Liver INTERNATIONAL
OFFICIAL JOURNAL OF THE INTERNATIONAL ASSOCIATION FOR THE STUDY OF THE LIVER

Liver International ISSN 1478-3223

CLINICAL STUDIES

Serum ferritin is a discriminant marker for both fibrosis and inflammation in histologically proven non-alcoholic fatty liver disease patients

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Keywords
ferritin – fibrosis – inflammation – NAFLD – NASH

Abbreviations
ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotranfrerase; BMI, body mass index; CRP, C-reactive protein; DM, diabetes mellitus; Fe, serum iron; γ-GT, gamma-glutamyl transferase; NS, non-significant; TBL, total bilirubin; TIBC, total iron-binding capacity.

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Received 11 April 2010
Accepted 31 January 2011

DOI:10.1111/j.1478-3231.2011.02488.x

Abstract

Introduction: Differentiation between steatosis and non-alcoholic steatohepatitis (NASH) in non-alcoholic fatty liver disease (NAFLD) is important as NASH progress to cirrhosis. No specific laboratory/imaging technique exists either to diagnose NASH or to select patients for liver biopsy. *Patients and methods:* We evaluated serum ferritin and the features of metabolic syndrome with respect to histological inflammation and/or fibrosis in NAFLD patients. The Kleiner scoring system was used to classify NAFLD in consecutive liver biopsies. One hundred and eleven patients: median age 52.6, 64 males, obesity 62, diabetes mellitus (DM) 58, arterial hypertension 26 and hyperlipidaemia 40%. *Results:* Histologically, 40.7 had fatty liver, 30.6% had borderline NASH, 28.7% had NASH and 11% had cirrhosis. Multivariate regression showed that diabetes, serum ferritin concentrations, body mass index (BMI) and AST were independently associated with NASH: together, the areas under the receiver operating characteristic (AUROC) was 0.91 (95% confidence interval 0.86–0.96); fibrosis was associated with ferritin concentrations and BMI: AUROC 0.87, portal inflammation with ferritin and DM: AUROC 0.82, while lobular inflammation was associated with BMI, DM and ferritin: AUROC 0.85. *Conclusion:* Serum ferritin concentrations and BMI are strongly associated with fibrosis, portal and lobular inflammation in NAFLD patients. Both ferritin and BMI are potential discriminant markers to select patients for liver biopsy and are associated with inflammation and fibrosis.

Non-alcoholic fatty liver disease (NAFLD), encompassing simple steatosis, non-alcoholic steatohepatitis (NASH) to cirrhosis, is emerging as one of the most common liver disorders in developed countries (1). In the UK, 34 and 32% of patients with unexplained abnormal liver function tests are subsequently diagnosed as having NASH or fatty liver, respectively, by liver biopsy (2). Although fatty liver is now established to have a benign clinical course (3), NASH can be a cause of progressive liver fibrosis leading to cirrhosis, liver failure and hepatocellular carcinoma (4, 5).

Severe fibrosis is noted in 7–49% of NASH patients and cirrhosis develops in 2–28% (6–10). Those at a high risk of progression include patients with histological advanced fibrosis (5, 11). The diagnosis of NAFLD is clinicopathological and, therefore, although the clinical component of the diagnosis is one of exclusion of significant alcohol ingestion and other causes of chronic liver disease, the histopathological examination is vital in diagnosing and staging suspected NAFLD (12). The important distinction is between fatty liver alone and NASH (i.e. inflammation with or without fibrosis). Given the prevalence of fatty liver, a liver biopsy cannot be considered for all patients. Indeed, in a recent study of an Italian group, fatty liver regressed in 50% of all cases examined and had a benign course (13). Therefore, there have been efforts to determine the predictive markers of fibrosis and/or steatohepatitis with the intention of avoiding biopsies, but to date, none have good clinical applicability (14).

The factors associated with NASH are documented in several studies: male gender, high levels of ALT and AST, γ-glutamyl transferase (γ-GT), triglycerides, type II diabetes mellitus (DM), systemic hypertension and serum acute-phase proteins (9, 15–18).

Non-alcoholic fatty liver disease is also associated with insulin resistance syndrome or recently characterized metabolic syndrome (19), which is associated with insulin resistance (15, 20), diabetes (7, 16), obesity (17) and hyperlipidaemia (7, 15, 18). There is a close relationship between NAFLD and several features of the metabolic syndrome (21, 22).

Serum ferritin is an acute-phase protein and concentrations are increased, in the absence of iron overload, where there is inflammation, liver necrosis and alcohol abuse (23). An increased concentration of serum ferritin in the presence of normal transferrin saturation and normal values of serum iron is usually considered to be iron independent even when the cause of the increase is unknown (24). With regard to NAFLD, increased values of serum ferritin have been reported in patients with DM (25, 26), in subjects with insulin resistance often exhibiting metabolic abnormalities (27) – i.e. high diastolic blood pressure, HDL levels, high glucose and insulin resistance (28) – and also in NASH patients (7, 29–32).

