Diagnosing and monitoring non-alcoholic fatty liver disease in adults

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Conflict of Interests:
All authors have no conflicts of interest to disclose

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Word count: 1199 words; **Boxes:** 8; **Figures:** 1; **References**: 39

At a routine health check arranged by his company, a 52 year-old sedentary male 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 levels were 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/m^2; waist circumference 102 cm and blood pressure 134/88 mmHg.

What is the next investigation?

His general practitioner requested a liver ultrasonography (confirming the presence of hepatic steatosis) and a repeat serum ALT measurement was 62 IU/L. Other blood tests (including serology for hepatitis B and C viruses, liver auto-antibodies and ferritin) excluded other causes of liver dysfunction. The patient is likely to have non-alcoholic fatty liver disease (NAFLD).

Box 1 describes how patients with NAFLD usually present. When LFTs (e.g. serum aminotransferases such as serum ALT levels) are increased (above the laboratory recommendation for the upper limit of normal), patients should be further investigated to diagnose (or exclude) NAFLD. **Figure 1** illustrates a potential investigative pathway for diagnosing NAFLD and for identifying other common causes of chronic liver disease.

When patients have any of the common cardiometabolic risk factors shown in **Box 2** *plus* abnormal LFTs, it is likely the diagnosis is NAFLD (in the absence of other risk factors for liver disease shown in **Box 3**).

Introduction

NAFLD encompasses a spectrum of progressive liver conditions, ranging from non-alcoholic fatty liver (NAFL) to steatohepatitis (NASH), fibrosis and cirrhosis. NAFLD is an important condition for clinicians and patients, because it not only has the potential for causing chronic serious liver disease, but it also may adversely influence diabetes and cardiovascular disease. 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 T2DM[1]. NAFLD is associated not only with liver-related morbidity and mortality, but also with an increased risk of developing fatal and nonfatal cardiovascular disease (CVD), T2DM and chronic kidney disease[2 3].

This rational testing article discusses the evidence for the different investigations currently available to diagnose and monitor NAFLD. The article highlights the variation in international guidelines (European, US and NICE) [4-6], particularly regarding the use of liver ultrasonography. Treatment options are beyond the scope of this article.

When to suspect NAFLD?

NAFLD is usually a 'silent' disease. Suspect NAFLD if the patient:

- is overweight or obese (although NAFLD can occur in lean individuals)
- has T2DM or metabolic syndrome [7]
- has incidentally discovered abnormal LFTs with mild-to-moderate elevations of serum aminotransferase levels. However, it should be noted that serum aminotransferase

levels are not sensitive or specific enough to exclude (or identify) NAFLD, especially in those with T2DM

- it is not recommended that patients with metabolic risk factors and normal LFTs are further investigated for NAFLD (although the European guidelines recommended that patients with type 2 diabetes or metabolic syndrome should undergo diagnostic procedures for the diagnosis of NAFLD[4].

How to investigate and diagnose NAFLD?

There are a number of methods to diagnose NAFLD (**Box 4**).

Liver biopsy is the reference method for diagnosing NAFLD, with most accurate assessment of disease grade and stage of liver fibrosis (0 to 4) [8 9]. However, liver biopsy is a risky, potentially painful and costly procedure[10]. Undertaking serial liver biopsies over time is therefore unacceptable to monitor disease progression. Nevertheless, biopsy is the only method for diagnosing NASH (as there are no good biomarkers for detecting NASH), and a biopsy may be necessary if other chronic liver diseases cannot be excluded.

Ultrasonography is the first-line imaging technique for diagnosing NAFLD. Compared to histology, ultrasonography has good sensitivity (~85%) and excellent specificity (~95%) for detecting moderate hepatic steatosis[11, 12]. However, ultrasonography has poor sensitivity for detecting low levels of liver fat infiltration (e.g. when <20% of hepatocytes are steatotic)[13]. In the UK the recent NICE guidelines concluded that ultrasonography was '*not* cost effective'[6] for detecting hepatic steatosis, despite there is widespread acceptance that ultrasonography is the most useful imaging technique to detect hepatic steatosis[4 5 14]. (N.B. European and US Guidelines recommend that the presence of hepatic steatosis should be always confirmed with imaging techniques such as ultrasonography).

Other imaging - Computed tomography (CT) and magnetic resonance imaging (MRI can be used to detect hepatic steatosis, but such imaging techniques are more expensive and less readily available[1,2]. Whereas MRI has much better sensitivity than ultrasonography to detect low levels of liver fat, CT has essentially similar sensitivity to ultrasonography, but exposes the patient to low levels of radiation. Therefore, ultrasonography should be considered the first-line imaging technique for detecting liver fat.

Non-invasive biomarkers of steatosis (e.g., fatty liver index) have a 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[15].

