alcoholic cirrhosis. Present algorithms allow either the exclusion or the exact definition of advanced fibrosis stages in ca. 95% of patients. The correct interpretation of liver stiffness requires a timely abdominal ultrasound and actual transaminase levels. Other non-invasive methods such as controlled attenuation parameter, serum levels of M30 or M65, susceptometry or breath tests are under current evaluation to assess the degree of steatosis, apoptosis and iron overload in these patients. Liver biopsy still remains an important option to rule out comorbidities and to confirm the prognosis namely for patients with AH.

Key words: Alcoholic hepatitis; Alcoholic steatohepatitis; Alcoholic liver disease; Non-invasive; Liver stiffness; Serum marker; Steatosis

Core tip: This review article summarizes recent advantages in non-invasive assessment of patients with alcoholic liver disease (ALD) such as elastographic techniques (Fibroscan), acoustic radiation force impulse imaging, shear wave elastography or serum marker and highlights future perspectives which may improve the early diagnosis of ALD.

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EPIDEMIOLOGY OF ALCOHOLIC LIVER DISEASE

Alcoholic liver disease (ALD) is, either alone or in association with other comorbidities such as obesity or viral hepatitis, the leading cause of liver disease. The liver is

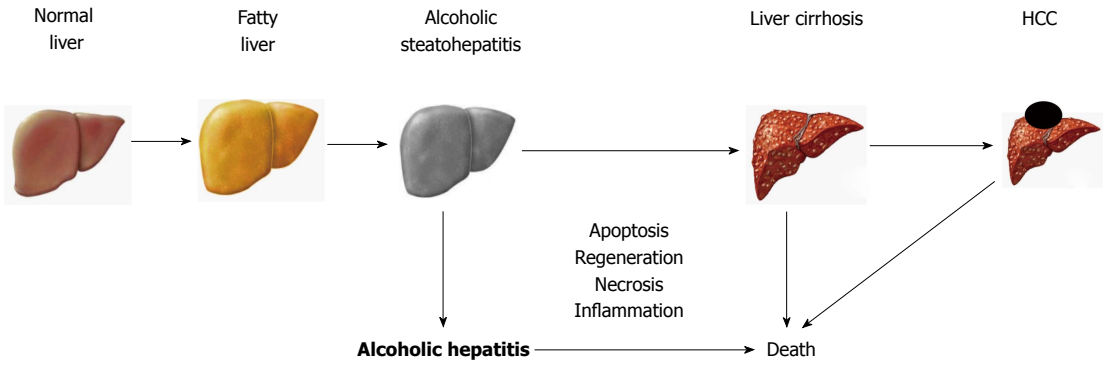

Figure 1 Natural course of alcoholic liver disease and major end points. HCC: Hepatocellular carcinoma.

also the most common target organ of chronic alcohol abuse. In Germany and the United States, chronic alcohol consumption is responsible for over 50% of chronic liver diseases[1]. In South Korea, 7%-31% of cirrhosis cases have been addressed to alcohol in a few single center studies[2]. The treatment of ALD causes huge costs for the health care system with nearly $3 billion per year[3]. Considering the sum of death and disability-adjusted life years (DALYs), in Portugal, liver diseases represented the main source of the burden attributable to alcohol with 31.5% of total DALYs, followed by traffic accidents and several types of cancer[4]. At present, China recorded a 40% increase in the annual per capita consumption of alcohol depending on the region and has therefore experienced the highest increase in alcohol associated health problems[5].

It is difficult to calculate alcohol related deaths because of imprecise or incomplete information about the actual drinking patterns. Moreover, patients with compensated liver cirrhosis have normal laboratory and ultrasound findings in ca. Forty percent and may often die by seemingly non-liver-related complications such as infections (*e.g.*, pneumonia). The consumption of 20 and 30 g of alcohol per day for women and men enhances the risk of developing ALD, respectively. Liver cirrhosis develops in a minority of ca. Fifteen percent of people consuming more than 80 g of ethanol daily[3] clearly indicating the importance of additional genetic factors for disease progression. Approximately 5% of the whole population show high risk drinking behavior in the United States[6] and Germany[7] and similar ca. Seven percent showed heavy alcohol consumption according to the Korean National Health and Nutrition Examination survey 2009[8]. In the global death statistics published in 2010, liver cirrhosis and hepatocellular carcinoma (HCC) are ranked at position 12 and 16[9] with one third directly attributable to alcohol. Liver cirrhosis accounts for over 170000 deaths per year in Europe and is in the fourth place in the so-called years of life lost statistics. In ALD patients with cirrhosis, HCC is the most common fatal complication ranking straight behind viral hepatitis B and C. Furthermore, in a global perspective HCC has the second highest cancer incidence rate after kidney tumors[10].

GENERAL DIAGNOSTIC ASPECTS OF ALD

The early and exact diagnosis of ALD and namely of fibrosis/cirrhosis is important since patients receive an explanation for their symptoms and complaints and get the opportunity to control disease progression through change of life style, avoidance of alcohol and other potentially harmful factors such as obesity. Furthermore, ALD should be separated from other comorbidities (*e.g.*, viral hepatitis) or disease modifying factors (*e.g.*, obesity, drugs) to provide detailed prognostic information. After diagnosis, a targeted search for potential complications such as varices or HCC can be started and surveillance intervals *e.g.*, for HCC can be defined. The diagnosis of ALD is complicated by a rather varied clinical presentation, underreporting by patients and the lack of good biomarkers for alcohol consumption. It is therefore routinely underestimated both by physicians and health statistics[11,12]. Therefore, its diagnosis has to rely on a combination of imaging, laboratory, clinical and elastographic findings.

The early detection of severe steatohepatitis and alcoholic cirrhosis is most important for several reasons: it safes lifes, prevents complications and may initiate follow up programs (Figure 1). Most critical and life threatening end points are (1) decompensated alcoholic liver cirrhosis; and (2) the rare and clinically defined alcoholic hepatitis (AH). AH should not be mismatched with the commonly and histologically detectable steatohepatitis (Figure 1). AH patients classically show not very high transaminase levels but rapidly become icteric[13]. Due to the jaundice they are rapidly diagnosed and presented to more specialized units. Nevertheless they show a poor prognosis usually assessed by the Maddrey discrimination function[14], the Glasgow ASH score[15] or the Lille model[16]. The nature of AH is still poorly understood. In contrast, the slow progression of ALD towards liver cirrhosis can be unnoticed for many years. For these reasons, patients who are sensitive to alcohol-mediated liver damage but diagnosed too late may have an unfavourable outcome. These patients are listed late for transplantation and are at high risk of dying from complications while waiting for a transplant.