In NAFLD, increased ferritin levels are considered to be an expression of metabolic syndrome and of hepatic damage because of inflammatory cytokine activation (33), although another group assumes that the association between ferritin and the components of the metabolic syndrome may be mediated by undiagnosed NAFLD (34). There are several studies evaluating NASH and increased serum ferritin levels (28, 35). However, there is no consensus as to whether increased ferritin concentrations are associated with severe fibrosis among NAFLD patients (Table 1), and indeed, a current review of non-invasive markers does not mention or consider serum ferritin (14).

The purpose of this study was firstly to establish the frequency of increased serum ferritin concentrations in patients with NAFLD and its association with the components of the metabolic syndrome, And secondly, to evaluate whether these associations (if established) could be used as predictive factors to identify patients with inflammation or fibrosis, to differentiate them from patients with simple fatty liver and thus be a guide as to which patients should have a liver biopsy.

Methods

Patients and liver biopsies

We evaluated consecutive patients whose liver biopsies had a database with the keywords steatosis and/or steatohepatitis as a pathological diagnosis compatible with NAFLD, and reviewed their clinical records. There were 355 such patients. The following variables were recorded: sex, age, renal and thyroid function, blood count, coagulation screen, iron studies, liver enzymes, fasting lipid profile, fasting glucose, HbA1c, alkaline phosphatase (ALP), albumin, creatinine kinase, serum ferritin and C-reactive protein (CRP) as well as urate and body mass index (BMI). Clinical data and blood test results for the patients included in our study were collected within 1 month from the time of the liver biopsy. Patients who did not have such data available were excluded from the study population.

Associations with specific histopathological findings were evaluated by univariate and multivariate logistic regression.

Other types of chronic liver disease were excluded using serological markers for viral hepatitis, autoantibodies, HFE testing and measuring copper and ceruloplasmin and α-1-antitrypsin concentrations. Patients were considered to have DM if they were receiving drug treatment for this disease or they had fasting glucose levels >7 mmol/l. They were defined as being hypertensive if their blood pressure was 130/85 mmHg or if they were receiving treatment for previously diagnosed hypertension. BMI was calculated as weight (kg) divided by squared height (m^2).

Patients with thyroid dysfunction or taking thyroxine and patients consuming >21 U (males) or 14 U (females) of alcohol per week were excluded. Patients were also excluded if they had a history of ingestion of drugs known to cause steatohepatitis such as corticosteroids, methotrexate or oestrogens.

The upper normal limit for ALT was considered to be 31 U/ml as given by our laboratory. Sensitivity analysis was also performed using the proposed upper normal limits of 30 U/ml for males and 19 U/ml for females (48). Similarly, serum ferritin differs between males and females, although we performed sensitivity analysis using the upper normal limits of 340 μg/l for males and 150 μg/l for females.

Histology was reviewed and histological variables were graded following the previously published Kleiner Score (49). Briefly, steatosis was graded 0–3 as follows: low- to medium-power evaluation of parenchymal involvement by steatosis <5, 5–33%, >33–66% and >66% representing 0, 1, 2 and 3 respectively. Fibrosis was staged as 0 representing none, 1, 1A, 1B and 1C representing perisinusoidal or periportal, mild zone 3 perisinusoidal, moderate zone 3 perisinusoidal portal/periportal retrospectively, perisinusoidal and portal/periportal 2, bridging fibrosis 3, cirrhosis 4.

Lobular inflammation was defined as an overall assessment of all inflammation: no foci as $0<2$ foci per $\times 200$ field as 1, 2–4 foci per $\times 200$ field as 2, >4 foci per $\times 200$ field as 3. Portal inflammation was assessed from low magnification: none to minimal as 0 and greater than minimal as 1. We did not consider microgranulomas or lipogranulomas as inflammation.

In our study, all cases were categorized by two pathologists (F. G. and J. W.), blinded to clinical findings, as NAFLD with only steatosis, NASH as having inflammation $+/-$ fibrosis and borderline cases that did not quite fulfil the diagnostic criteria for NASH (50). Patients were placed into two groups: the first group including the

Table 1. List of studies evaluating the correlation between serum ferritin and severe fibrosis among non-alcoholic fatty liver disease patients

