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PRACTICE

RATIONAL TESTING

Tests for diagnosing and monitoring non-alcoholic fatty liver disease in adults

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This series of occasional articles provides an update on the best use of key diagnostic tests in the initial investigation of common or important clinical presentations. The series advisers are Steve Atkin, professor of medicine, Weill Cornell Medical College Qatar; and Eric Kilpatrick, division chief, clinical chemistry, Sidra Medical and Research Center, Qatar; honorary professor, department of clinical biochemistry, Hull Royal Infirmary, Hull York Medical School. To suggest a topic for this series, please email us at practice@bmj.com.

What you need to know

- Liver ultrasonography is a pragmatic first line test to diagnose hepatic steatosis and exclude other liver pathology in those with non-alcoholic fatty liver disease (NAFLD)
- In patients with confirmed hepatic steatosis, use simple non-invasive markers of fibrosis (such as an enhanced liver fibrosis blood test (ELF)) and/or FibroScan to investigate for liver fibrosis
- Offer patients with hepatic fibrosis referral for specialist opinion, as hepatic fibrosis is the strongest predictor of overall and liver related mortality in those with NAFLD

At a routine work health check, a 52 year old sedentary computer programmer was found to have a serum alanine aminotransferase (ALT) concentration of 68 IU/L (normal 0-40 IU/L), and a triglyceride concentration of 1.9 mmol/L. His fasting plasma glucose level was 5.8 mmol/L and other basic liver, renal, and lipid blood tests were normal. He had an unremarkable medical history and took no regular medications, did not smoke, and consumed <7 units of alcohol/week. Clinical examination was unremarkable. His body mass index was 29 kg/m2; waist circumference 102 cm, and blood pressure 134/88 mmHg. A repeat serum ALT measurement remained raised some months later, at 62 IU/L.

Non-alcoholic fatty liver disease (NAFLD) is a metabolic liver disease that encompasses a spectrum of progressive pathological conditions, ranging from non-alcoholic fatty liver (NAFL) to steatohepatitis (NASH), fibrosis, and cirrhosis. When hepatic steatosis occurs in the absence of excessive alcohol consumption and other recognised causes of liver fat, and with cardiometabolic risk factors, it is likely that the diagnosis is NAFLD as NAFLD is principally a diagnosis of exclusion.

NAFLD is the commonest liver disease in high income countries, and is estimated to affect at least 25%-30% of adults in the general population and up to 70%-90% of persons with obesity or type 2 diabetes.[1]NAFLD is associated not only with liver related morbidity and mortality, but also with an increased risk of developing cardiovascular disease and type 2 diabetes.[2 3] Liver biopsy remains the reference method for diagnosing NAFLD, as it provides the most accurate assessment of disease grade and stage.[4 5] However, undertaking a liver biopsy is costly, risky, and potentially painful. Moreover, interpretation of NAFLD severity can be compromised by sampling errors in what can be a patchy disease.[6 7]

In this article, we discuss the diagnosis of NAFLD, testing for liver fibrosis in those with NAFLD, and monitoring of those most likely to develop advanced liver disease. We examine the evidence and guidelines from Europe, the United States, and the UK's National Institute for Health and Care Excellence (NICE)[8-10] for and against the use of specific diagnostic tests. Our approach to the use of liver ultrasound in establishing a diagnosis of hepatic steatosis differs from the recent NICE guidelines,[10] but complements British Society of Gastroenterology guidelines.[11] Treatment options are beyond the scope of this article.

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What are the next investigations?

After performing a history and examination, the next investigations to establish whether the patient has NAFLD or another liver condition are:

- A non-invasive liver screen, which includes tests such as serology for hepatitis B and C viruses, and measurement of liver auto-antibodies, immunoglobulins, caeruloplasmin, alpha 1 anti-trypsin, and ferritin concentrations
- A liver ultrasound to look for features suggestive of NAFLD (hepatic steatosis) and to rule in or out other pathology.

