Elevated serum ferritin in non-alcoholic fatty liver disease is not predictive of fibrosis

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ABSTRACT

BACKGROUND: Non-alcoholic fatty liver disease (NAFLD) is common with widely ranging severity. Non-invasive risk scores for risk stratification are recommended but misclassify a significant proportion of patients. In situations where non-invasive risk scores do not provide guidance, referral is typically made to a Hepatologist for transient elastography or liver biopsy. Serum ferritin is elevated in many patients with NAFLD related to dysmetabolic and inflammatory hyperferritinemia. Ferritin is widely available and part of a standard workup for chronic liver disease.

METHODS: To explore the association of ferritin and risk of fibrosis in NAFLD, we reviewed patients diagnosed with NAFLD at the hepatology clinic of the Vancouver General Hospital between the years of 2015 and 2018. We collected data on 317 patients retrospectively assessing for a relationship between serum ferritin and elastography score.

RESULTS: Two hundred twenty-four patients were included in the final analysis. Median ferritin was 145 µg/L (IQR 62–311). Median liver stiffness was 5.2 kPa with 14.3% of patients having liver stiffness ≥8.7 kPa and 17.4% ≥ 8.0 kPa. ROC curve analysis using a liver stiffness ≥8.0 kPa as a cutoff for F2 fibrosis showed an AUROC of 0.54 for serum ferritin levels. At a cut-off of both 300 µg/L; and 450 µg/L median liver stiffness did not differ significantly in those with ferritin above the cutoff (ferritin ≥300 µg/L; p = 0.099, ferritin ≥450 µg/L; p = 0.12). Ferritin was significantly higher in male patients (198 versus 91 µg/L; p = 0.001). There was a weak linear association between AST and ferritin levels.

CONCLUSION: In this cohort of 224 patients with NAFLD, serum ferritin was not predictive of significant liver fibrosis.

KEYWORDS: disease; fatty; ferritin; fibrosis; liver; non-alcoholic; steatohepatitis

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INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) is defined as evidence of hepatic steatosis, either by imaging or histology with no cause for secondary hepatic fat accumulation including significant alcohol consumption, steatogenic medications, or hereditary disorders (1). Results from a meta-analysis in 2016 demonstrated a global prevalence of NAFLD of 25.2%, which is estimated to reach 33.5% by 2030 (2).

Higher prevalence is associated with higher rates of obesity and metabolic disease (3). Importantly, a large body of evidence suggests that only a subset of NAFLD patients develop non-alcoholic steatohepatitis (NASH) and of those, only some develop progressive liver dysfunction, cirrhosis, and hepatocellular carcinoma (3). However, given the large number of patients with NAFLD/NASH, a significant number progress to end-stage disease and malignancy. Identification of patients with high risk of progression is therefore crucial to managing NAFLD.

The most recent AASLD guidelines recommend risk stratification using non-invasive tests for fibrosis (NAFLD fibrosis score, FIB-4, or transient elastography) or liver biopsy in patients considered at risk of progressive liver disease (1). Accurately predicting risk of fibrosis in an undifferentiated population of patients with NAFLD with non-invasive tests reduces unnecessary hepatology referrals and liver biopsies with a reduction in biopsy associated complications and allows for more efficient resource use (4–8).

Serum ferritin is a potential biochemical marker of NAFLD severity that is widely available, relatively inexpensive, and frequently included as a part of the standard workup for chronic liver disease. Ferritin is an iron storage molecule found in the liver, serum, bone marrow, and, to a lesser extent, in other tissues (9). Levels of ferritin in serum are measured as a proxy for total body iron stores but also increase with systemic and liver specific inflammation as well as in metabolic disease such as obesity and diabetes mellitus (10,11). Dysmetabolic hyperferritinemia is the elevation of serum ferritin levels in the absence of iron overload caused by metabolic syndrome and is common in patients with NAFLD (12). Dysmetabolic iron overload is a related, though rarer condition with a mild increase in hepatic iron accumulation (12). Dysmetabolic hyperferritinemia has been reported to represent non-iron related causes of liver disease more commonly than iron overload in some populations (13). Hyperferritinemia has been reported to correlate with histologic severity of NAFLD and with presence of NASH independent of hepatic iron stores (10,14). Furthermore, at least one study of 628 patients showed an association between elevated serum ferritin and fibrosis on biopsy though with a wide confidence interval (10).