Table 1 Diagnosis of confirmed advanced fibrosis/cirrhosis (F3-4) (histology, elastography) by conventional clinical parameters (ultrasound, laboratory) from Salem Medical Center (n = 364)

Parameter	Pathologic	F0-2 elevated	F3-4 normal
Bilirubin	> 1.3 mg/dL	7.6%	58.3%
INR	> 1.27	1.2%	74.8%
Platelets	< 150 /nL	18.5%	49.6%
Spleen size	> 11.5 cm	6.4%	70.4%
Signs of liver cirrhosis	> 0	1.6%	59.1%
Bilirubin, INR, signs of liver cirrhosis	> 0	10.0%	43.5%
Bilirubin, INR, platelets, spleen size, signs of liver cirrhosis	> 0	27.7%	22.6%

The table shows typical findings of fibrosis/cirrhosis as indicated in the ultrasound (spleen size, signs of cirrhosis) or laboratory [bilirubin, international normalized ratio (INR) and platelets] in patients with no or low fibrosis stages (F0-2) *vs* advanced fibrosis stages (F3-4). In the last two rows, cirrhosis was considered if one of 3 or 5 parameters was pathologic. As an example, ca. 43% of F3-4 cirrhosis are not diagnosed by a combination of bilirubin, INR and ultrasound signs of liver cirrhosis.

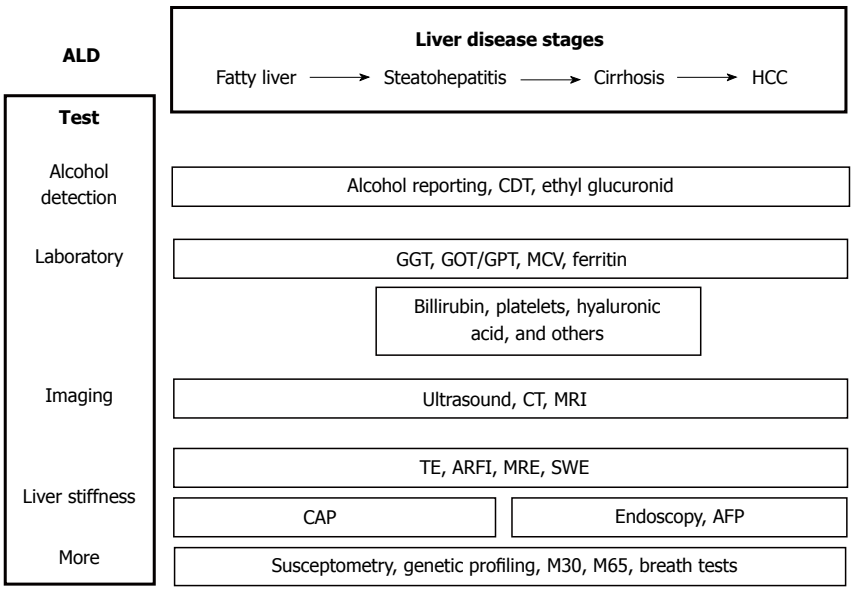

Figure 2 General non-invasive approaches for patients with suspected alcoholic liver disease. Combination of different tests will help to establish alcohol as underlying reason and to assess the stage of liver disease. ALD: Alcoholic liver disease; HCC: Hepatocellular carcinoma; CDT: Carbohydrate deficient transferrin; MCV: Mean corpuscular volume; CT: Computed tomography; MRI: Magnetic resonance imaging; TE: Transient elastography; ARFI: Acoustic radiation force impulse imaging elastography (Siemens); CAP: Controlled attenuation parameter (Echosens); MRE: Magnetic resonance elastography; SWE: Shear wave elastography (Supersonic imaging); GGT: γ-glutamyl transpeptadase; GOT: Glutamic-oxal(o)acetic transaminase; GPT: Glutamate pyruvate transaminase; AFP: α-fetoprotein.

The increasing use of transient elastography (TE) as novel ultrasound-based technique has significantly improved early diagnosis and follow up. It is a widespread misconception that conventional approaches such as routine imaging studies or blood tests are able to rule out fibrosis/cirrhosis. Our experience on over 364 patients at Salem Medical Center in Heidelberg indicates that approximately 40% of manifest cirrhosis is overlooked by routine ultrasound and lab tests which are clearly seen with elastography or histology (Table 1). Thus, 22.6% with established F3-4 cirrhosis by histology or elastography have normal bilirubin, INR, platelets, spleen size and no signs of liver cirrhosis. If only ultrasound, bilirubin and INR are considered, 43.5% are normal. Although no long-term prognostic studies have been performed on cirrhotic patients solely identified by elastography, they are certainly at an increased risk of developing HCC or complications of portal hypertension.

In addition, many other non-invasive approaches to detect various stages of ALD are currently under investigation, such as controlled attenuation parameter (CAP) for fatty liver disease, susceptometry to detect cancerogenic hepatic iron accumulation or serum markers of liver damage or apoptosis such as M65 and M30 (Figure 2). Of course, modern imaging techniques are absolutely essential for HCC screening and are continuously improved. Finally, genetic tests *e.g.*, for *PNPLA3* mutations are potential options in the near future since such mutations are increasingly recognized as risk factors for cirrhosis progression.

CLINICAL APPROACH TO ALD

The diagnosis of ALD has first to establish the consumption of alcohol as cause of the liver disease. Beside serum alcohol concentration measurements as indicator for alcohol consumption within the last 20 h, no serologic marker can be used to monitor chronic alcohol consumption on its own. Ethyl glucuronide levels in the

Table 2 Fibrosis stages of alcoholic liver disease patients undergoing alcohol detoxication as determined by liver biopsy *n* (%)

Fibrosis stage	Histology	TE	All
F0	5	162	167 (45.9)
F1-2	46	36	82 (22.5)
F3	17	26	43 (11.8)
F4	21	51	72 (19.8)
Total	89	275	364

Preliminary data from Salem Medical Center (n = 364). TE: Transient elastography.

Table 3 Relative distribution of histological features in alcoholic liver disease patients

Kleiner score (range)	Considered elevated	Percentage
Kleiner steatosis 0-3	> 1	69.4%
Lobular inflammation 0-3	> 1	38.8%
Portal inflammation 0-1	> 0	15.3%
Ballooning 0-2	> 1	15.3%
Megamitochondria 0-1	> 0	1.6%
Mallory hyaline 0-1	> 0	25.9%
Classification steatohepatitis 0-2	> 0	75.3%

Preliminary data from Salem Medical Center (n = 89).

urine (up to 3 d) and, more widely, carbohydrate deficient transferrin (CDT) are being used to detect alcohol consumed previously (4 -21 d). CDT is only a reliable marker if more than 50 g alcohol are consumed per day and even then shows a moderate sensitivity of 60%. A rather new and longer tracking of alcohol consumption is provided by determination of ethyl glucuronide in the hair, which is especially useful in the transplant setting[17]. Next, the pathologic stages of ALD should be ascertained such as steatosis, steatohepatitis, fibrosis/cirrhosis (Figure 2)[11]. Sometimes, the diagnosis of ALD is not so obvious because alcohol consequences may manifest for example in the brain (Wernicke Korsakov Syndrome), in the peripheral nerves (polyneuropathy) or as alcoholic cardiomyopathy. Therefore, they may need a more extended clinical view on the patient symptoms from experienced physicians. Along with rib fractures commonly seen on X ray images, other clinical symptoms such as parotid enlargement, Dupuytren's contracture, and clinical findings are highly associated with ALD.