An ultrasound scan of the liver is the most typical imaging test requested by non-specialists in patients in whom NAFLD is suspected. This is because it can help to confirm and exclude other causes of liver disease such as gall stones or metastasis, as well as confirm hepatic steatosis. Although the NICE guidelines state that ultrasonography is "not cost effective,"[3] it is important to understand why such a statement was made, despite widespread acceptance that the most useful imaging technique to detect steatosis is ultrasonography.[1 2 12] In estimating the cost effectiveness of any test or intervention, what is considered is not only the cost, but also the relationship between the measured factor (ie, hepatic steatosis) and the outcome. Ultrasonography enables an accurate diagnosis of hepatic steatosis, but the presence of steatosis does not predict risk of end-stage liver disease. Rather, it is advanced fibrosis (which is not accurately detected by ultrasonography) that more accurately predicts the risk of developing end-stage liver disease and hepatocellular carcinoma.[13-16] Ultrasonography is, however, a pragmatic approach to investigating the possibility of NAFLD because it also allows the exclusion of other liver conditions and so is central to the approach laid out in this article.

A validated, widely accepted procedure for the diagnosis and monitoring of NAFLD does not yet exist. We propose a potential approach (infographic) including "red flags" and when to seek specialist advice. The infographic gives an overview of testing for NAFLD and excluding other pathologies in a person with abnormal liver function test results. The graphic includes features of abnormal liver function tests and factors that are useful to note in the history and examination. It provides information about further testing in those identified with NAFLD to diagnose liver fibrosis, and about how to monitor those found to have advanced fibrosis.

Is it NAFLD or something else?

When hepatic steatosis occurs in the absence of recognised causes of liver fat (table 1), and with cardiometabolic risk factors (table 2), it is likely that the diagnosis is NAFLD.

NAFLD might be suspected because the patient is overweight or obese, has type 2 diabetes, or has other metabolic syndrome features.[17] More often, a diagnosis of NAFLD is suspected when liver blood tests show mild to moderate elevations of serum aminotransferase levels. However, serum aminotransferase levels are not sensitive or specific to make or rule out a diagnosis of NAFLD. Table 2 describes these risk factors. Be aware that NAFLD can also occur in non-obese or lean individuals (termed "lean NAFLD").

An alternative pathology might be more likely if a non-invasive liver screen of other factors in the history, such as high alcohol intake, suggests another cause (table 1).

What techniques can be used to test for hepatic steatosis?

The presence of hepatic steatosis, and therefore NAFLD, can be diagnosed by various methods (table 3).

Ultrasonography is the first line imaging technique for diagnosing hepatic steatosis. Compared with histology, it has a good sensitivity (~85%) and specificity (~95%) for detecting moderate steatosis,[15 16] and traditionally its sensitivity is thought to be poor when <20%-30% of hepatocytes are steatotic.[22]

Combining standard ultrasonography with computer software technology (MATLAB) (eg, combined ultrasound hepatic/renal ratio and hepatic echo-intensity attenuation rate evaluation)[18] improves the sensitivity of ultrasonography further. In this methodology, the ultrasound hepatic/renal echo-intensity ratio and ultrasound hepatic echo-intensity attenuation rate were obtained from ordinary ultrasound images using the MATLAB program. Compared with proton magnetic resonance spectroscopy (ie, the gold standard for detecting low levels of liver fat content) (see table 3), at levels of <15% liver fat content, the sensitivity and specificity of the ultrasound quantitative model was 81.4% and 100%.

Computed tomography, magnetic resonance imaging, and magnetic resonance spectroscopy can be used, but such imaging techniques are more expensive and less readily available.[8 9]

Some non-invasive biomarkers of steatosis (eg, fatty liver index) have been proposed, but they have limited clinical utility, as they often do not accurately quantify steatosis as assessed histologically. Controlled attenuation parameter (CAP, assessed by transient elastography) can also be used, although it remains uncertain what CAP thresholds should be adopted to diagnose steatosis.[23]

Liver biopsy remains the reference method for diagnosing and staging NAFLD, but is not a practical first line investigation. Undertaking serial liver biopsies over time is fraught with difficulties, and is unacceptable to monitor disease. Nevertheless, biopsy is the only method for diagnosing inflammation in NAFLD (ie, NASH), and should also be also considered when other chronic liver diseases cannot be definitively excluded.

For those with NAFLD, what further investigations are offered?