Transient elastography (TE) is a non-invasive test of liver stiffness and is beginning to replace liver biopsy as the first-line confirmatory test of fibrosis (15). Due to potential for sampling error with biopsy it also provides a potentially more accurate picture of the degree of fibrosis in NAFLD (16). Transient elastography, however, remains difficult to access for the majority of patients with NAFLD and is usually limited to hepatology specialists. Given the value of identifying non-invasive, widely available predictors of fibrosis in the primary care setting, we sought to evaluate whether serum ferritin values were associated with significant fibrosis identified on transient elastography.

MATERIALS AND METHODS

This study was an observational, retrospective cohort study of patients referred for NAFLD evaluation to the Vancouver General Hospital hepatology clinic from January 2015 to August 2018. This clinic sees a high volume of hepatology consults covering the full spectrum of liver diseases. Approval was obtained prior to chart review from the Clinical Ethics Review Board of the University of British Columbia.

Patients aged 18 years or greater, with liver disease compatible with NAFLD who underwent transient elastography were included in the study. Liver disease compatible with NAFLD was defined as hepatic steatosis with no identified cause on global assessment, including:

- Hypertension (≥140/90 mmHg), high serum triglyceride levels (≥1.7 mmol/L), low serum HDL (≤0.9 mmol/L), glucose intolerance/diabetes (Fasting plasma glucose >6.0 mmol/L, hemoglobin A1c ≥6.0%, 2-hour oral glucose tolerance test >7.7 mmol/L), truncal obesity (waist to hip ratio >0.9 or waist circumference >102 cm in men and >0.85 or >88 cm in women) or ethnic risk factors (Asian or Latin American descent).
Exclusion criteria included genetic disorders of metabolism and other causes of liver disease including hepatitis B, hepatitis C, Wilson’s disease, autoimmune hepatitis, primary biliary cholangitis, primary sclerosing cholangitis, drug-induced liver injury, alpha-1-antitrypsin deficiency, and those with known hereditary hemochromatosis (HFE heterozygous or homozygous) or other diseases of iron overload (eg, transfusion associated). We also excluded those with presence of ascites, hepatocellular carcinoma, or intrahepatic cholangiocarcinoma or large (>5 cm) benign liver tumours including hemangiomas, adenoma, focal nodular hyperplasia, direct hyperbilirubinemia (total bilirubin >34 umol/L with >50% direct fraction), liver disease secondary to post-hepatic vascular flow obstruction (right heart failure, Budd-Chiari syndrome), concomitant diagnosis of sinusoidal obstruction/portal-sinusoidal disease) and finally alcohol consumption of >15 units weekly in men or >10 units weekly in women.

All patients’ weights and heights were measured before the TE. All TE were performed by experienced operators with more than 100 measurements of experience and all patients were instructed to fast for a minimum of 3 hours prior to their test. A minimum of 10 readings in each patient were performed and median results with an interquartile range/median score of <0.3 were considered valid. We used the closest ferritin value measured within the last three months prior to the TE as a reference. Presence of hypertension, triglycerides, hemoglobin A1c, AST, ALT, platelet count, bilirubin, INR, albumin, ferritin measured within 3 months of TE were collected. TE was performed using a FibroScan 530 Compact device (Echosens, France) with the appropriate probe based on patient size. Low likelihood of fibrosis (>F1) was defined as <8.0 kPa and high likelihood of significant fibrosis was defined as a TE score >8.7 kPa (F3-F4).

Medians and interquartile ranges were calculated for all continuous variables. We compared ferritin levels against liver stiffness visually using a scatter plot using both ferritin and log (ferritin). Receiver operating characteristic curve analysis was performed using ferritin and log (ferritin) to predict TE score ≥8.0 kPa. Gender, age, ALT, AST, BMI, bilirubin, presence of hypertension, and presence of impaired fasting glucose or diabetes were also tested as potential predictors of higher ferritin levels. Median TE scores were also compared to elevated ferritin at cutoffs of ≥300 µg/L and ≥450 µg/L. Median values were compared using the Kruskal-Wallis test. Statistical analyses were performed using Stata 15 software (College Station, Texas). An alpha level of significance was defined as a p value <0.05 in a two-tailed t-test.