Monitoring for hepatic fibrosis

Liver fibrosis cannot be accurately detected by ultrasonography and diagnosis requires the use of either liver biopsy or non-invasive tests[5 16] (summarized in **Box 5**). As many as a third of patients with NAFL develop liver fibrosis[17 18], and the annual fibrosis progression rate is about one histological stage (from 0 to 4) over 14.3 years for patients with NAFL and 7.1 years for those with NASH, respectively. Advanced hepatic fibrosis (histological stage 3 or 4) strongly predicts risk of developing end-stage liver disease and hepatocellular carcinoma [19-22] and these patients therefore need identifying and referring to secondary care. This level of hepatic fibrosis can now be detected by non-invasive biomarker tests with a sufficiently high sensitivity and specificity, and therefore these non-invasive tests should be implemented and used in primary care to identify those patients with advanced hepatic fibrosis who require

referral to secondary care specialists. The NICE Guidelines recommended the use of the ELF test (see Figure 1), but where this blood test is not available other non-invasive biomarker score tests could be easily used such as Fibrosis-4 score (FIB-4) or NAFLD Fibrosis score (see **Box 5**). (These two scores can be calculated using freely available simple online calculators, which require input of simple anthropometric and biochemical measurements). The performances of the ELF test, FIB-4 and NAFLD Fibrosis score to diagnose advanced fibrosis (≥F3 stage) are summarised from several studies in NAFLD[23] and are shown in **Box 6**.

When levels of hepatic fibrosis are below those indicated in **Figure 1 and Box 5**, re-testing after ~3 years is recommended in line with the NICE Guidelines.

Pragmatic approach

Figure. 1 is a suggested *pragmatic* flow diagram for the diagnosis and monitoring of NAFLD in asymptomatic adult patients in primary and non-specialist secondary care. The diagram contains 'red flag' boxes, boxes to guide investigation and boxes to indicate points in the flow diagram at which we would recommend seeking a Specialist opinion. However, it is important to underline that a validated, widely accepted, flow diagram for the diagnosis and monitoring of NAFLD does not yet exist.

Evidence into Practice Box

- When and in whom should NAFLD be suspected?
- How should the presence of liver fat and liver fibrosis be diagnosed?
- Which patients with NAFLD need to be referred for specialist opinion and care?

Acknowledgments

The authors thank Ms Irene McGill and Ms Jane Putsey Mull for their helpful comments and advice during the writing of this article. Ms Irene McGill and Ms Jane Putsey Mull are expert patients with NAFLD, who previously participated as Committee members in the writing of the NICE NAFLD guidelines (ng 49).

Contribution.

All authors fulfil all four ICMJE criteria for authorship.

Competing interests statement

All authors have completed the ICMJE uniform disclosure form and declare: no support from any organisation for the submitted work, no financial relationships with any organisations that might have an interest in the submitted work in the previous three years, and no other relationships or activities that could appear to have influenced the submitted work.

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FIGURE LEGEND

Fig. 1 - A pragmatic flow diagram for diagnosing and monitoring NAFLD in adults in clinical practice.

Staging of hepatic fibrosis can be undertaken with the use of either biopsy (the "gold standard") or various non-invasive tests. The Enhanced Liver Fibrosis (ELF) blood test (recommended by the NICE Guidelines) has an excellent accuracy in distinguishing advanced fibrosis and cirrhosis. In the case of a patient with an intermediate ELF blood test score (ranging from 7.8 to 10.51), further investigation with vibration controlled transient elastography (FibroScan) could be also considered. The use of the ELF blood test is not recommended or adopted in any country outside the UK or in any international society practice guidelines. So, if the ELF blood testing is not available, a Fibrosis-4 (FIB4) score >2.67; a NAFLD fibrosis score (NFS) score >0.676 or a second-line test such as the vibration controlled transient elastography (FibroScan) (see **Box 5**) can be used (singly or in combination) to identify those individuals with advanced hepatic fibrosis that must be referred to specialists in hepatology. The combination of the FibroScan with FIB4/NFS measurements has shown an excellent accuracy in distinguishing advanced fibrosis[16]. Comprehensive review articles of drug treatment options for NAFLD have been published elsewhere[4 5].

Box 1

How do patients with NAFLD usually present?

Common presentations potentially suggestive of NAFLD
Incidental finding of increased levels (above the upper limit of normal) of serum ALT or AST or both (with other LFTs otherwise normal) and alcohol intake <21 standard drinks per week in men or <14 standard drinks per week in women
LFTs noted to be abnormal as above prior to treatment with a statin for increased cardiovascular risk
Fatigue and LFTs noted to be abnormal as above
LFTs checked as part of investigation of patients with other diseases (e.g. diabetes, obesity, metabolic syndrome, cardiovascular disease)
LFTs being monitored for other conditions or treatments
Incidental finding of hepatic steatosis noted on ultrasound examination of abdomen

Box 2

Common cardiometabolic risk factors for non-alcoholic fatty liver disease (NAFLD).