Previously thought to be a harmless condition, hepatic steatosis is now increasingly being recognised as a cause of progressive and advanced liver disease. Recent follow-up studies showed that, contrary to conventional paradigm, patients with NAFL (ie, simple steatosis on histology) can develop progressive liver fibrosis.[24] Hepatic steatosis (detected by ultrasonography) is also strongly associated with an increased risk of fatal and non-fatal cardiovascular disease, type 2 diabetes, and chronic kidney disease.[2 3] After a diagnosis of hepatic steatosis has been established, strong evidence[8-10] now indicates that it is clinically more important to stage liver fibrosis than to ascertain the presence of NASH.

Characterise the severity of NAFLD

Once steatosis has been diagnosed, the presence and severity of liver fibrosis should be assessed using combined non-invasive tests to identify those individuals with advanced fibrosis who should be referred to specialists in hepatology for further investigations. Staging of liver fibrosis can be undertaken with the use of biopsy or various non-invasive tests[9 25] (table 4). Choice of test will depend on local availability. The infographic outlines two possible approaches. Tests such as Fibrosis-4 score

(FIB4), NAFLD fibrosis score (NFS), and enhanced liver fibrosis (ELF) can be conducted by non-specialists. How they are calculated is outlined at the foot of table 4.

The ELF test (a commercial blood test using three direct fibrosis biomarkers) has good performance for diagnosing significant and advanced fibrosis, and it is now strongly recommended by the NICE guidelines, although it is not used worldwide. Other "biochemical" score systems (eg, the NFS and FIB4 scores, which are both cost effective and highly sensitive tools to exclude patients with advanced fibrosis) and second line "physical" techniques (liver stiffness measurements assessed with transient elastography [FibroScan] or with newer imaging techniques) are frequently used to assess the severity of liver fibrosis. The combination of FibroScan with FIB4/NFS measurements has shown excellent accuracy in distinguishing advanced fibrosis.[25]

All non-invasive tests for liver fibrosis are better at excluding advanced fibrosis than diagnosing it. They have only modest positive predictive value for advanced fibrosis, but a much stronger negative predictive value. Furthermore, none is good at detecting intermediate stages of fibrosis. As such, no test can fully replace liver biopsy. For example, the NAFLD fibrosis score, the most widely validated non-invasive test, has good performance for identifying patients without fibrosis, but poorer performance for diagnosing clinically significant and advanced fibrosis. As recommended by the European[8] and American[9] practice guidelines, current non-invasive tests of fibrosis should be used in a staged approach, utilising their high negative predictive value to rule out patients who are unlikely to have advanced fibrosis, and so reserving liver biopsy for patients who are most likely to have substantial (clinically significant) fibrosis or when there is diagnostic uncertainty.

Outcome

The man's general practitioner requested a liver ultrasound scan (confirming the presence of hepatic steatosis) together with a repeat serum ALT measurement of 62 IU/L. Other blood tests (including serology for hepatitis B and C viruses, liver auto-antibodies, immunoglobulins, caeruloplasmin, alpha-1 antitrypsin and ferritin levels) excluded other causes of liver disease.

The patient is likely to have NAFLD.

How this article was made

We searched PubMed for original articles and reviews using the keywords "nonalcoholic fatty liver disease," or "fatty liver" combined with "diagnosis," "prognosis," or "mortality" published between 1990 and 2018. Articles published in languages other than English were excluded from the analysis.

How patients were involved in the creation of this article

Several of our patients have told us that doctors are inconsistent in their approaches to investigating their liver disease. Two patient representatives (Irene McGill, who has NAFLD, and Jane Putsey, who has cared for her father with NAFLD) participated in the NICE NAFLD NG 49 Guideline Development Group and contributed to the guideline. Ms McGill and Ms Putsey advised Professor Byrne of what they thought was important for patients with NAFLD, which influenced the writing of this manuscript. Both representatives commented on the article and gave helpful suggestions to drafts of the manuscript to improve its clarity. For example, they asked for clear information on how NAFLD could be diagnosed.