**RESULTS**

Data was obtained from 317 patients on initial screening. After removing patients with incomplete data, 224 remained and were used for the study. The baseline characteristics of patients included in this cohort are shown in Table 1. Median age was 52 years (IQR 43–60), 50% were male, 27% were of East Asian ethnicity, median BMI was 28.0 (IQR 25.3–31.8), 21.9% had diabetes or impaired fasting glucose, and 37% had hypertension. The median grams of alcohol per week was 0 (IQR 0–13). Median AST and ALT were 30 IU/L (IQR 22–45) and 43 IU/L (IQR 27–65) respectively. Median HbA1c was 5.8% (IQR 5.3–6.3), median ferritin was 145 µg/L (IQR 62–311). Median liver stiffness was 5.2 kPa with 14.3% of patients having liver stiffness ≥8.7 kPa and 17.4% ≥ 8.0 kPa. Receiver operating characteristic curve (shown in Figure 1) analysis using a liver stiffness ≥8.0 kPa as a gold-standard showed an AUROC of 0.54 for serum ferritin levels. At a cut-off of both 300 µg/L and 450 µg/L, fibrosis score was not significantly different for those with elevated ferritin compared with non-elevated ferritin. For those with ferritin ≥300 µg/L, median TE score was 5.35 kPa (IQR 4.6–7.68) versus 5.1 kPa (IQR 4.3–6.55) for those with ferritin <300 µg/L (p = 0.099). For those with ferritin ≥450 µg/L, median TE score was 5.3 kPa (IQR 4.6–7.9) versus 5.1 kPa (IQR 4.3–6.6) for those with ferritin < 450 (p = 0.12). Figure 2 illustrates using a boxplot the distribution of the transient elastography score using a cutoff of ferritin at 300 µg/L. Although not shown, a similar distribution was seen with a cutoff value of 450 µg/L. Ferritin was significantly higher in male patients (198 versus 91 µg/L, p < 0.001). There was a very weak linear association between AST and ferritin levels ($R^2 = 0.099$, $p < 0.001$). Using logarithm transformation of ferritin values did not yield different results (not shown).
DISCUSSION

Given the growing prevalence of NAFLD, cost-effective and easily accessible risk stratification is critical to identifying patients at risk of significant liver disease. While current non-invasive scoring systems such as the NAFLD fibrosis score and the FIB-4 score are effective and well-validated, they leave a significant percentage of patients unclassified and in need of further testing. The NAFLD fibrosis score (NFS) misclassifies or provides no guidance for up to 32.5% of patients. Using the sensitivity and specificity of the authors defined optimal upper and lower cut-offs leaves 25.0% of patients classified as indeterminate and leaves a further 7.5% miscategorized (4). Similar analyses of the NFS and other predictive scores

Table 1: Summary of patient characteristics

| Characteristic                                      | Overall; N = 224 | Fibrosis (kPa ≥ 8.0); n = 39 | No Fibrosis (kPa < 8.0); n = 185 |
|----------------------------------------------------|-----------------|-------------------------------|---------------------------------|
| Demographics                                       |                 |                               |                                 |
| Age, y, median (IQR)                               | 52 (43–60)      | 57 (52–63)                    | 50 (41–59)                      |
| Male sex, no. (%)                                  | 112 (0.50)      | 17 (0.43)                     | 95 (0.51)                       |
| East Asian, no. (%)                                | 61 (0.27)       | 9 (0.23)                      | 52 (0.28)                       |
| Non-East Asian, no. (%)                            | 163 (0.73)      | 30 (0.77)                     | 133 (0.82)                      |
| Comorbidities                                       |                 |                               |                                 |
| Impaired fasting glucose or diabetes, no. (%)      | 49 (0.22)       | 20 (0.51)                     | 29 (0.15)                       |
| Hypertension, no. (%)                              | 83 (0.37)       | 18 (0.46)                     | 65 (0.35)                       |
| Alcohol intake, g/wk, median (IQR)                 | 0 (0–13)        | 0 (0–7)                       | 0 (0–14)                        |
| Physical measurements, median (IQR)                |                 |                               |                                 |
| Weight, kg                                         | 80 (70–91)      | 85 (73–105)                   | 79 (70–90)                      |
| Height, m                                          | 1.68 (1.60–1.75)| 1.68 (1.57–1.73)              | 1.68 (1.6–1.76)                 |
| BMI, kg/m²                                         | 28.0 (25.3–31.8)| 30.9 (27.4–36.6)              | 27.5 (25–31)                    |
| Transient elastography, kPa                        | 5.2 (4.4–6.8)   | 14.5 (9.3–21.65)              | 4.9 (4.3–5.8)                   |
| NAFLD fibrosis score                               | –2.21 (–3.05 to –1.07) | –1.03 (–1.85 to –05)        | –2.37 (–3.29 to –1.61)          |
| Laboratory values, y, median (IQR)                 |                 |                               |                                 |
| ALT, IU/L                                          | 43 (27–65)      | 54 (34–95)                    | 41 (26–64)                      |
| AST, IU/L                                          | 30 (22–45)      | 47 (36–68)                    | 28 (21–38)                      |
| Albumin, g/L                                       | 44 (42–46)      | 42.5 (38–45)                  | 44 (42–46)                      |
| Bilirubin, mmol/L                                  | 9 (7–13)        | 8 (7–15)                      | 9 (7–13)                        |
| Ferritin, µg/L                                     | 145 (62–311)    | 161 (82–365)                  | 135 (60–304)                    |
| Iron saturation                                    | 0.29 (0.21–0.36)| 0.28 (0.21–0.33)              | 0.29 (0.21–0.37)                |
| INR                                                | 1.0 (0.9–1.0)   | 1.0 (0.9–1.0)                 | 1.0 (0.9–1.0)                   |
| Platelets                                          | 241 (210–283)   | 248 (201–283)                 | 241 (211–283)                   |
| HbA₁c, %                                           | 5.8 (5.3–6.3)   | 6.4 (5.8–7.2)                 | 5.6 (5.3–6.1)                   |
| Triglycerides, mmol/L                              | 1.5 (1.0–2.2)   | 1.4 (1.1–2.0)                 | 1.5 (1.0–2.2)                   |
| LDL, mmol/L                                        | 2.74 (2.1–3.4)  | 2.41 (2.1–3.1)                | 2.89 (2.17–3.40)                |