HISTOLOGY AND ALD

Liver biopsy is still considered the gold standard for assessment of fibrosis/cirrhosis particular in the context of ALD. This is especially the case when in doubt or when non-invasive tests are unreliable. Liver biopsy can be done percutaneous, transjugular or laparoscopic with the latter having probably the safest risk profile. In ALD patients with severe steatohepatitis or AH, which require certain medication, *e.g.*, corticosteroids and/or pentoxifylline or in patients with suspected comorbidities such as HCV or NASH, biopsies are highly indicated. Furthermore, liver biopsy may be necessary to establish the nature of hepatic lesions.

In the daily clinical routine, however, liver biopsy is often limited in ALD patients due to technical requirements (cylinder size larger than 15 mm), inter-observer variability and sampling errors with regard to fibrosis staging which can reach 30%[18-22] or mild (pain and small bleedings in 6%) or severe complications (fatal perforations and bleedings in 0.1%)[23,24]. Because of newly introduced elastographic techniques, liver biopsy should no longer be regularly performed to quantitate fibrosis stage or steatosis except in complex cases or studies. Elementary histological features in ALD include steatosis, with macro- and micro-vesicles, hepatocellular ballooning, inflammatory infiltrates (neutrophils) that predominate in the lobules and variable degrees of fibrosis including pericellular fibrosis and lobular distortion perhaps progressing to cirrhosis[25].

Patients symptoms may range from a single lesion or combinations of elementary lesions[26,27]. Until today, the prevalence and distribution of histological lesions among heavy drinkers is not well known. Naveau *et al*[28] showed in a large study of 1604 patients diagnosed with ALD undergoing liver biopsy that 14% of patients had normal liver, 28% steatosis without fibrosis, 20% presented with fibrosis (with or without steatosis), 8.5% with acute AH, and 29% indicated cirrhosis. Table 2 shows number and percentage of fibrosis stages of the Heidelberg cohort of patients undergoing alcohol detoxification. In the biopsy-proven group (n = 89), ca. 30% were F3-4 while almost no one was F0. In patients staged by transient elastography after alcohol withdrawal (n = 275) 60.7% were classified as F0 and 41.8% were F3-4. These data indicate that biopsy-proven studies are naturally biased missing many patients without liver cirrhosis. Table 3 shows the distribution of histological features in the biopsy proven cohort. More than 70% showed steatosis and 75.3% steatohepatitis.

Steatosis represents the early phase of ALD and is most frequently seen in injured livers[29]. Nevertheless, it is still not clear whether simple steatosis is a benign condition, a prerequisite for further progression towards steatohepatitis or even a compensatory protective reaction. Alcoholic steatohepatitis (ASH) is characterized by steatosis in combination with hepatocyte ballooning, hepatocellular damage and tissue inflammation represented by infiltrates of polymorphonuclear cells[12]. Among ASH, steatosis and the extent of fibrosis, ASH demonstrated the highest risk for cirrhosis development in at least 40% of cases[30-35]. The assessment of the fibrosis degree should be performed by special techniques, such as trichrome or Sirius red staining. Reticulin is commonly used to assess the extent of fibrosis and liver architecture in parallel. Despite missing validation in the setting of ALD, semi-quantitative methods such as the Metavir scale are also used. The Kleiner-Brunt score, originally developed for NAFLD, has been recently used

Table 4 Typical routine blood tests in alcoholic liver disease

Parameter	Pathologic	F0-2 (< 8 kPa)	F3-4 (> 8 kPA)	Mean F0-2	Mean F3-4
GOT (U/L)	> 50	56.9%	78.4%	89.5	135.4
GPT (U/L)	> 50	50.6%	48.6%	75.0	70.6
GGT (U/L)	> 60	69.4%	97.3%	238.8	792.8
AP (U/L)	> 130	9.0%	50.5%	90.2	152.0
Bilirubin total (mg/dL)	> 1.3	7.8%	43.2%	0.8	2.9
INR	> 1.27	0.8%	25.9%	1.2	1.1
Platelets (/nL)	< 150	17.5%	48.6%	222.5	172.0
Ferritin (ng/mL)	> 1000	15.6%	35.4%	524.7	830.7
Ferritin (ng/mL)	> 400	39.8%	60.4%	524.7	830.7
Triglycerides (mg/dL)	> 200	29.4%	25.7%	187.9	206.5
Cholesterine (mg/dL)	> 200	65.5%	52.8%	225.7	202.8
Albumin (g/dL)	< 3.8	4.3%	37.3%	5.4	5.2
Transferrin (g/A)	< 2	14.8%	38.6%	2.5	2.2
Transferrin saturation	> 45%	32.0%	45.9%	42.0	48.0
Hepatic steatosis (US)	> 1	70.9%	82.4%	1.9	2.2
Spleen size (cm)	> 11.5	7.5%	35.5%	9.6	11.1
Ascites	> 0	0.0%	20.7%	0.0	0.2
Signs of cirrhosis (US)	> 0	1.7%	43.9%	0.0	0.4
Liver stiffness (final)	> 8	0.0%	100.0%	4.8	32.7
CAP (dB/m)	> 300	42.2%	64.9%	287.0	308.3

The table shows percentage and means of pathologic routine blood tests in patients with alcoholic liver disease (ALD) (preliminary data from Salem Medical Center n = 275) solely characterized by non-invasive transient elastography (TE). US: Ultrasonography; CAP: Controlled attenuation parameter (Echosens); GGT: γ-glutamyl transpeptadase; GOT: Glutamic-oxal(o)acetic transaminase; GPT: Glutamate pyruvate transaminase; AFP: α-fetoprotein; AP: Alkaline phosphatase.

in ALD studies since NAFLD and ALD show common features if not to the same extent[36,37].

NON-INVASIVE DIAGNOSIS OF ALCOHOLIC STEATOSIS

Early screening for steatosis can be carried out using ultrasonography (US), computed tomography (CT) and magnetic resonance imaging (MRI)[38]. Among those methods, hepatic steatosis assessment *via* US, especially in patients below 30% fat deposition has poor analytical sensitivity and specificity. MRI and MR technique are the imaging tools of choice allowing for accurate steatosis assessment but limited by the lack of established standardization of sequence characteristics and their high cost[39,40]. Recent ultrasound based techniques such as CAP are promising. CAP is run on the Fibroscan platform and so far restricted to the M probe. CAP is reproducible and quantitative with an AUROC up to 90% for fatty liver[41]. However, the histological validation of alcoholic steatosis and CAP in large studies with ALD patients is still pending. More details are given below. In clinical practice, US can be proposed in heavy drinkers as a screening procedure for steatosis[42].

