Research Letter

Spleen size does not correlate with histological stage of liver disease in people with non-alcoholic fatty liver disease

Short Title: Splenomegaly in NAFLD

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Splenomegaly in the context of liver disease is classically associated with advanced cirrhosis and portal hypertension[1]. More recently, we observed an increasing number of patients with splenomegaly and non-alcoholic fatty liver disease (NAFLD), but in whom intensive work-up revealed no evidence of advanced liver disease or portal hypertension. Interestingly, splenomegaly has been reported in association with obesity and the metabolic syndrome[2–4], both contributors to the pathogenesis and progression of NAFLD. In this retrospective study, we sought to test the correlation between splenomegaly and histological stage of liver disease, as well as a number of anthropometric and biochemical characteristics.

All patients undergoing a liver biopsy for NAFLD staging at Cambridge University Hospitals, UK, between January 2015 and December 2019, and who had a recorded sonographic measurement of longitudinal spleen size within one year of biopsy, were included in this study. Splenomegaly was defined as a longitudinal spleen length greater than 13 cm[5]. Patients with alternative causes of splenomegaly were excluded. Liver biopsies were scored by an expert histopathologist (SED) using the Clinical Research Network (CRN) scoring system for steatosis (S0-3), ballooning (B0-2), inflammation (I0-I4) and fibrosis (F0-F4). Body height and weight were measured using standard methods, and body mass index (BMI) calculated as the ratio of weight to height squared. Fasting lipid profile and glycated haemoglobin (HbA1c) were measured using standard laboratory methods. Prior to assessing for correlations, the data was assessed for statistical outliers in order to avoid leverage points. Data was analysed using Fisher's exact test (categorical data), simple linear regression (continuous data) and multiple linear regression using GraphPad Prism v8.

We identified 275 patients who attended our centre between 2015 and 2019, and had both a liver biopsy and recorded spleen size. Five had alternative causes of splenomegaly and were excluded from further analysis (acromegaly n = 1; follicular lymphoma n = 2, spherocytosis n = 1, chronic lymphocytic leukemia n = 1). Sixty per cent of subjects were male, with a mean BMI of 35.5 ± 6.2 kg/m^2, and mean age of 56.0 ± 10.4 years (Table 1). All histological stages of fibrosis were represented (F0 = 4%, F1 21.9%, F2 18.9%, F3 43.0%, F4 = 12.2%). Mean spleen size was 11.9 ± 2 cm, with a mean platelet count of 215.3 x10^9/L.

There was no correlation between stage of liver disease and spleen size in NAFLD.

Splenomegaly, conventionally defined as a longitudinal spleen length greater than 13 cm[5], was found in 27.4% of subjects across all histological grades of fibrosis (F0 = 6.8%, F1 = 16.2%, F2 = 24.3%, F3 = 31.1%, F4 = 21.6%). There was no association between spleen size and degree of steatosis, ballooning degeneration of hepatocytes or lobular inflammation (Table 1, Supplementary Figure 1). None of the subjects with F0 – F3 fibrosis had sonographic features of portal hypertension (no ascites or collateral vessels). There was evidence of portal hypertension in 4/33 (12.1%) of patients with biopsy-proven cirrhosis.

Body weight is the strongest predictor of spleen size, followed by high density lipoprotein (HDL).

We found a positive correlation between spleen size and body height, body weight and BMI, as well as triglycerides and the ratio of triglycerides to HDL cholesterol, considered a marker of insulin resistance. There was a negative correlation between spleen size and age, and separately HDL-cholesterol. Parameters with a positive correlation with spleen size were taken forward in a multivariate analysis to predict spleen size from age, height, body weight, HDL and triglycerides. The model statistically significantly predicted spleen size ($F(5,257) = 13.6$, $p < 0.0001$, adj. $R^2 = 0.19$). Body weight was the greatest determinant of spleen size ($p = < 0.0001$), followed by HDL-cholesterol ($p = 0.0004$) (Table 1). There was a negative correlation between spleen size and platelet count ($p = 0.0006$) (Table 1).

In this study, we found no correlation between splenomegaly and the degree of underlying liver disease in a large population of people with biopsy proven NAFLD. We did find a strong correlation between spleen size and body weight, suggesting an alternative mechanism for splenomegaly in the context of obesity, such as increased visceral deposition of lipid in the spleen. This finding could avoid unnecessary investigation in patients with a clinical diagnosis of NAFLD and reassuring non-invasive assessment of fibrosis. Similarly, these findings suggest that splenomegaly is not a good indicator of progressive liver disease in patients with fatty liver disease. Bassegoda *et al* recently demonstrated that hepatic venous pressure gradient (HVPG) is not a good predictor of decompensating events in NAFLD compared to hepatitis C related cirrhosis, possibly due to increased presinusoidal resistance[6]. Here, we demonstrate that extra-hepatic factors – namely obesity – contribute to splenomegaly in NAFLD, making it a poor biomarker of progressive liver disease. Both observations support the need for better

understanding of the mechanisms underpinning progressive disease in NAFLD, and for the development of alternative biomarkers for disease monitoring and prognostication specific to this patient group.