Author	Study population	Aim of the study	Conclusion
Bugianesi *et al*. (36)	132 HCV patients with a matched cohort of 132 NAFLD patients	Identify the possible serological determinants of histological differences	Severe fibrosis was predicted by steatosis, ferritin, insulin resistance
Bugianesi *et al*. (37)	263 prospectively enrolled NAFLD patients	Define the impact of iron overload, genetic mutations of HFE and insulin resistance on the severity of liver fibrosis in NAFLD patients	Ferritin independently associated with fibrosis after adjusting for age, sex and BMI
Ledinghen *et al*. (38)	67 patients retrospectively included	Evaluate the prevalence of fibrosis and NASH in patients with accidentally detected elevated ALT	Ferritin was significantly different between $F \geq 2$ and $F < 2$ groups (Metavir)
Giostra *et al*. (39)	505 obese patients at the time of gastric by-pass surgery	Identification of factors to select patients at risk of NASH for liver biopsy	Insulin resistance, DM, ferritin could predict patients with NASH
Loguercio *et al*. (40)	Prospectively enrolled 305 NAFLD patients	Define the characteristics of Italian NAFLD patients	Ferritin correlated univariately with liver fibrosis and inflammation
Moon *et al*. (41)	39 NAFLD patients	Evaluate the association between serum and hepatic iron deposition with hepatic fibrosis or inflammation	Ferritin and BMI associated with inflammation and fibrosis univariately
Canbakan *et al*. (42)	105 NAFLD patients studied, follow-up 6 months	Comparative analysis between clinical, biochemical and histological variables of NAFLD	Serum ferritin has prognostic significance in liver damage and fibrosis. DM is predictive of advanced fibrosis and inflammation
Shimada *et al*. (43)	81 patients with biopsy-proven NASH	Identify features of NASH and risk factors for severe fibrosis	Significant difference of serum ferritin between mild and severe fibrosis but in an adverse way
Angulo *et al*. (44)	144 biopsy-proven NAFLD patients	Identify independent predictors of severe hepatic fibrosis in NASH	Older age, obesity and DM help identify those NASH patients at risk of severe liver fibrosis
Koruk *et al*. (45)	18 NASH patients compared with 16 normal controls	Levels of acute-phase proteins in NASH patients and relation to histopathology	No relation between ferritin and the degree of steatosis, inflammation, liver fibrosis in NASH patients
Albano *et al*. (46)	167 NAFLD patients and 59 controls	Association between immune reactions (because of oxidative stress) and stage of NAFLD	Immune reactions triggered by oxidative stress can be an independent predictor of advanced fibrosis
Fierbinteanu-Braticevici *et al*. (47)	80 NASH patients	Assess the risk factors of fibrosis in NASH	Age, BMI, ALT, glucose and triglycerides, Perls' grade and the serum index of oxidative stress (MDA and GSH) are independent risk factors for fibrosis in NASH

ALT, alanine aminotransferase; BMI, body mass index; DM, diabetes mellitus; GSH, glutathione; HCV, hepatitis C virus; MDA, malondialdehyde; NAFLD, non-alcoholic fatty liver disease; NASH, non-alcoholic steatohepatitis.

NASH patients and the second group including the non-NASH patients. The latter presented with either NAFLD or those cases that were thought to be borderline. This was done so as to have a definitive diagnosis of NASH and hence have a basis for predictive evaluation, as clinically, these patients would be the ones to be considered for liver biopsy. Additionally, we assigned two groups of fibrosis, basing our assessment on the Kleiner score: group 1 (none or mild fibrosis), which included 0, 1 and 1A, and group 2 (moderate fibrosis to cirrhosis), which included 1B, 1C, 2, 3 and 4.

Statistical analysis

Descriptive statistics were performed on all study parameters [mean, standard error of means (SEM), range]. All continuous variables were tested for normality. All normally distributed data were analysed using a *t*-test. Categorical data and derived proportions were analysed using the chi-square test to compare NASH vs no NASH, fibrosis none-mild vs severe-cirrhosis and inflammation vs minimal inflammation.

Diagnostic values for diagnosis, fibrosis and inflammation in NASH were assessed using sensitivities, specificities, positive and negative predictive values and the areas under the receiver operating characteristic (AUROC) curves. Stepwise logistic regression was performed to assess the variables associated with diagnosis, fibrosis and inflammation. A P value <0.05 was considered to be significant in the univariate and multivariate analysis.

A normogram was derived using the predictors from the multivariate analysis for fibrosis and inflammation separately, so as to relate the risk of fibrosis to increasing concentrations of serum ferritin.

The SPSS version 12.0 was used for the statistical analysis.

Results

Patients

There were 111 biopsies from 111 patients who fulfilled the inclusion criteria. The median age was 52.6 years (range: 21–72 years) and 71 patients were men (64%). Obesity was present in 73 (66%) (BMI > 25) while 64 (58%) had DM, 29 (26%) arterial hypertension, 60 (54%) abnormal cholesterol (> 5.2 mmol/l) and 47 (42%) had increased triglycerides (> 2.3 mmol/l). Ferritin was abnormal in 27 (24.5%) of our population (> 340 ng/ml) and CRP in 48 (43%) (> 5). With regard to liver function tests, ALT was normal in 12 (11.1%), AST in 23 (20.6%) and γ-GT in 15 (13.9%). Total bilirubin was within the normal range in 91 (82.2%), albumin in 102 (92.5%) and ALP in 92 (82.5%). Only one male patient had ALT between 30 and 31 IU/l and three females between 19 and 31 IU/l, so that using the cutoff by Prati made no difference to the analyses below (48).

Histologically, the diagnosis was fatty liver in 45 (40.7%), borderline NASH in 34 (30.6%) and NASH in 32 (28.7%); regarding fibrosis, 14 (13%) had perisinusoidal and portal/periportal fibrosis, 10 (9.3) bridging fibrosis and 12 (11.1%) had established cirrhosis. Minimal portal inflammation was present in 56 (50.9) and 15 (13.9%) had prominent ballooning. Lobular inflammation was scored as 3, 2 and 1 in 0.9, 20.4 and 50% of our study group respectively. There was no lobular inflammation in 28.7% of our patients (Table 2).