NON-INVASIVE DIAGNOSIS OF ALD BY BLOOD TESTS

ALD patients are represented by characteristic laboratory findings depending on the stage of liver disease. One of the best marker in ALD is elevated γ-glutamyl transpeptadase (GGT) representing an induction of enzymatic activity with a combined sensitivity and specificity of > 70%[43,44]. In addition, GGT activity is not very specific for alcohol intoxication and can be also caused by other conditions namely cholestastic liver disease, cardiac insufficiency, drugs and many more. Furthermore, serum GGT looses its alcohol specificity in more advanced stages[12,45]. Glutamic-oxal(o)acetic transaminase (GOT) is typically elevated in severe AH while GOT levels > 300 U/L are rarely detected. In about 70% of patients with non-viral liver disorders, the GOT/glutamate pyruvate transaminase (GPT) ratio is higher than two[46]. In cirrhotic stages, transaminases may normalize and GOT levels are slightly continuously increased in the absence of alcohol consumption. The blood test also shows alterations in patients with ALD, such as an increased mean cell volume (MCV) being equally sensitive as elevated transaminases, low numbers of platelets as indicator for cirrhosis or elevated leukocytes as marker for acute alcoholic steatohepatitis[47]. The combination of GGT, MCV, IgA, CDT, and GOT/GPT ratio increases the diagnostic accuracy for ALD with a sensitivity and specificity > 90%[48]. Elevated direct bilirubin levels are also increasingly noted in ALD patients either due to cirrhosis or severe steatohepatitis[48]. Table 4 illustrates typical routine blood tests together with some common ultrasound parameters in patients with ALD from our Heidelberg cohort (n = 364). The fibrosis profile of this population for F0, F1-2, F3 and F4 cirrhosis is 61.7%, 10%, 10% and 18.1%. Changes in iron metabolisms and iron related proteins may be also detected and can be easily mixed up with *e.g.*, hereditary hemochromatosis. In the Heidelberg population, serum ferritin levels are above normal (> 400 ng/mL) in 37% and higher than 1000 ng/mL in 16% (see also Table 4). Transferrin saturation is also often elevated (> 45%) in 36% and > 60% in more than ca. 20% of patients which indicates that transferrin saturation is not indicative for hereditary iron overload (see also Table 4). Therefore, alcohol withdrawal for at least four weeks is recommended since liver iron parameters will change slowly.

NON-INVASIVE DIAGNOSIS OF ALCOHOLIC LIVER FIBROSIS/CIRRHOSIS

Hepatic imaging techniques

US, MRI and CT may allow the assessment of steatosis or more advanced stages, help to exclude other causes of chronic liver disease and its complications independent of the etiology[49]. Imaging techniques could help to exclude other causes of abnormal liver tests, such as obstructive cholestasis, infiltrative or neoplastic liver diseases. With respect to fibrosis assessment, all imaging techniques have to rely on so called sure morphological signs of cirrhosis such as nodular aspects of the liver or recanalization of the umbilical vein while splenomegaly or ascites are not specific. Despite high diagnostic accuracy for the detection of ALD under study conditions,

Table 5 Serum fibrosis markers in alcoholic liver disease

Serum marker	*n*	Outcome	Ref.
PⅢNP	44	Correlation of PⅢNP with fibrosis, but not inflammation or steatosis; PⅢNP also positively correlated with ALP and GGT	Gabrielli *et al*[106], 1989
ApoA1	482	Correlation with fibrosis (r = -0.70; $P \leq 0.001$)	Bedossa *et al*[107], 1989
PGA (GGT PT ApoA1) PT	624	AUROC not significant	Poynard *et al*[108], 1991
PⅢNP, Laminin	44	PⅢNP, PⅢNP-Fab and laminin measured by RIA were 21 ± 19 μg/L, 90 ± 42 μg/L and 2.5 ± 0.8 U/mL in alcoholic cirrhosis	Lotterer *et al*[109], 1992
PⅢNP Type I col	69	Correlation PⅢNP and score of alcoholic hepatitis (r = 0.60, $P \leq 0.0001$) Correlation type I collagen and fibrosis score (r = 0.34, $P \leq 0.001$)	Trinchet *et al*[110], 1992
TIMP1, PⅢNP	44	Correlation TIMP1 and fibrosis (r = 0.70, $P < 0.001$ AUROC 0.96 ± 0.03)	Li *et al*[111], 1994
CDT	74	Sensitivity of CDT for alcohol consumption 57% with 100% specificity	Seitz *et al*[112], 1995
HA, PⅢNP	45	AUROC for PⅢNP 0.867 ± 0.054	Pares *et al*[55], 1996
7S-Ⅳ col, TH-Ⅳ col, Laminin, TIMP	58	TH-Ⅳ concentration as best marker to distinguish ALD from non-ALD; good correlation between hepatic type V collagen and serum TH-Ⅳ, but not 7S-Ⅳ collagen; TIMP, may be useful in evaluating the degree of hepatic fibrosis	Tsutsumi *et al*[113], 1996
HA PT	160	Accuracy for cirrhosis diagnosis from 89.5% to 95%	Oberti *et al*[114], 1997
YKL-40	20	YKL-40 was significantly increased in patients with alcoholic cirrhosis (median, 523 μg/L; $P < 0.001$)	Johansen *et al*[115], 1997
YKL-40, HA, PGA, Tran index	146	Threshold of 330 μg/L gave sensitivity of 50.8% with specificity 88.5% Correlation HA and prothrombin index	Tran *et al*[116,117], 2000
HA	70	Significant correlation ($P < 0.01$) between HA and albumin, platelets and bilirubin, but not with ALT	Plevris *et al*[118], 2000
TPS	77	TPS correlated significantly with liver cell necrosis and Mallory's hyaline degeneration	González-Quintela *et al*[119], 2000
Laminin Type-Ⅳ col	80	Cut-off for Laminin 4.1 UI/mL gave 90% sensitivity, 77% specificity, cut-off for CⅣ 150 ng/mL gave 89% sensitivity 77% specificity	Castera *et al*[120], 2000
Type-VI col	61	CⅥ and CⅩⅣ as sensitive marker in fibrosis progression in alcoholics	Stickel *et al*[121], 2001
Type-ⅩⅣ col	13		
PT	243	Correlation PT and fibrosis score; r = -0.70, $P < 0.0001$	Croquet *et al*[122], 2002
YKL-40, PⅢNP	370	Serum levels of YKL-40 and PⅢNP are elevated in alcoholic patients; related to fibrosis	Nøjgaard *et al*[123], 2003
HA	87	Correlation HA and histological stage of ALD (r = 0.54, $P < 0.0001$); AUROC for HA and fibrosis 0.76	Stickel *et al*[124], 2003
ELF panel	64	AUROC 0.94 ± 0.056	Rosenberg *et al*[125], 2004

ALD: Alcoholic liver disease.

imaging techniques are especially limited in the daily routine in diagnosing compensated liver cirrhosis (sensitivity < 70%) (see also Table 3). Conventional grey scale US is one imaging modality in screening for liver cirrhosis and relies on liver parenchyma abnormalities and morphological changes. Colour Doppler US provides further information on haemodynamics of portal venous system, the hepatic artery and the hepatic veins, but the reliability and reproducibility are limitations for its daily usage as screening tool[50]. US findings can be considered to confirm cirrhotic livers but a negative result cannot fully rule out cirrhosis. Although acoustic structure quantification is a promising new ultrasound software program which provides encouraging results in the diagnosis of cirrhosis/fibrosis, it has to date not attained the same diagnostic performance as Fibroscan[51]. The diagnostic accuracies for cirrhosis detection using MRI and CT were reported with 70% and 67% with sensitivities and specificities of 87%, 84%, 52% and 54%, respectively[52].