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Tables

Table 1| Tests and factors which do not suggest NAFLD

Risk factors for liver disease	Factors that do not suggest NAFLD
Alcohol >21 standard drinks per week in men and >14 standard drinks per week in women *	History of excessive alcohol consumption
Drugs Valproic acid, oestrogens, tamoxifen, corticosteroids, tetracycline, amiodarone, perhexiline maleate, methotrexate, 4,4′-diethylaminoethoxyhexesterol, chloroquine, L-asparaginase	History of drug exposure
Viral hepatitis	Serological positivity for HBsAg and anti-hepatitis C antibodies/HCV-RNA
Haemochromatosis	High transferrin saturation (>45%); high serum ferritin (>1000 µg/L)
**Autoimmune hepatitis ** **	Serum: Immunoglobulins (IgG) raised; anti-mitochondrial antibodies+ve; smooth muscle cell antibodies strongly+ve; anti-nuclear antibodies+ve; anti-liver kidney microsomal+ve
Wilson's disease * **	Low level of caeruloplasmin (<200 mg/L)
Alpha 1 anti-trypsin deficiency	Low level of α 1 anti-trypsin protein (<260 micromol/L)
Coeliac disease	Anti-tissue transglutaminase antibodies+ve
Others	Occupational exposure to hepatoxins; malnutrition (especially Kwashiorkor); total parenteral nutrition; rapid weight loss; surgically altered bowel anatomy (eg, jejuno-ileal bypass, extensive small-bowel resection); lipodystrophy; hypobetalipoproteinaemia

* The alcohol thresholds for liver disease reported in table 1 are not entirely congruent with the UK current thresholds for safe alcohol consumption, which are >14 units (standard drinks) per week in both men and women. ** Low titres of anti-nuclear, anti-smooth muscle, and anti-mitochondrial antibodies can be noted in patients with NAFLD (in the absence of autoimmune hepatitis) ***Slightly lower caeruloplasmin levels can also be found

Table 2| **Common cardiometabolic risk factors for NAFLD**

Risk factor	Metabolic abnormalities	Diagnostic criteria of the risk factor
Overweight/obesity		Apply ethnic specific cut-offs for body mass index, eg, white European ≥25/ ≥30 kg/m^2
Metabolic syndrome features	Abdominal obesity	Apply ethnic specific cut-offs for waist circumference, eg, white European >94/80 cm (M/F)
	High serum triglycerides	≥1.7 mmol/L or lipid lowering treatment
	Increased blood pressure/hypertension	≥130/85 mmHg or anti-hypertensive treatment
	Low HDL cholesterol	<1.0/1.3 mmol/L (M/F)
	Impaired fasting glycaemia	Fasting glucose levels ≥5.6 mmol/L
Type 2 diabetes mellitus		Fasting glucose levels ≥7 mmol/L or glucose lowering treatment

Other modifiable risk factors for NAFLD are cigarette smoking (due to its pro-fibrotic hepatic effect), excessive dietary intakes of fructose, carbohydrates, and saturated fatty acids

Table 3| Invasive and non-invasive techniques for diagnosing hepatic steatosis in NAFLD

Technique	Result compatible with NAFLD	Pros and cons of technique
Biopsy	Histological examination shows lipid droplets in at least 5% of hepatocytes	Reference method for diagnosing NAFLD and where there is diagnostic uncertainty. Expensive, invasive, substantial morbidity and even mortality (rarely). Not suitable for detailed monitoring of disease
Ultrasonography	Liver echogenicity exceeds that of renal cortex and spleen and there is attenuation of the ultrasound wave, loss of definition of the diaphragm, and poor delineation of the intrahepatic architecture	The sensitivity of ultrasound is poor below levels of fat infiltration <20%-25%; however, the technique is highly sensitive and specific at higher levels of fat infiltration. Combining standard ultrasound with computer software technology (MATLAB) (eg, combined ultrasound hepatic/renal ratio and hepatic echo-intensity attenuation rate evaluation,[18] improves the sensitivity of ultrasound even further
Fatty liver index (FLI) (algorithm derived score using body mass index, waist circumference, fasting serum triglycerides, and gamma-glutamyltransferase concentrations)	FLI ≥60 suggestive of hepatic steatosis and validated against ultrasound,[19] or magnetic resonance spectroscopy (MRS)[20]	Inexpensive, but requires waist circumference measurements. Not validated against liver histology
NAFLD liver fat score (algorithm derived score using the presence of metabolic syndrome and type 2 diabetes, fasting serum insulin, AST, and the AST/alanine aminotransferase ratio)	Optimal cut-off point=-0.640 for diagnosing hepatic steatosis on MRS[21]	Inexpensive, but requires serum insulin and AST measurements. Not validated against liver histology
Transient elastography (FibroScan)	Optimal controlled attenuation parameter (CAP) thresholds ≥248, ≥268 dB/m for those above stage 1 steatosis grade, respectively[12]	Transient elastography is a promising technique, but further evidence and validation of its utility for diagnosing hepatic steatosis (by CAP measurement) is required. The signal can be affected in severely obese patients
Computed tomography	Attenuation of the liver is at least 10 Hounsfield units (HU) less than that of the spleen, or attenuation of the liver less than 40 HU[13]	Good for investigating other potential abdominal pathologies. Computed tomography has limited sensitivity to detect low levels (<30% liver fat) and exposes the patient to substantial levels of radiation
Magnetic resonance imaging (MRI) or magnetic resonance spectroscopy (MRS)	MRI: Chemical shift gradient echo imaging with in-phase and opposed-phase acquisitions identifying ≥5.5% liver fat accumulation. MRS: Proton MRS identifying ≥5.5% liver fat accumulation[14]	MRI and MRS are very sensitive non-invasive techniques for diagnosing liver fat, but are currently expensive techniques for this indication

