Research Article

EASL EUROPEAN ASSOCIATION FOR THE STUDY OF THE LIVER | JOURNAL OF HEPATOLOGY

Serum immunoglobulin levels predict fibrosis in patients with non-alcoholic fatty liver disease

Stuart McPherson[1,2,*], Elsbeth Henderson[1], Alastair D. Burt[2,3], Christopher P. Day[1,2], Quentin M. Anstee[1,2]

[1]*Liver Unit, Newcastle Upon Tyne Hospitals NHS Trust, Freeman Hospital, Newcastle Upon Tyne, United Kingdom;* [2]*Institute of Cellular Medicine, Faculty of Medical Sciences, Newcastle University, Newcastle Upon Tyne, United Kingdom;* [3]*The School of Medicine, University of Adelaide, Australia*

Background & Aims: A third of the population are estimated to have NAFLD of varying severity. Serum immunoglobulins are frequently elevated in patients with chronic liver disease, but little is known about serum immunoglobulin levels in patients with NAFLD. Aim of this study was to evaluate serum immunoglobulin levels (IgA, IgG, and IgM) in a large cohort of patients with biopsy-proven NAFLD and determine if immunoglobulin levels are associated with clinical or histological features.

Methods: Patients seen in a tertiary fatty liver clinic between 1999 and 2009 were included. Liver biopsies were assessed using the Kleiner score. Immunoglobulin levels and other blood tests were taken at time of biopsy.

Results: 285 patients (110 simple steatosis and 175 NASH) had serum immunoglobulins measured within 6 months of liver biopsy. 130 (46%) patients had elevated (>1× upper limit of normal) serum IgA levels, 28 (10%) patients had elevated IgG and 22 (8%) raised IgM. Serum IgA levels were elevated more frequently in patients with NASH compared with subjects with simple steatosis (55% *vs.* 31%, p <0.001). Overall, 55 (19%) patients had advanced liver fibrosis (Kleiner stage 3–4). There was a significant positive association between serum IgA levels and the stage of fibrosis (p <0.001). Serum IgA, age, platelets, AST/ALT ratio and BMI were all independently with advanced fibrosis following multivariate analysis. A model constructed from these independent predictors accurately predicted advanced fibrosis (AUROC 0.87).

Conclusions: The serum IgA level was frequently elevated in patients with NAFLD and was an independent predictor of advanced fibrosis.

Keywords: NASH; IgA; IgG; Fibrosis; Steatohepatitis; Fatty liver; Immunoglobulins; Diabetes.

Received 24 October 2013; received in revised form 11 December 2013; accepted 6 January 2014; available online 18 January 2014

* Corresponding author. Address: Liver Unit, Level 6, Freeman Hospital, Freeman Road, Newcastle upon Tyne NE7 7DN, United Kingdom. Tel.: +44 (0) 191 233 6161.

E-mail address: stuart.mcpherson@nuth.nhs.uk (S. McPherson).

Introduction

Non-alcoholic fatty liver disease (NAFLD) is an important manifestation of obesity and the metabolic syndrome [1,2]. With increasing rates of obesity, NAFLD has become one of the most common causes of liver dysfunction worldwide, affecting 20–30% of the population in developed countries [3–5]. NAFLD is defined as fatty infiltration affecting >5% of liver tissue (in the absence of excessive alcohol consumption) and can range in severity from simple steatosis (fat without hepatocellular injury), to Steatohepatitis (NASH; fat with hepatocellular injury and hepatic inflammation), which can progress to fibrosis and cirrhosis [2,6]. The natural history of NAFLD is variable with simple steatosis having a good long term prognosis with low rates of liver-related morbidity [7–10]. However, approximately 30% of patients with NASH will progress to cirrhosis [10,11] and be at risk of subsequent life threatening liver related complications such as hepatocellular carcinoma, portal hypertension and liver failure [12–15].

It is important to identify patients with advanced fibrosis and cirrhosis to screen for liver-related complications. Several previous studies have identified clinical and laboratory factors that are associated with fibrosis in patients with NAFLD including age, the presence of diabetes, raised BMI, AST/ALT ratio, platelet count, serum albumin, and ferritin [16–18]. Some of these variables are included in simple non-invasive scoring systems to predict fibrosis in NAFLD such as the FIB-4 score and the NAFLD fibrosis score [17,19,20]. When used clinically, these scores have excellent negative predictive ability and can reliably exclude advanced fibrosis [21,22].

Serum immunoglobulins are tested routinely as part of the investigation of subjects with suspected liver disease and a polyclonal increase in serum immunoglobulin levels is frequently seen in patients with liver cirrhosis [23]. In addition, characteristic patterns of elevation in serum immunoglobulins are observed in specific liver diseases such as autoimmune hepatitis (raised IgG), primary biliary cirrhosis (raised IgM), and alcoholic liver disease (raised IgA). These can be used clinically to aid diagnosis [24,25]. In alcoholic liver disease (ALD) raised serum IgA levels are associated with more advanced liver fibrosis [26]. Deposition of IgA in the liver has also been described in ALD, suggesting it might have a pathogenic role [27]. Previous studies have shown

that serum IgA levels are increased in subjects with the metabolic syndrome and type 2 diabetes [28,29].

To date, little is known about how serum immunoglobulin levels are altered in patients with NAFLD. In view of the shared pathogenic mechanisms and similar histological appearances in NAFLD and ALD, we hypothesised that serum IgA will be elevated in subjects with NAFLD, and that higher levels will be seen in those with more advanced fibrosis.

Our aim was to a evaluate serum immunoglobulin levels (IgA, IgG, and IgM) in a large cohort of patients with biopsy-proven NAFLD of varying severity to determine how frequently elevated serum immunoglobulin levels occur and whether there are any relationships between immunoglobulin levels and histological or clinical features.