Table 2. Histological findings in a consecutive cohort of biopsies with non-alcoholic fatty liver disease

Grade/ stage	Steatosis (%)	Fibrosis (%)	Inflammation (%)		Diagnostic classification (%)
			Lobular	Portal	
0	3.7	41.7	28.7	49.1	40.7
1	30.6	25	50	50.9	30.6
2	43.5	13	20.4		28.7
3	22.2	9.3	0.9		
4		11.1			

Histological variables scored according to Kleiner and colleagues (%).
We used the descriptive scoring system for histological variables in NAFLD established by Kleiner and colleagues. Steatosis was graded 0–3 as follows: low- to medium-power evaluation of parenchymal involvement by steatosis < 5%, 5–33%, > 33–66%, > 66% representing 0, 1, 2, 3 respectively. Fibrosis was staged as 0 representing none, 1, 1A, 1B and 1C representing perisinusoidal or periportal, mild zone 3 perisinusoidal, moderate zone 3 perisinusoidal portal/periportal retrospectively, perisinusoidal and portal/periportal 2, bridging fibrosis 3, cirrhosis 4. Lobular inflammation was defined as overall assessment of all inflammation: no foci as 0 < 2 foci per × 200 field as 1, 2–4 foci per × 200 field as 2, > 4 foci per × 200 field as 3. Portal inflammation was assessed from low magnification: none to minimal as 0, greater than minimal as 1: categories were assigned as: not steatohepatitis (NASH) (1), borderline/possible steatohepatitis (NASH) (2) and definite steatohepatitis (NASH) (3) for the study.

Non-alcoholic steatohepatitis diagnosis

There were no statistical differences between NAFLD patients with or without NASH with regard to age, gender, arterial hypertension, hyperlipidaemia and cholesterol. There were also no differences for serum urate, ALT, γ-GT, total bilirubin, albumin, ALP, CRP, serum iron and TIBC (Table 3).

Interestingly, in those with normal LFTs, serum ferritin was higher than normal in 15% with simple fatty liver and 24% with NASH. Univariate analysis revealed that BMI, DM, ferritin and AST were significantly associated with the diagnosis of NASH. Forward regression analysis showed that diabetes status, ferritin concentrations, BMI and AST were independently associated with a diagnosis of NASH (Table 4). ROC curve combining these four variables (BMI, ferritin, diabetes, AST) was derived from regression analysis for the diagnosis of NASH. The area under the ROC curve was 0.91 [95% confidence interval (CI) 0.86–0.96] (Fig. 1).

Fibrosis

In relation to fibrosis, patients' characteristics are shown in Table 5. BMI, ferritin, DM, γ-GT, AST and CRP showed a significant association with fibrosis in univariate analysis. Interestingly, the AST was significantly higher in NASH patients with severe fibrosis. In the multivariate analysis, forward regression analysis showed that ferritin concentrations and BMI were the only two variables independently associated with fibrosis (Table 4). The area under the ROC curve of independent predictors, BMI and ferritin, for the diagnosis of fibrosis was 0.87 (95% CI 0.80–0.93) (Table 6).

When patients with cirrhosis (11%) were excluded from our study group, then BMI, ferritin as well as DM were the three variables predicting fibrosis in the multivariate analysis. The area under the ROC curve for these three predictors was 0.85 (95% CI 0.78–0.93) (Table 6).

Inflammation

In the univariate analysis, portal inflammation correlated with ferritin, DM, total bilirubin, triglycerides and total iron-binding capacity. In the multivariate analysis, ferritin and DM were again significant. The ROC curve of ferritin and DM showed that the area under the curve was 0.816 (95% CI 0.736–0.896) (Tables 4 and 6).

Lobular inflammation showed a univariate correlation with AST, BMI, DM and ferritin. Logistic regression showed that ferritin, DM and BMI were significantly associated with lobular inflammation. The area under the ROC curve was 0.848 (95% CI 0.778–0.918) (Tables 4 and 6).

Interestingly, serum ferritin at a cut-off value of 240 ng/ml and above (derived from the ROC curve), in a

Table 3. Anthropometric, clinical and laboratory data of patients with fatty liver and steatohepatitis