Serum marker

In the last decades, serum markers have been intensively studied to assess fibrosis and inflammation. Table 5 shows important serum fibrosis markers and their outcome in ALD studies. So-called indirect markers correlate with the hepatic function, but not directly with the deposition of extracellular matrix. Indirect markers are *e.g.*, platelet count, parameter of liver synthesis, such as INR or albumin and transaminase levels. In contrast, direct markers are tightly associated with matrix deposition, the key feature of liver cirrhosis. Examples of such markers are hyaluronic acid, procollagen Type I and Ⅲ and TIMP1. Some more complex systems combine direct and indirect markers (see Table 5).

Four fibrosis serum marker systems have been extensively studied: FibroTest/FibroSure, Hepascore, FibroSpect and the ELF test ("European Liver Fibrosis Study Group panel"). The ideal serum marker should be specific, non-invasive, reproducible, be correlated with disease severity and prognosis and unaffected by drugs and other (metabolic) conditions. Today available markers do not meet all of these requirements because they are not liver-specific, may represent impaired hepatic clearance or are affected by inflammation rather than fibrosis stage. Some liver disease specific markers *e.g.*, the APRI score is widely used in viral hepatitis but useless in ALD[53].

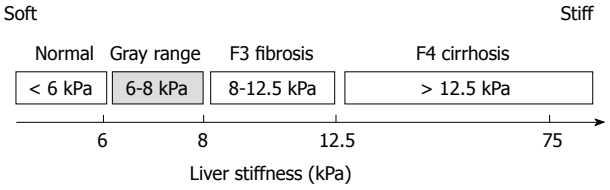

Figure 3 Liver stiffness scale with cut-off values for various fibrosis stages in alcoholic liver disease patients without pronounced inflammation, congestion, tumors or mechanic cholestasis.

In principle, serum markers allow a good differentiation between F0-1 and F2-4 and no special equipment is required. For some patented tests (*e.g.*, Fibrotest), however, serum needs to be sent to special institutions and the real algorithm (exclusion criteria) cannot be validated. Fibrotest has been evaluated in ALD and has reached a diagnostic accuracy of 0.8[54]. Other markers include the ELF test or determination of cytokeratin 18 (CK18). Unfortunately, the possible interference of these markers with steatohepatitis has not been studied and they were not applied in clinical practice. Interestingly, the best single serum marker was hyaluronic acid formerly introduced by Parés *et al*[55] showing a significant correlation with the histological fibrosis independent of the inflammatory status. Future studies are required to better define which serum markers should be used in cases where no liver stiffness (LS) can be obtained and to which extent it is modified by co-existing inflammation.

Fibrotest®, a marker panel analysing alpha-2-macroglobulin, haptoglobin, GGT, ApoA1 and bilirubin and corrected for age and sex[56] has high diagnostic potential for the detection of significant fibrosis in patients with ALD. In a study of 221 patients with biopsy-proven ALD, the mean Fibrotest® value ranged from 0.29 in patients with F0 to 0.88 in those with F4 cirrhosis. For the diagnosis of F4 cirrhosis, the AUROC was very high (0.95)[57]. FibrometerA®, combining PT, alpha-2-macroglobulin, hyaluronic acid and age has similar diagnostic accuracy in ALD with an AUROC of 0.962[58]. The diagnostic value of Hepascore® combining bilirubin, GGT, hyaluronic acid, alpha-2-macroglobulin, age and sex did not differ from that of FibrometerA® or Fibrotest® and was significantly greater than those of non-patented biomarkers (APRI, Forns, FIB4)[54]. The combination of any of these tests did not improve diagnostic accuracy[54]. In addition to their diagnostic performance in the screening of fibrosis, non-invasive tests may be useful in predicting liver-related mortality as shown in a study of patients with ALD followed-up for more than 8 years, where survival was correlated with baseline non-invasive fibrosis score[54]. The so-called ELF® test may also predict clinical outcomes in patients with chronic liver disease[59] but its efficacy needs further evaluation in larger ALD cohorts. Preliminary comparative analysis from the Heidelberg Center suggests that the formerly introduced single hyaluronic acid[55] is quite useful and could well serve as backup marker in those ALD patients that cannot be measured by elastography.

Table 6 Liver stiffness and fibrosis stages in alcoholic liver disease (biopsy proven studies)

Ref.	Patients (*n*)	Correlation	AUROC F4	Cut-off F4
Nahon *et al*[126], 2008	174	0.70, $P < 0.0001$	0.87	22.6
Nguyen-Khac *et al*[62], 2008	103	0.72, $P < 0.014$	0.92	19.5
Kim *et al*[127], 2009	45		0.97	25.8
Mueller *et al*[37], 2010	101	0.72; $P < 0.001$	0.92	11.5
Janssens *et al*[128], 2010	49		0.86	21.1

Assessment of fibrosis stage by elastographic techniques via LS

The new approaches to assess LS have significantly improved the diagnosis of liver fibrosis[60,61]. TE (Fibroscan®) was the first technique to be introduced. Consequently, most published LS studies have been performed with TE. In the last year, Fibroscan was also approved by the FDA in the United States. Acoustic radiation force impulse imaging (ARFI, Siemens) and shear wave elastography (SWE, Supersonic Imaging) are additional competing ultrasound-based techniques that are commercially available. Magnetic resonance elastography (MRE) hold great promises for three-dimensional assessment of stiffness in various organs not restricted to the liver. However, it is routinely used only in few centers. First comparative studies indicate that ARFI, SWE and MRE are matching with TE with regard to accuracy. Future studies will identify individual limitations and strengths. We here consider the interpretation of LS in general and independent of the methodology. Unfortunately, different non-standardized units are used by the above mentioned techniques that may lead to confusion when comparing different studies.