LDL = low-density lipoprotein; IQR = interquartile range
Figure 1: ROC curve using serum ferritin to predict significant fibrosis
Serum ferritin performed poorly as a predictor of significant fibrosis defined as ≥8.0 kPa on transient elastography
AUROC = 0.54

Figure 2: Boxplot showing distribution of transient elastography scores based on ferritin levels
Elevated serum ferritin in NAFLD is not predictive of fibrosis

(FIB-4 and Enhanced Liver Fibrosis score) in a large database of patients recruited for clinical trials found reasonable sensitivity and specificities for significant fibrosis of 74.0%–89.0% and 89.0%–98.0% respectively using a dual cut-off method. Unfortunately, this approach still left 43.0%–51.0% of patients with an indeterminate risk rating, requiring further investigations to identify or exclude significant fibrosis (6). In the same study, an additional 11.0%–19.0% of patients were misclassified using liver biopsy as a gold standard, thus yielding a negative predictive value of only 60.0%–68.0% (6).

In dedicated hepatology clinics, patients are commonly risk stratified using TE as a non-invasive measurement of liver fibrosis as opposed to liver biopsy or risk score. TE is a well-validated method of measuring liver stiffness and correlates well with fibrosis stage (5,6). TE, however, requires expensive and specialized equipment and is not usually available in primary care.

Abnormalities in markers of iron metabolism in non-hemochromatosis-associated liver disease are well-described (9,10,13,16). Metabolic disease and inflammation related hyperferritinemia is the most common manifestation, but increased stainable iron in liver tissue, increased transferrin, and inappropriate serum hepcidin levels have also been shown to occur in patients with non-hemochromatosis related liver disease (10). While serum ferritin >1.5 x ULN was found to be associated with advanced fibrosis and histologic severity of inflammation in one study, there was no linear association between ferritin level and risk of fibrosis, namely those with serum ferritin >3.5 x ULN were not more likely to have fibrosis than those >1.5 x ULN (9). Additionally, iron depletion does not improve markers of either metabolic or liver disease in a large meta-analysis suggesting against a pathophysiologic role for iron deposition as a significant cause of liver fibrosis for most patients (17).

Although serum ferritin has been correlated to increased histologic activity in NAFLD and to fibrosis in previous published cohort studies, the relationship was weak (OR 1.66, \( p = 0.028 \)) (9). In this retrospective cohort, we did not find a significant correlation between elevated ferritin and liver fibrosis as measured by transient elastography. We found no correlation despite using high sensitivity cutoffs for fibrosis and a range of ferritin values suggesting against utility of ferritin in predicting significant fibrosis. Strengths of this study include a relatively large sample size. However, we lacked histology for the majority of patients, although in clinical practice, it would be difficult to justify an invasive biopsy for most patients. We had a relatively small number of patients with significant fibrosis suggesting that the study may have been underpowered to detect a significant difference. However, the relatively large cohort of patients is representative of the patients with NAFLD seen in a Canadian community outpatient setting and is generalizable to what is encountered by most Canadian physicians. The lack of utility of serum ferritin as an indicator of fibrosis from NAFLD suggests that it would be of little value in this role, to Canadian physicians and patients.

Although this study is a negative one, our experience strongly suggests that future clinical research efforts and resources exploring non-invasive markers of fibrosis in NAFLD would be better expended studying biomarkers other than the serum ferritin.

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