	Normal values	Total	Fatty liver	NASH	*P*
Number, *n* (%)		111 (100%)	47 (42.3%)	64 (57.7%)	
Age (at biopsy)		54 ± 14	53 ± 13	54 ± 12	NS
Gender (male)		63.9%	72.7%	60%	NS
BMI*	< 28 kg/m^2	28.2 ± 5	25.3 ± 2.8	29.2 ± 3.9	0.000
DM*		58.3%	27.3%	73%	0.001
Arterial hypertension	130/85 mmHg	26.2%	36.1%	40.6%	NS
Serum biochemical markers					
Glucose	< 6.1 mmol/l	4.9 ± 1.2	4.6 ± 1.1	5.2 ± 2.1	NS
Triglycerides	< 2.3 mmol/l	2.4 ± 1.3	2.3 ± 1.5	2.4 ± 1.0	NS
HDL	> 1 mmol/l	1.4 ± 0.5	1.3 ± 0.4	1.5 ± 0.6	NS
LDL	< 3 mmol/l	2.9 ± 0.9	2.9 ± 0.9	3.1 ± 0.9	NS
Cholesterol	< 5 mmol/l	5.4 ± 1.2	5.3 ± 1.3	5.5 ± 1.1	NS
AST*	< 31 U/l	49 ± 24	36 ± 9	55 ± 8	0.002
ALT	< 31 U/l	70 ± 39	65 ± 11	72 ± 13	NS
γ-GT	5–36 U/l	98 ± 58	84 ± 17	113 ± 13	NS
TBL	< 19 μmol/l	13.5 ± 9	151 ± 0	12 ± 8	NS
ALP	35–129 U/l	94 ± 46	93 ± 19	97 ± 11.8	NS
Albumin	35–50 g/l	45 ± 4.4	45 ± 4	45 ± 4.5	NS
CRP	0–5 mg/l	5.3 ± 4.4	4.6 ± 3.5	6.3 ± 3.1	NS
Ferritin*	40–340 μg/l	228 ± 100	146 ± 26	309 ± 26	0.000
Fe	11–36 μmol/l	20 ± 7	19.6 ± 6	19.8 ± 6.9	NS
TIBC	53–85 μmol/l	58 ± 10	58.2 ± 10	57.8 ± 10	NS
Urate	0.1–0.4 mmol/l	0.31 ± 0.1	0.28 ± 0.1	0.37 ± 0.1	NS

Values are expressed in mean ± SE or number (*n*) or percent of patients (%).

*Statistically significant in univariate analysis.

ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; CRP, C-reactive protein; DM, diabetes mellitus, Fe, serum iron; γ-GT, gamma-glutamyl transferase; HDL, high-density lipoprotein; LDL, low-density lipoprotein; NS, non-significant; TBL, total bilirubin; TIBC, total iron-binding capacity.

Table 4. Independent predictors of non-alcoholic steatohepatitis (diagnosis), liver fibrosis and inflammation in patients with non-alcoholic fatty liver disease in the multivariate logistic regression analysis

					95% CI for Exp (*B*)	
Diagnosis	*B*	SE	Sig.	Odds ratio	Lower	Upper
BMI	0.157	0.098	0.001	1.398	1.229	2.279
DM	0.007	0.002	0.002	1.007	1.001	1.021
AST	− 2.536	0.659	0.000	0.079	0.020	0.721
Ferritin	0.033	0.017	0.05	1.034	1.003	1.161
Constant	− 8.095	2.779	0.004	0.000		
Fibrosis						
BMI	0.236	0.103	0.022	1.266	1.035	1.550
Ferritin	0.016	0.004	0.000	1.016	1.007	1.024
Constant	− 10.119	3.162	0.001	0.000		
Portal inflammation						
DM	1.101	0.420	0.009	3.007	1.319	6.853
Ferritin	0.019	0.009	0.035	1.019	1.008	1.022
Constant	− 4.726	1.983	0.017	0.009		
Lobular inflammation						
BMI	0.187	0.077	0.014	1.066	1.038	1.401
DM	2.163	0.626	0.001	0.115	0.034	0.392
Ferritin	0.054	0.020	0.007	1.056	1.015	1.099
Constant	− 7.706	2.933	0.009	0.000		

The independent effects of significant variables on diagnosis, fibrosis and inflammation were assessed by multiple regression analysis with forward stepwise selection procedures.

In our study, we defined NASH as steatohepatitis, whereas borderline NASH and fatty liver were defined as no NASH.

AST, aspartate aminotransferase; *B*, estimated co-efficient; BMI, body mass index; CI, confidence intervals; DM, diabetes mellitus; NASH, non-alcoholic steatohepatitis; SE, standard error; sig., significance.

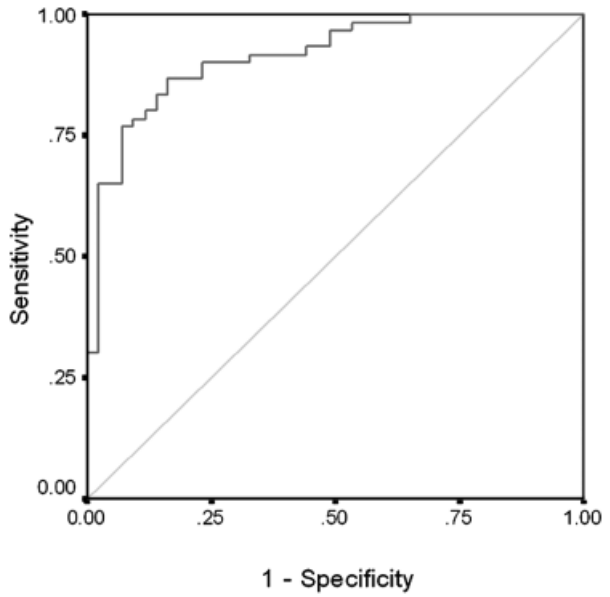

Fig. 1. ROC curve for the diagnosis of NASH using serum ferritin, BMI, diabetes and serum AST. ROC curve of independent predictors derived from regression analysis for the diagnosis of NASH. The diagonal line represents what is achieved by chance alone. The area under the ROC curve is 0.91 (95% CI 0.86–0.96). We defined NASH as steatohepatitis, whereas borderline NASH and fatty liver were defined as no NASH. AST, aspartate aminotransferase; BMI, body mass index; CI, confidence interval; NASH, non-alcoholic steatohepatitis; ROC, receiver operating characteristic curve.