LS is an excellent surrogate marker of advanced fibrosis (F3) and cirrhosis (F4) in ALD and superior to all serum markers[62]. LS scale with cut-off values for various fibrosis stages in ALD are shown in Figure 3. LS values below 6 kPa are generally considered as normal and exclude even mild fibrosis (Figure 3). Although severe fat deposition may lower LS, it rarely has an impact on fibrosis stage determined by LS. Due to the narrow "gray range" from 6 to 8 kPa and potential interferences (positioning, breathing or eating), an exact discrimination between F1 and F2 stages is not recommended for clinical purpose. Finally, LS highly correlates with portal pressure and esophageal varices and HCC are likely at LS > 20 kPa[60,61]. However, LS can be also elevated by inflammation[63,64], liver congestion[65], and mechanic cholestasis[66] in the absence of fibrosis. Since all these conditions may be present in ALD patients, LS should always be interpreted in the context of imaging, laboratory and clinical findings. Table 6 lists all biopsy-proven studies on patients with ALD so far. Although an excellent performance could be shown in all studies, they differ quite drastically with regard to the cut-off values. In our opinion, this is mainly due to the presence of inflammation as assessed by transaminase levels[37]. In this study, we demonstrated that LS decreases in patients with ALD dur-

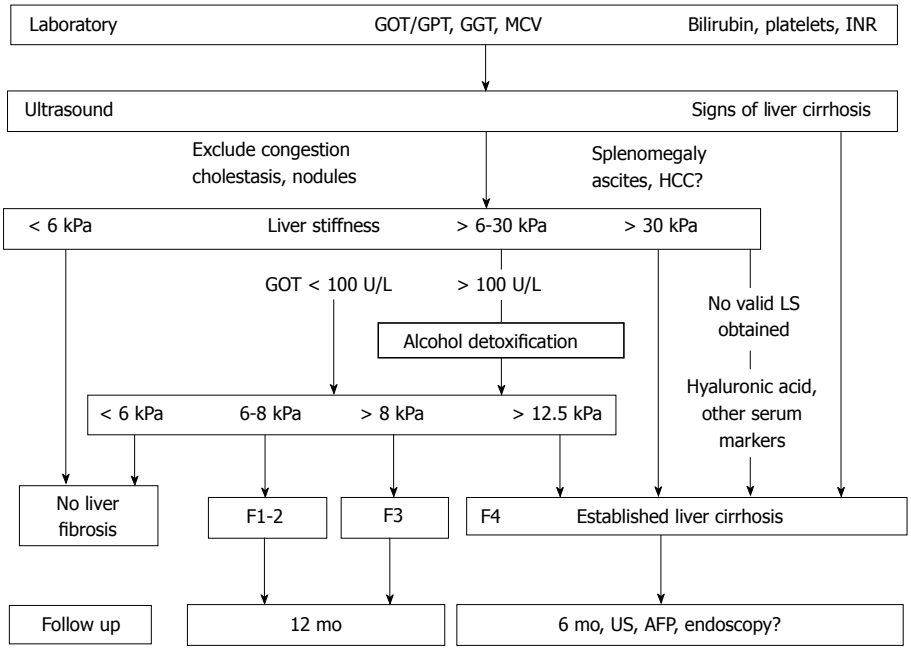

Figure 4 Complete non-invasive diagnostic work plan for patients with alcoholic liver disease at Salem Medical Center Heidelberg with follow up. Flow scheme allowed diagnosis of fibrosis in 95% of patients. In the remaining 5% of patients without valid LS measurements, the role of serum markers need to be settled but single hyaluronic acid looks promising. In patients with LS > 30 kPa, cirrhosis is established despite increased transaminase levels. MCV: Mean corpuscular volume; HCC: Hepatocellular carcinoma; LS: Liver stiffness; US: Ultrasonography; GGT: γ-glutamyl transpeptadase; GOT: Glutamic-oxal(o)acetic transaminase; GPT: Glutamate pyruvate transaminase; AFP: α-fetoprotein.

ing alcohol withdrawal[37]. The decrease of LS was best estimated based on GOT levels. Thus, GOT levels higher than 100 U/L were predictive for an inflammation-associated elevation of LS. When only considering patients with low or normal transaminase levels, cut-off values were comparable to those observed in patients with viral hepatitis[37], *e.g.*, 12.5 kPa for F4 cirrhosis. In addition, the diagnostic accuracy of LS could be improved when considering the GOT levels. These data have also been confirmed by others[67]. In our present cohort of 364 patients undergoing alcohol withdrawal, the overall mean decrease of LS was 10%, which transformed into overestimation of fibrosis stage in 27%. In some patients, fibrosis stage changed up to three degrees after alcohol withdrawal. For these reasons, we require actual laboratory testing for correct LS interpretation. More practical algorithms are provided below.

Transfer into clinical practice

In Figure 4, the work up plan is shown as applied daily at the Salem Medical Center. After suspicion of ALD either by patients reporting, clinical or laboratory signs, TE is performed directly after the abdominal ultrasound and routine blood tests. 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[68,69]. If LS was elevated and patients had GOT > 100 U/mL, alcohol withdrawal for at least 2 wk is recommended followed by a second LS measurement. The following practical setting is applied in Heidelberg: (1) we always perform the LS measurement right after the abdominal ultrasound. By doing so, direct and indirect ultrasound criteria for cirrhosis are seen and important other non-cirrhotic factors for an increased LS (congestion, cholestasis, tumors, others) are diagnosed; (2) a LS < 6 kPa excludes cirrhosis and even mild fibrosis; (3) if the LS > 12.5 kPa, the patient has compensated cirrhosis in case of GOT levels < 100 U/L. Transaminases typically normalize within 1-3 wk, so LS can always be re-measured after 1-3 wk of abstaining from alcohol; and (4) in patients with LS > 30 kPa, the diagnosis of cirrhosis is settled despite steatohepatitis as measured by elevated transaminase levels. At these levels, the development of ascites is very likely.

This approach allows definitive non-invasive assessment of fibrosis stage in ca. 95%. Compared to conventional routine ultrasound, TE identifies twice as many patients with advanced fibrosis/cirrhosis and has a smaller sample error as compared to histology (3%-5% *vs* 20%-50%). In a recent French elastography screening study on more than 1000 apparently healthy people older than 45 years, 7.5% had a pathologically increased liver stiffness > 8 kPa with 36% of them eventually being due to ALD[70]. 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 GOT-adapted cut-off values should be used *e.g.*, for ad hoc decisions in patients with no time or options to withdraw from alcohol, remains still a matter of debate.