Combining standard ultrasonography with computer software technology (MATLAB) (eg, combined ultrasound hepatic/renal ratio and hepatic echo-intensity attenuation rate evaluation)[18] improves the sensitivity of ultrasonography. Compared with proton-magnetic resonance spectroscopy (ie, the gold standard for detecting low levels of liver fat content), at levels of <15% liver fat content, the sensitivity and specificity of the ultrasound quantitative model was 81.4% and 100%.

Table 4| **Invasive and non-invasive techniques for diagnosing advanced fibrosis in NAFLD**

Technique	Result compatible with NAFLD
Biopsy	Advanced fibrosis thresholds=F3 or F4 stages Fibrosis may vary from no fibrosis (F0), portal fibrosis without septa (F1), portal fibrosis with few septa (F2), bridging fibrosis between portal and central veins (F3), and cirrhosis (F4)
Liver fibrosis tests (biochemical variables+/−anthropometry)	Advanced fibrosis thresholds Fibrosis-4 score (FIB4) >2.67[26] NAFLD fibrosis score (NFS) >0.676[27] ELF blood test score ≥10.51[28]
Transient elastography eg, FibroScan with M or XL probes (measurement of liver stiffness)	Advanced fibrosis threshold Vibration controlled transient elastography >8.7 kPA[29 30]
Acoustic radiation force impulse elastography (ARFI)	Advanced fibrosis threshold ARFI >1.4 m/s[31]
Magnetic resonance imaging techniques eg, magnetic resonance elastography (MRE)	Advanced fibrosis threshold MRE >3.64[32]

1 The FIB4 score is calculated as (age×AST)÷(platelet count×√ALT) 2. The NFS is calculated as follows:−1.675+0.037×age+0.094×BMI+1.13×IFG or diabetes (yes=1, no=0)+0.99×AST/ALT ratio−0.013×platelet count−0.66×serum albumin 3. The ELF score is a commercial blood test that combines quantitative measurements of three serum direct fibrosis biomarkers (ie, tissue inhibitor of metalloproteinase 1, procollagen III N-terminal peptide, and hyaluronic acid) to a single value. In a recent meta-analysis, the summary sensitivities and specificities of ELF score for detecting significant fibrosis were 83% and 73%, respectively; those for detecting advanced fibrosis were 78% and 76%, whereas those for detecting cirrhosis were 80% and 71%, respectively.[33] 4. In a recent meta-analysis, the summary sensitivities and specificities of FibroScan with the M probe (threshold of 8.7-9.0 kPA) for detecting advanced fibrosis were 87% and 79%, respectively.[30] A Fibroscan with the XL probe has also been validated for severely obese patients, and has a diagnostic accuracy substantially comparable with that of the standard M probe 5. Magnetic resonance elastography has the highest diagnostic accuracy for staging fibrosis in NAFLD. Patients with NASH might or might not have substantial liver fibrosis. The “gold standard” for diagnosis of NASH is only liver biopsy, with evidence of hepatocellular ballooning and Mallory bodies.