multivariate logistic regression, was significantly associated with stage ($P = 0.034$), lobular inflammation ($P = 0.009$) and portal inflammation ($P = 0.043$). Indeed, above a serum ferritin of 240 ng/ml, 62% of those patients with normal liver function tests had NASH. The cut-off value of serum ferritin at 240 ng/ml ferritin showed a sensitivity of 91% and a specificity of 70% in ascertaining the presence of fibrosis and/or inflammation in NAFLD patients. The area under the ROC curve with respect to a cutoff of 240 ng/ml ferritin evaluating the combination of fibrosis and the two features of inflammation, i.e. NASH was 0.82 (95% CI 0.73–0.90) (Fig. 2).

When females were evaluated separately from males, as the normal range of serum ferritin is lower, there were no differences with males, possibly because most females were post-menopausal (83%). In addition, the components of the metabolic syndrome were distributed without significant differences between males and females.

Discussion

As it is not practical to perform a liver biopsy in every patient with suspected NAFLD, patients are selected for biopsy, sometimes based on ultrasound appearances, lower than the normal platelet count (suggesting possible portal hypertension) or particular features of the metabolic syndrome (e.g. diabetes or obesity) as these features are potentially associated with worse disease (51). It is important to identify NAFLD patients with fibrosis or solely inflammation in association with fatty liver because long-term studies suggest that the presence of NASH is of prognostic importance (52).

Despite the use of non-invasive markers of chronic liver disease, such as serum markers and transient elastography, liver biopsy remains the gold standard for the assessment of fibrosis in NAFLD (14, 53). The NAFLD patients included in our retrospective study were similar to other cohorts in the literature. In particular, those with normal liver function tests had simple fatty

Table 5. Anthropometric, clinical and laboratory data of patients with none to mild fibrosis or moderate fibrosis to cirrhosis

	Normal values	Fibrosis none-mild	Fibrosis moderate-cirrhosis	*P*
Number, *n* (%)		60%	40%	
Age (at biopsy)		46.7 ± 14	49.5 ± 7.7	NS
Gender (male)		63.6%	66.6%	NS
BMI*	< 28 kg/m^2	26.4 ± 3.3	30.3 ± 4.4	0.001
DM*		42.4%	71.4%	0.006
Arterial hypertension	130/85 mmHg	39.4%	40.5%	NS
Serum biochemical markers				
Glucose	< 6.1 mmol/l	5.5 ± 0.7	6.1 ± 0.9	NS
Triglycerides	< 2.3 mmol/l	2.4 ± 1.1	2.5 ± 1	NS
HDL	> 1 mmol/l	1.5 ± 0.6	1.4 ± 0.5	NS
LDL	< 3 mmol/l	2.9 ± 0.9	2.9 ± 1.0	NS
Cholesterol	< 5 mmol/l	5.1 ± 0.8	5.3 ± 1.3	NS
AST*	< 31 U/l	44 ± 16	64 ± 22	0.000
ALT	< 31 U/l	72 ± 22	73 ± 21	NS
γ-GT*	5–36 U/l	72 ± 11	182 ± 38	0.003
TBL	< 19 μmol/l	11 ± 6	12 ± 5	NS
ALP	35–129 U/l	68 ± 18	77 ± 28	NS
Albumin	35–50 g/l	47 ± 3	49 ± 6	NS
CRP*	0–5 mg/l	5.5 ± 2.1	6.1 ± 2.7	0.04
Ferritin*	40–340 μg/l	117 ± 19	377 ± 27	0.000
Fe	11–36 μmol/l	18.8 ± 7.3	21.3 ± 2.12	NS
TIBC	53–85 μmol/l	57.8 ± 11.3	60.5 ± 0.7	NS
Urate	0.1–0.4 mmol/l	0.3 ± 0.1	0.4 ± 0.1	NS

Values are expressed in mean ± SD or number (*n*) or per cent of patients (%).

*Statistically significant in univariate analysis.

For abbreviations, see footnote in Table 3.

Table 6. Characteristics of receiver operating characteristic curves for fibrosis, portal and lobular inflammation

State variable	Test variable	AUROC	95% CI
Fibrosis	Ferritin and BMI	0.87	0.80 – 0.93
Fibrosis (excluded cirrhotic patients)	Ferritin, BMI, DM	0.85	0.78 – 0.93
Portal inflammation	Ferritin and BMI	0.82	0.74 – 0.90
Lobular inflammation	Ferritin, BMI, DM	0.85	0.78 – 0.92

AUROC, area under the ROC curve; BMI, body mass index; CI, confidence intervals; DM, diabetes mellitus; ROC, receiver operating characteristic curve.

liver in 24% and NASH in 15%. Thus, our patients are representative of NAFLD patients seen in other studies including prospective ones from various countries (36, 40, 44, 47).