Overweight/obesity		Apply ethnic-specific cut offs for body mass index (BMI) *e.g.*, white European ≥25/≥30 kg/m^2
Metabolic Syndrome features	Abdominal obesity	Apply ethnic-specific cut offs for waist circumference *e.g.*, 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.

N.B. NAFLD can also occur in lean individuals (the so-called “lean NAFLD”).

Box 3

Risk factors for liver disease to exclude in diagnosing NAFLD.

Risk factors for liver disease	History/initial test results suggestive of alternative diagnoses
>21 standard drinks per week in men and >14 standard drinks per week in women (see footnote*)	History of excessive alcohol consumption
Drugs *e.g*. 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-HCV antibodies/HCV-RNA
Haemochromatosis	High transferrin saturation (>45%) High serum ferritin (>1000 mcg/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 alpha 1 anti-trypsin protein (<260 micromol/l)
Coeliac disease	Anti-tissue transglutaminase antibodies +ve
Others	Occupational exposure to hepatoxins Malnutrition (especially Kwashiokor) Total parenteral nutrition Rapid weight loss Surgically altered bowel anatomy (*e.g*. jejuno-ileal bypass, extensive small-bowel resection) Lipodystrophy Hypobetalipoproteinemia

* According to the US National Institute on Alcohol Abuse and Alcoholism (NIAAA), a standard alcoholic drink is any drink that contains about 14 grams of pure alcohol. A unit of alcohol = 8 grams of alcohol. Of note, the alcohol thresholds for liver disease reported in the Table are not congruent with the UK current thresholds for safe alcohol consumption, which are <14 units per week in both men and women.

N.B.: Low titers of anti-nuclear, anti-smooth muscle and anti-mitochondrial antibodies can be noted in patients with NAFLD (in the absence of autoimmune hepatitis). Similarly, slightly lower caeruloplasmin levels can be also found.

Box 4 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, significant morbidity and even mortality. Not suitable for 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	Whilst the sensitivity of ultrasonography is poor below levels of fatty liver infiltration <20-25% on liver histology, ultrasonography is highly sensitive and specific at higher levels of fat infiltration. Combining standard ultrasonography with computer software technology (MATLAB) e.g. combined ultrasound hepatic/renal ratio and hepatic echo-intensity attenuation rate evaluation[24], improves the sensitivity of ultrasonography even further
Fatty liver index (FLI) (Algorithm-derived score utilising body mass index, waist circumference, fasting serum triglycerides and gamma-glutamyltransferase concentrations)	FLI ≥60 suggestive of hepatic steatosis and validated against ultrasonography[25], or MRS[26].	Inexpensive, but requires waist circumference measurements. Not validated against liver histology
NAFLD liver fat score (Algorithm-derived score utilising the presence of metabolic syndrome and type 2 diabetes, fasting serum insulin, aspartate aminotransferase (AST), and the AST/alanine aminotransferase ratio	Optimal cut-off point = -0.640 for diagnosing hepatic steatosis on MRS[27]	Inexpensive, but requires serum insulin and AST measurements. Not validated against liver histology
Vibration controlled transient elastography (FibroScan)	Optimal Controlled Attenuation Parameter (CAP) thresholds ≥248, ≥268 dB/m for those above stage 1 hepatic steatosis grade, respectively[28]	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[29]	Good for investigating other potential abdominal pathologies. Computer tomography has limited sensitivity to detect low levels (<30% liver fat infiltration on liver histology) and exposes the patient to significant 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 MR spectroscopy identifying ≥5.5% liver fat accumulation[30]	MRI and MRS are very sensitive non-invasive techniques for diagnosing hepatic steatosis, but are presently expensive techniques for this indication

N.B. Ultrasonography was not recommended in the NICE NAFLD guidelines (ng49) based on a failure of the test (for diagnosing hepatic steatosis) to meet the NICE Quality Adjusted Life Years (QALY) thresholds. However, such a cost-effectiveness analysis was not able to take into account that clinicians use liver ultrasonography not only to diagnose liver fat (in the investigation of abnormal liver function tests among suspected cases of NAFLD), but also to exclude other common causes of abnormal liver function tests, such as gallstones or hepatic metastases. Moreover,

combining standard ultrasonography with computer software technology (MATLAB) e.g. combined ultrasound hepatic/renal ratio and hepatic echo-intensity attenuation rate evaluation[24], improves the sensitivity of ultrasonography even 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 (i.e., the gold standard for detecting low levels of liver fat content) (see Box 3), at levels of <15% liver fat content, the sensitivity and specificity of the aforementioned ultrasound quantitative model was 81.4% and 100%. Of note, the use of liver ultrasonography has also been strongly recommended in the recent British Society of Gastroenterology guidelines for investigating abnormal liver function tests[31].