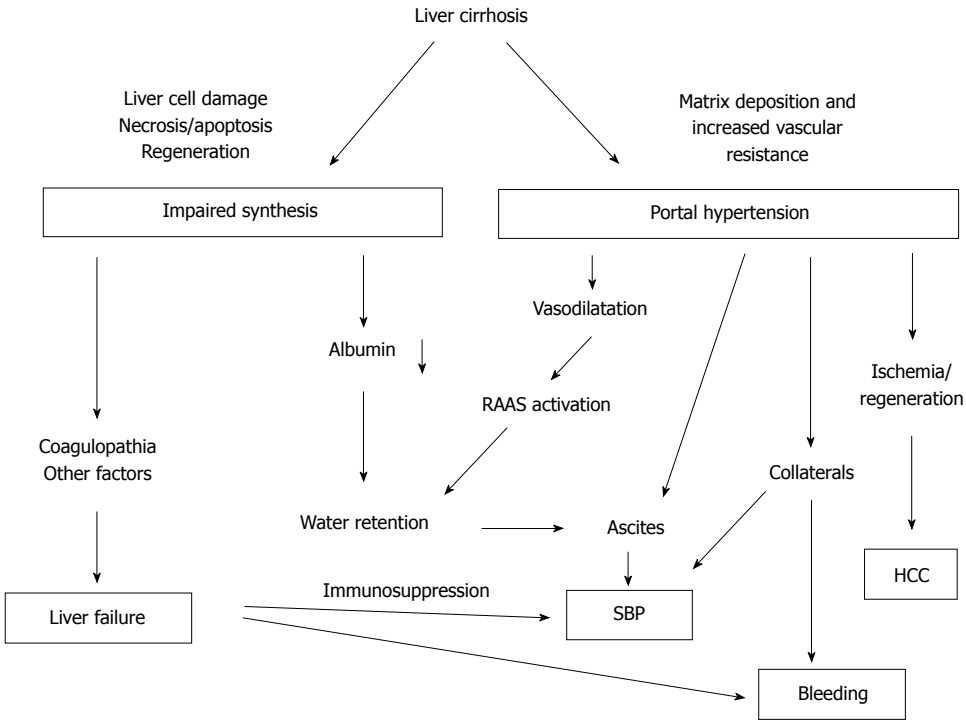

Figure 5 Clinical significance of synthesis impairment and portal hypertension in cirrhotics. Both factors are independently and individually occurring in cirrhotic patients and determine the individual risk of severe complications (framed). While synthesis is easily assessed by lab tests, elastographic techniques are the future highly sensitive method of choice to identify patients with portal hypertension. HCC: Hepatocellular carcinoma; SBP: Systolic blood pressure.

FUTURE CHALLENGES

Importance to discriminate between impaired liver synthesis and portal hypertension in cirrhotic patients

One problem in discussing the term liver cirrhosis is the fact that histomorphological features of liver cirrhosis (gold standard) are associated with a broad variety of clinical symptoms and complications. Dependent on their clinical specialization, physicians will be confronted with different aspects of the liver disease and, consequently, will have a distinct look on liver cirrhosis *per se*. This sometimes causes a different usage of terminology. Figure 5 demonstrates that liver cirrhosis manifests in every patient individually *via* clinically different but diagnostically accessible routes mainly due to impaired synthesis, metabolic activity and detoxification or portal hypertension. Although both impairments, portal hypertension or reduced synthesis, can coexist in every patient and are highly associated with each other, patients exist in which one or the other impairment is dominant and determines prognosis and survival.

Thus, the degree of synthesis impairment and portal hypertension should be evaluated separately to better determine the natural course and potential complications. In practice, patients can be seen with normal synthesis parameters but pronounced portal hypertension and *vice versa*. Despite normal INR and albumin levels, they can develop massive ascites and may later die from spontaneous bacterial peritonitis or varical bleeding. Such patients have a stiff liver and show vast matrix deposition in the biopsy. In contrast, other patients show rather early signs of icterus and impaired coagulation tests but portal hypertension is less pronounced. More research needs to be performed to better understand genetic determinants of these individual natural courses. The different aspects of liver cirrhosis and the absence of standardized usage of terminology are challenged by novel elastographic methods. It can be expected that liver cirrhosis will be evaluated differently in the near future. The new perspective may easily explain why conventional laboratory based scores rather detect the synthesis-impaired cirrhotics but overlook patients with portal hypertension. By contrast, we think that elastographic techniques are highly sensitive to identify patients with portal hypertension as also suggested in a recent Korean study by Hong *et al*[71].

AH

AH is characterized by a high mortality rate and typically affects younger patients with a shorter drinking history[72]. Despite much effort, invasive and non-invasive methods for early AH detection are limited and diagnosis by serum markers or histology is still a matter of controversies. So far, liver transplantation is the therapy of choice with a success rate of 90%[73,74]. However, it is not allowed in most countries before 6 mo of abstinence. In addition, only a small group of patients with early bilirubin response and no contraindications are candidates for steroids or pentoxifylline[72]. At the moment, there is a huge controversy of using non-invasive clinical *vs* histological scores the latter being recommended by most guidelines. Table 7 shows three more recently introduced histological scores to assess alcoholic hepatitis with an AUROC of

Table 7 Histological scores for alcoholic hepatitis

Ref.	Score	*n*	AUROC	Day survival	Histological parameters	INR	Bilirubin	Age	Albumin	Urea	Leuko	MELD	DF
Forrest *et al*[15], 2005	GAHS	241/195 (137)	0.65-0.71	28 and 84 d	Steatohepatitis	-	9	-	-	-	-	-	41
Mookerjee *et al*[129], 2011	ASH Grade	68	0.80	-	Fibrosis Cholestasis Cholangiolitis Steatosis Ballooning Steatosis	1.7	13.3	51	25	13.5	13.3	12.5	38
Affò *et al*[130], 2013	ASH score	121+205	0.74	90 d	Fibrosis Bilirubinostasis Megamitochondria PMN infiltration	1.6	9.7	49	-	-	-	18	-

ASH: Alcoholic steatohepatitis; PMN: Polymorphonuclear.

Table 8 Clinical scores and alcoholic hepatitis

Ref.	Score	*n*	AUROC			Parameters							
			30 d	90 d	6 mo	INR	Bilirubin	Creatinin	Age	Leukocytes	Urea	Albumin	Bili decrease
Maddrey *et al*[14], 1978	DF	55				+	+						
Dunn *et al*[131], 2005	MELD	73	0.83	0.86		+	+	+					
Forrest *et al*[15], 2005	GAHS	241/195	0.81	0.78		+	+		+	+	+		
Louvet *et al*[16], 2007	Lille model	295/115			0.89	+	+	+	+			+	+
Dominguez *et al*[132], 2008	ABIC	103/80				+	+	+	+				

ca. 0.8 to predict 90-d survival. Moreover, biliary features seem to be of high interest to early recognize signs of infection, sepsis and poor prognosis.

It is interesting to note, that the well-established clinical scores (Table 8) show a comparable AUROC to predict survival. As shown in Table 8, major non-invasive routine markers that have been identified in various studies include INR, bilirubin, creatinine, age, leukocytes, urea, albumin and decrease of bilirubin over 7 d. Unfortunately, both clinical and histological scores are not yet accurate enough and none of the studies really compared all clinical scores *vs* all histological scores. Preliminary first observations also suggest that liver stiffness will not add any new and helpful information with regard to prognosis of AH. Interestingly, transaminase levels are usually only slightly increased and are also not predictive. Recent data suggest that serum CK18 fragments (M65 and M30) are highly sensitive and more significant markers of the histological degree of inflammation and liver damage clearly exceeding transaminase levels. In addition, a recent study on ALD patients undergoing alcohol detoxification showed an unexpected increase of M30 while M65 and transaminases decreased or even normalized. These data could give a first hint on the role of dysregulated apoptotic events during AH[75].