In this study, we showed that increased concentrations of serum ferritin are an independent predictor of fibrosis (78% sensitivity, 50% specificity) and inflammation, both portal (78% sensitivity, 60% specificity) and lobular (85% sensitivity, 67% specificity). In this context, it is the sensitivity that is useful, as in clinical practice, other forms of liver disease are excluded by routine laboratory assays. Thus, these findings could aid the clinician in the selection of which NAFLD patients should have a liver biopsy.

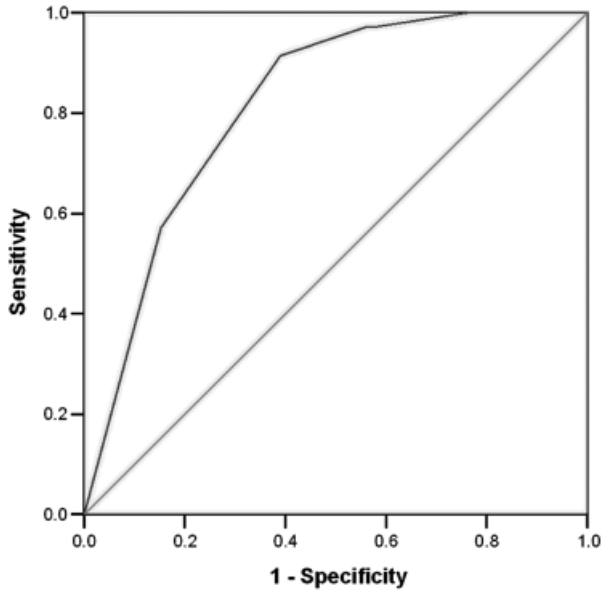

Fig. 2. ROC curve using a cutoff for serum ferritin of 240 µg/l or more with respect to a histological diagnosis of NASH, i.e. fibrosis and/or inflammation in NAFLD patients. The area under the ROC curve is 0.82 (95% CI 0.73–0.90). In our study, we defined NASH as steatohepatitis, whereas borderline NASH and fatty liver were defined as no NASH. CI, confidence interval; NAFLD, non-alcoholic fatty liver disease; NASH, non-alcoholic steatohepatitis; ROC, receiver operating characteristic curve.

Using the cutoff of serum ferritin 240 ng/ml or more, and a BMI >28.2, this combination identified patients at risk of having fibrosis with an 82% sensitivity and a 79% specificity. Interestingly, when patients with cirrhosis (11%) were excluded from the study group, and applying the same cutoffs, fibrosis was predicted with a 90% sensitivity and a 72% specificity in diabetic patients and 86% sensitivity and 77% sensitivity in the entire study group.

Our results could be used to test the clinical utility of serum ferritin concentrations in decision making regarding a liver biopsy in patients with clinically diagnosed NAFLD (Figs 3 and 4). In particular, the association of an increased BMI >28.2 and increased ferritin >240 ng/ml seems to indicate a particularly high risk of NASH, and in the face of a normal ALT, being obese but with a ferritin of 240 ng/ml or more is associated with NASH in 62% of cases. Moreover, using four variables consisting of serum ferritin, DM, BMI and AST, we could predict NASH with a sensitivity of 92 and a specificity of 80%.

In a recently published study (54), the characteristics of 823 patients with biopsy-proven NAFLD were analysed retrospectively. The authors compared two groups of patients similar to our comparison groups and concluded that BMI ≥ 28, AST/ALT ratio ≥ 0.8, and the presence of diabetes are independent predictive factors for severe fibrosis. Combining these variables, a clinical scoring system was devised for predicting severe fibrosis in NAFLD patients (54).

However, in a recently published review (53), commenting on the use of non-invasive tests to predict hepatic fibrosis in NAFLD patients, it was highlighted that prospective validation of these clinical scoring systems is lacking.

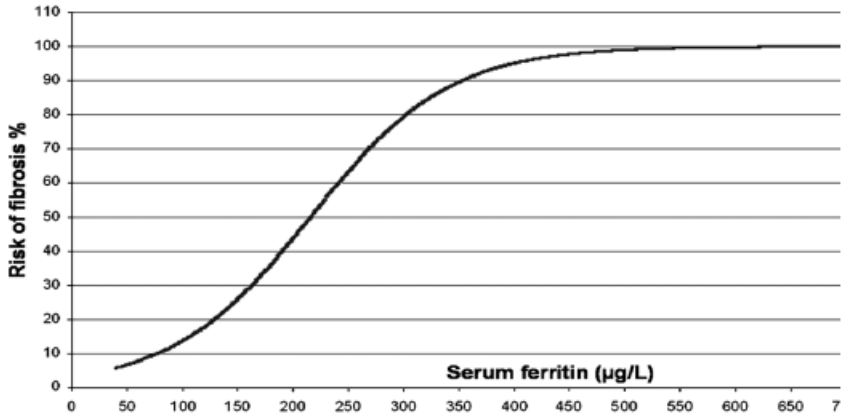

Fig. 3. Nomogram of the risk of fibrosis in liver biopsies of 111 NAFLD patients adjusted for BMI. The independent predictors were derived from the logistic regression analysis to calculate this nomogram. We used the mean value of BMI in our study group (28.2). The probability of fibrosis was calibrated using the equation: $P = e^{\text{odds of fibrosis}}/(1+e^{\text{odds of fibrosis}})$. BMI, body mass index; NAFLD, non-alcoholic fatty liver disease.