Box 5

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[32] NAFLD fibrosis score (NFS) >0.676[33] Enhanced Liver Fibrosis (ELF) blood test score ≥10.51[34]
Transient elastography *e.g.* FibroScan with M or XL probes (measurement of 'liver stiffness')	Advanced fibrosis threshold Vibration controlled transient elastography >8.7 kPA[35 36]
Acoustic radiation force impulse elastography (ARFI)	Advanced fibrosis threshold ARFI >1.4 m/s[37]
Magnetic resonance imaging techniques *e.g.* magnetic resonance-based elastography (MRE)	Advanced fibrosis threshold MRE >3.64[38]

N.B.: It should be noted that all non-invasive tests for liver fibrosis are better at excluding advanced fibrosis that diagnosing it. N.B.: Patients with NASH may, or may not have significant liver fibrosis. The 'gold standard' for diagnosis of NASH is only liver biopsy, with evidence of hepatocellular ballooning and Mallory bodies.

1. The FIB4 score is calculated as (age × AST) ÷ (platelet count × $\sqrt{}$ALT)

2. The NFS is calculated as follows: −1.675 + 0.037 × age + 0.094 × BMI + 1.13 × IR or diabetes (yes = 1, no = 0) + 0.99 × AST/ALT ratio − 0.013 × platelet count − 0.66 × serum albumin
   New thresholds for use of FIB-4 and the NFS have been recently proposed (and validated) for patients aged ≥65 years to exclude the presence of advanced fibrosis (F3 and F4 stages) (FIB-4 <2.0, sensitivity 77% and specificity 70%; NFS <0.12, sensitivity 80% and specificity 70%)[28]. Conversely, the thresholds of FIB-4 and NFS used in these older patients for identifying the presence of advanced fibrosis remained the same (FIB-4 >2.67)[39]. The NFS is not accurate for excluding advanced fibrosis in individuals aged <30 years

3. The ELF score is a commercial blood test that combines quantitative measurements of three serum direct fibrosis biomarkers (i.e. tissue inhibitor of metalloproteinase 1, procollagen III amino terminal peptide and hyaluronic acid) to a single value[34]. In distinguishing moderate fibrosis, a threshold of ⩾10.51 has an acceptable balance of good sensitivity, specificity, and positive and negative predictive values.

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[36]. A Fibroscan with the XL probe has been also validated for severely obese patients, and has a diagnostic accuracy substantially comparable with that of the standard M probe

5. Magnetic resonance-based elastography (MRE) has the highest diagnostic accuracy for staging fibrosis in NAFLD

Box 6

Performance of common non-invasive biomarker scores for advanced hepatic fibrosis (≥F3 histological stage).

Biomarker score	Advanced fibrosis thresholds (F3 or F4 histological stages)				
	AUROC	Sens (%)	Spec (%)	PPV (%)	NPV (%)
ELF score	0.90	80	90	71	94
NAFLD Fibrosis score (NFS)	0.84	77	70	55	90
FIB-4 score	0.86	85	65	36	95

Abbreviations: Sens, sensitivity; Spec, specificity; PPV, positive predictive value; NPV, negative predictive value.

NB: Performance figures are summary estimates from several studies summarised in[23].

Box 7

"What you need to know"

- When NAFLD is suspected in the presence of abnormal serum liver enzyme levels (in the absence of other competing causes of chronic liver disease), investigate for hepatic steatosis and exclude other liver pathology firstly using liver ultrasonography to diagnose hepatic steatosis and exclude other coexisting liver pathology.
- Once hepatic steatosis has been diagnosed, assess the presence and severity of hepatic fibrosis with the use of non-invasive tests (i.e., biomarker scores and/or vibration controlled transient elastography).
- Refer individuals with suspected advanced hepatic fibrosis to specialists in hepatology for further investigations.

Box 8

“How this article was made”

Search strategy

We searched PubMed for original articles and reviews using the keywords “nonalcoholic fatty liver disease” or “fatty liver” combined with “diagnosis”, “prognosis” or “mortality” between 1990 and 2017. Articles published in languages other than English were excluded from the analysis.

CDB and GT wrote the first draft and all authors reviewed and contributed to the writing of the article.

“How patients were involved in the creation of this article”

Several of our patients have told us of the problem of an inconsistent approach amongst their doctors to investigating their liver disease. Two patient representatives (Ms Irene McGill and Ms Jane Putsey) Mull who have NAFLD and who participated in the NICE NAFLD ng 49 Guideline Development Group commented on the article and gave helpful suggestions to drafts of the manuscript.

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