Future non-invasive tests for ALD

Various aspects of ALD could be potentially assessed in a non-invasive manner and a broad and diverse array of promising techniques are currently under investigation. This paragraph is far from being complete and only a few novel methods are mentioned for the lack of space.

CAP

With regard to hepatic steatosis, CAP (controlled attenuation parameter) looks very encouraging and is already commercially available. CAP uses a sophisticated process based on vibration control transient elastography (VCTE, Fibroscan) but is so far restricted to the M probe. CAP was first validated as an estimate of ultrasonic attenuation at 3.5 MHz using Field II simulations and tissue-mimicking phantoms. Although ALD was not addressed specifically, CAP correlated well with the histological degree of steatosis (Spearman rho = 0.81, $P < 10^{-16}$) and the AUROC was equal to 0.91 and 0.95 for the detection of more than 10% and 33% of steatosis, respectively[41]. Factors significantly associated with elevated CAP were BMI (> 30 kg/m^2), metabolic syndrome, alcohol consumption of higher than 14 drinks per week and an elevated liver stiffness[76]. Comparative studies in patients with NAFLD, HCV and HBV indicated that CAP seems to work independent of the etiology of the liver disease[77] and ethnic origin[78].

Susceptometry

ALD patients often show pathological high iron deposits in the liver. Iron could lead to progressive liver disease because of its high cancerogenicity due to Fenton-like reactions thereby determining outcome[79]. Both the underlying mechanisms and potential therapeutic approaches are still unresolved. In addition, it is often overlooked that routine iron parameters do not reliably reflect

hepatic iron overload namely in patients with ALD. Techniques such as the SQUID technology are only on few places worldwide available and are too expensive for screening purposes. Although modified MRI techniques can principally be used to quantitate hepatic iron and are used in some centers to measure liver iron in *e.g.*, patients with heavy iron overload such as β-thalassemia, they have not been really explored in ALD patients. Furthermore, their potential interferences and detection limits in such common metabolic liver diseases have not been carefully studied. The recently developed room temperature susceptometer[80,81] seems to be an alternative approach and first preliminary data on ALD patients at Salem Medical Center are encouraging.

Breath tests

It is surprising that information from the exhaled air have not been more intensively explored given the enormous technical progress *e.g.*, such as mass spectroscopy. A few studies have been published so far. Millonig *et al*[82] tested if ion-molecule-reaction mass spectrometry combined with a new statistical modality could be used for the diagnosis of liver diseases including some individuals with alcoholic fatty liver disease. Characteristic exhalation patterns could be identified reaching an AUROC for individual liver diseases between 0.88 and 0.97. Other authors tested whether volatile compounds from breath samples as detected by selected-ion flow-tube mass spectrometry correlate with the diagnosis of AH and the severity of liver disease in patients with AH. In this study six compounds (2-propanol, acetaldehyde, acetone, ethanol, pentane and trimethylamine) were identified whose levels were increased in patients with liver disease compared with control subjects[83].

Apoptosis markers

Quantification of hepatocyte cell death by circulating CK18 levels and its caspase-cleaved fragments has been recently explored to evaluate the progression of ALD[84]. M30 and M65 antibodies can be used for monitoring liver cell death in heavy alcoholics[85,86]. CK18 was higher in the serum of heavy drinkers as compared to controls and also increased in patients with alcoholic hepatitis when compared to patients with fatty liver[87]. Furthermore urinary levels of full length CK18 are enhanced in alcoholics[88]. Recently, Lavallard *et al*[84] quantified and correlated CK18 and its fragments in the serum of 143 heavy alcoholics with disease severity. They reported a strong correlation of CK18 and its fragments with Mallory-Denk bodies, ballooning, fibrosis and with hepatic TNF-α and TGF-β assessed in the liver of 24 patients. Elevated levels of serum hepatocyte death and apoptotic markers were independent risk factors in predicting severe fibrosis in a model combining alkaline phosphatase, bilirubin, prothrombin index, HA, hepatocyte death and apoptotic markers (AUROC 0.84 and 0.76). Recent data suggest that M65 and M30 are highly sensitive and more significant markers of the histological degree of inflammation and liver damage clearly exceeding transaminase levels. In addition, a recent study on ALD patients undergoing alcohol detoxification showed an unexpected increase of M30 while M65 and transaminases decreased or even normalized[75]. These data could give a first hint on the role of dysregulated apoptotic events during AH. Another study examined the tumor necrosis factor related apoptosis inducing ligand (TRAIL) as essential factor involved in apoptosis in liver injury animal models after alcohol consumption. They showed that after alcohol consumption in the livers of animals virally transfected with TRAIL, TRAIL expression led to hepatic steatosis, without hepatocyte cell death, indicating that TRAIL-mediated apoptosis and steatosis may be independently modulated after viral infection and alcohol intake. Therefore, TRAIL was proposed as a new mediator of hepatic steatosis after alcohol intake[89]. An additional approach could be the analysis of Stat3 DNA-binding in ALD patients, because *in vitro* and animal studies suggest that alcohol might interfere with Stat3 signaling, a regulator of hepatocyte cell death and proliferation[90-92]. Stärkel *et al*[93] assessed Stat3 expression, binding activity and the apoptotic-proliferation balance in ALD patients and found no detectable Stat3 DNA-binding activity in all ALD samples. This was also associated with high Pias3 expression, but not with increased Socs3 levels. Bcl-2 was upregulated in ALD together with decreased Caspase-3 activity. They concluded from the results that alcoholic cirrhosis is characterized by impaired Stat3 DNA-binding activity and this might contribute to disturbed liver regeneration and repair and the fatal outcome.

Genetic profiling (PNPLA3)

Since only 15% of heavy drinkers will develop cirrhosis, it has been conceived for a long time that genetic factors are important disease modifiers in ALD[94]. Studies examining ethnic factors, familial history or twin studies point also to ALD as a genetically determined disease[95,96]. Only recently, a genome-wide association study (GWAS) identified a small nucleotide polymorphism (SNP; rs738409 C->G) in the patatin-like phospholipase domain containing 3 (*PNPLA3*/Adiponutrin) gene as genetic variant associated with steatosis[97]. Several studies confirmed that this variant predisposes towards all stages of liver damage starting from simple steatosis to steatohepatitis and progressive fibrosis and is also linked to increased risk of ALD (steatohepatitis to cirrhosis)[98,99]. In a well-characterized cohort of ALD patients, a significant correlation was found between the GG allelic variant with histological signs of hepatocyte damage (microgranulomas and ballooning $r > 0.3$, $P < 0.005$) but less with histological steatosis ($r = 0.24$, $P < 0.05$)[100]. Therefore, the determination of SNP status and following consequences will reveal novel mechanisms involved in ALD development and progression and may possibly help to establish new treatment options.

Other markers

Further intensively discussed markers of diagnostic potential include miRNA[101-103] and osteopontin[104,105] just to name a few. It is quite conceivable that the intensive search for novel physical or molecular markers will drastically improve the non-invasive management of ALD in the upcoming decade.

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