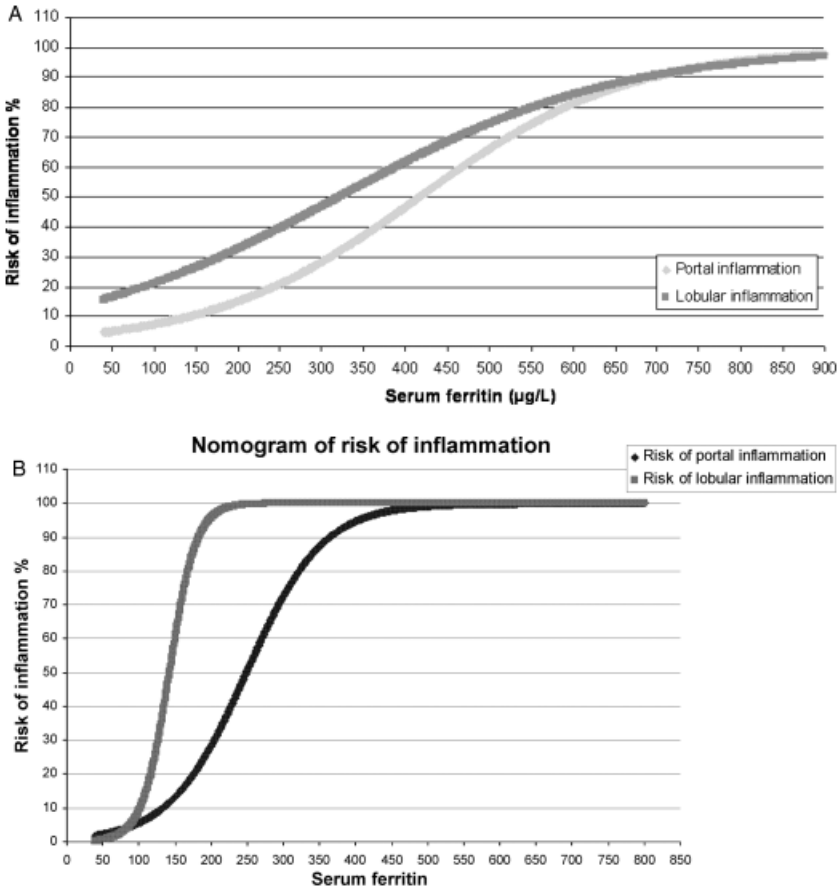

Fig. 4. (a) Nomogram of the risk of portal and lobular inflammation as components of NASH in liver biopsies of NAFLD patients without diabetes. The predicted probability of portal and lobular inflammation as components of NASH related to serum ferritin concentrations. The probability of lobular inflammation is adjusted for BMI (mean = 28.2 in our population). Predicted probability was calculated as $e^{\text{odds of inflammation}}/(1+e^{\text{odds of inflammation}})$ derived from logistic regression analysis. Patients were considered to have diabetes mellitus if they were receiving drug treatment for this disease or if they had fasting glucose levels > 6.1 mmol/l. (b) Nomogram of the risk of portal and lobular inflammation as components of NASH in liver biopsies of NAFLD patients with diabetes. The predicted probability of histological inflammation related to serum ferritin concentrations. The probability of lobular inflammation is adjusted for BMI (mean = 28.2 in our population). Predicted probability was calculated as $e^{\text{odds of inflammation}}/(1+e^{\text{odds of inflammation}})$ derived from logistic regression analysis.

This also applies to our study, but it has the advantage of evaluating both inflammation and fibrosis separately as well as together, making our data on serum ferritin much more robust. Our findings are similar and in accordance to those of a recently published study (55) evaluating 458 NAFLD patients, in which serum ferritin was an independent factor for predicting severe fibrosis, but not in patients with normal ALT values ($n = 63$).

Our findings suggest that serum ferritin can be used in the decision making to perform a biopsy in a patient who by exclusion has NAFLD, and identify as a group those who have either inflammation or fibrosis or both. Its potential utility as assessed by AUROC is as good as other proposed markers, and, when combined with BMI, diabetes and AST, is better than models in the literature. Our results confirm those of Bugianesi and colleagues (36, 37, 55), de Ledinghen (38) that serum ferritin is an independent factor associated with NASH in NAFLD patients. Validation of our results as for other studies is needed, and should be relatively easy as serum ferritin is a routine test that is already obtained when assessing chronic liver disease.

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