The significant negative association between splenomegaly and HDL-cholesterol is interesting, and may be due to increased sequestration of HDL in splenic lymph. If this is the case, given that low levels of HDL are a risk factor for cardiovascular disease[7–9], we speculate that splenomegaly may be a predictor of cardiovascular events in NAFLD, and merits further evaluation in larger cohorts.

We recognize the limitations of this study, namely, the retrospective observational design and the sonographic measurement of spleen size in a predominantly obese population. Lamb *et al* demonstrated that spleen length, measured by ultrasound, correlated well with splenic volume as assessed by three-dimensional computed tomography (CT)[10]. Prospective studies correlating splenic volume assessed by cross-sectional imaging and histologic stage of fatty liver disease could address this question more accurately.

References

1. Berzigotti A, Seijo S, Arena U, et al. Elastography, spleen size, and platelet count identify portal hypertension in patients with compensated cirrhosis. Gastroenterology 2013;144.
2. Tsushima Y, Endo K. Spleen enlargement in patients with nonalcoholic fatty liver: Correlation between degree of fatty infiltration in liver and size of spleen. Dig Dis Sci 2000;45:196-200.
3. Abd El-Aziz R, Naguib M, Rashed LA. Spleen size in patients with metabolic syndrome and its relation to metabolic and inflammatory parameters. Egypt J Intern Med 2018;30:78-82.
4. Chow KU, Luxembourg B, Seifried E, et al. Spleen Size Is Significantly Influenced by Body Height and Sex: Establishment of Normal Values for Spleen Size at US with a Cohort of 1200 Healthy Individuals. Radiology 2016;279:306-313.
5. Pozo AL, Godfrey EM, Bowles KM. Splenomegaly: Investigation, diagnosis and management. Blood Rev 2009;23:105-111.
6. Bassegoda O, Olivas P, Turco L, et al. Decompensation in Advanced Nonalcoholic Fatty Liver Disease May Occur at Lower Hepatic Venous Pressure Gradient Levels Than in Patients With Viral Disease. Clin Gastroenterol Hepatol October 2021.
7. Kannel WB, Dawber TR, Friedman GD, et al. Risk factors in coronary heart disease. An evaluation of several serum lipids as predictors of coronary heart disease; The Framingham Study. Ann Intern Med 1964;61:888-899.

8. Toth PP, Barter PJ, Rosenson RS, et al. High-density lipoproteins: A consensus statement from the National Lipid Association. J Clin Lipidol 2013;7:484-525.
9. Goff DC, Lloyd-Jones DM, Bennett G, et al. 2013 ACC/AHA guideline on the assessment of cardiovascular risk: A report of the American college of cardiology/American heart association task force on practice guidelines. Circulation 2014;129.
10. Lamb PM, Lund A, Kanagasabay RR, et al. Spleen size: how well do linear ultrasound measurements correlate with three-dimensional CT volume assessments? Br J Radiol 2002;75:573-577.

Table 1: Correlation between splenomegaly and histological, clinical and metabolic features of 270 patients with biopsy-proven NAFLD. The association between splenomegaly and biopsy features was tested using a Chi squared test. The association between spleen size and anthropometric and metabolic parameters as well as platelet count was tested using simple linear regression. Associated variables were tested in a multiple regression analysis. (SD = standard deviation, OR – odds ratio, p = p value, * denotes statistically significant results, defined as p-value < 0.05)

	Univariate Analysis			Multivariate analysis	
BIOPSY		***OR***	***p***		
Fibrosis Stage (F1-F2 v F3-F4)		0.87	0.68		
Steatosis (S0-S1 v S2-S3)		0.89	0.67		
Ballooning (B0 v B1-2)		0.92	0.81		
Inflammation (I0-I1 v I2 - I3)		0.59	0.18		
ANTHROPOMETRY	***Mean ± SD***	***R^2***	***p***	***t statistic***	***p***
Age (years)	56.0 ± 10.4	0.05	0.0005*	1.9	0.06
Height (m)	1.7 ± 0.1	0.08	<0.0001*	0.7	0.5
Weight (kg)	103.2 ± 19.8	0.13	<0.0001*	4.9	<0.0001*
BMI (kg/m^2)	35.5 ± 6.2	0.05	0.0002*		
METABOLIC PARAMETERS	***Mean ± SD***	***R^2***	***p***	***t statistic***	***p***
Total Cholesterol (mmol/L)	4.7 ± 1.1	0.002	0.4		
LDL-Cholesterol (mmol/L)	2.5 ± 0.9	0.001	0.6		
HDL-Cholesterol (mmol/L)	1.2 ± 0.3	0.07	<0.0001*	3.6	0.0004*
Triglycerides (mmol/L)	2.3 ± 1.3	0.02	0.01*	0.8	0.4
HbA1c (mmol/mol)	53.5 ± 18.6	0.003	0.4		
Triglycerides / HDL	2.2 ± 1.8	0.05	0.0004*		
HYPERSPLENISM	***Mean ± SD***	***R^2***	***p***		
Platelet Count (x10^9/L)	216 ± 62	0.04	0.0006*		

Supplementary Figure 1: Distribution of liver biopsy characteristics describing (A) Fibrosis stage, (B) Steatosis grade, (C) Ballooning score and (D) Inflammation score according to spleen size.