Patients and methods

This study included consecutive patients with biopsy-proven NAFLD who attended the Newcastle Hospitals fatty liver clinic between 1999 and 2009. Liver biopsies were performed as part of investigation of abnormal liver function tests or to stage disease severity in patients with ultrasound evidence of NAFLD. Clinical and laboratory data were collected from the time of liver biopsy. This was a retrospective study of prospectively collected data. Alternate diagnoses were excluded, including increased alcohol intake (males and females consuming greater than 21/14 units of alcohol per week [>30/20 g/day ethanol] respectively were excluded), as were any individuals with chronic viral hepatitis (hepatitis B and hepatitis C), autoimmune liver diseases, hereditary hemochromatosis, α1-antitrypsin deficiency, Wilson's disease and drug induced liver disease. In addition, patients in whom immunoglobulins were not available within 6 months of the liver biopsy were excluded. The study was approved by the local ethics committee.

Relevant clinical details including gender, age, weight, height, and average current and previous alcohol intake (g/day) were obtained from all patients at the time of liver biopsy. The body mass index (BMI) was calculated by the formula: weight (kg) /height $(m)^2$. Waist circumference (in centimetres) was measured at the midpoint between the lower costal edge and upper iliac crest following a normal expiration. Patients were identified as having diabetes if they had been diagnosed with diabetes according to the 2004 ADA criteria or if they were taking an oral hypoglycaemic drug or insulin [30]. Blood test results were taken from the time of liver biopsy or within 6 months. The normal ranges for serum IgA, IgG, and IgM were 0.64–2.97 g/L, 5.8–15.4 g/L, and 0.71–2.3 g/L respectively. Immunoglobulin levels were considered elevated in this study if the value was >1$\times$ the upper limit of normal (ULN). The NAFLD fibrosis score and FIB-4 score were calculated as previously described [17,19].

Percutaneous liver biopsies were performed using a Menghini needle or an 18G BioPince liver biopsy system (Medical Devices Technologies, Gainville, Florida, USA). Liver biopsy specimens were assessed by an experienced hepatopathologist (ADB). Histological scoring was performed according to the NIH NAFLD Clinical Research Network criteria [31]. NASH was defined as a score of 5 or more with at least 1 for ballooning degeneration.

All statistical analyses were performed using SPSS software version 19.0 (SPSS Inc, Chicago, USA). Continuous normally distributed variables were represented as mean ± standard deviation (SD). Categorical variables were represented as percentages and non-normal variables were summarised as median and interquartile range (IQR). Chi square tests were used to determine the distribution of categorical variables between groups. To compare the means of normally distributed variables between groups the Student's *t* test was performed. To determine differences between groups for continuous non-normally distributed variables, medians were compared using the Mann-Whitney U or Kruskal-Wallis test.

To identify variables independently associated with the presence or absence of advanced fibrosis a backward stepwise logistic regression analysis was conducted correcting for age, gender, BMI, presence of diabetes, gamma-glutamyl transferase, platelets, AST/ALT ratio, serum IgA and total serum immunoglobulins. Significant variables from the logistic regression analysis were included in a model (the BAAAP score) for clinical use to predict patients with advanced fibrosis. A further logistic regression analysis was conducted to determine the independent predictors of the presence or absence of steatohepatitis (correcting for age, BMI, ALT/AST ratio, platelets, presence of diabetes, GGT, serum IgA, serum total immunoglobulins, and ferritin). Independent predictors of steatohepatitis were included in a model to determine steatohepatitis. The accuracy of the scores was assessed by receiver operating characteristic (ROC) curves. The area under the ROC (AUROC) was used as an index to compare the accuracy of several tests. The lower and upper cut-off points for sensitivity and specificity for the score were chosen to give 95% negative predictive value (NPV) and 75% positive predictive value (PPV) for advanced fibrosis respectively. AUROCs were also displayed for the BAAAP score when comparing other stages of fibrosis (e.g., Stage 0 *vs.* Stage 1–4). Previously published cut-offs for the NAFLD fibrosis and FIB-4 score were used [17,21]. *p* values <0.05 were considered significant.

Results

Patient characteristics

From a total of 405 patients biopsied for NAFLD between 1999 and 2009, 285 patients had serum immunoglobulins measured within 6 months of liver biopsy and were included in the analysis. Eighty-one of the patients also had a follow up liver biopsy. The baseline demographic, clinical and biochemical data for all patients is summarised in Table 1. One hundred and ten patients (39%) had evidence of simple steatosis and 175 (61%) had evidence of steatohepatitis on biopsy. Patients exhibiting all 5 stages of fibrosis were present in the cohort (see Table 1). Fifty-five (19%) patients had advanced liver fibrosis (Kleiner stage 3–4) on biopsy.

Overall, 130 (46%) patients had elevated serum IgA levels (>1$\times$ ULN [>2.97 g/L]) and 2 (0.7%) patients had evidence of IgA deficiency. The serum IgG was elevated (>15.4 g/L) in 28 (10%) patients and the serum IgM elevated (>2.3 g/L) in 22 patients (8%). A graph showing the distribution of serum IgA levels in the patient cohort is shown in Fig. 1. A comparison of the clinical and demographic factors between subjects with normal serum IgA levels and patients with raised serum IgA is shown in Table 1.

Relationship between serum IgA levels and histology

Serum IgA levels were more frequently elevated (>1$\times$ ULN) in patients with NASH compared with subjects with simple steatosis (55% *vs.* 31%, p <0.001) and the overall median serum levels of IgA were higher in patients with NASH *vs.* those with simple steatosis (3.1 [2.2–4.5] *vs.* 2.3 [1.7–3.3] g/L, p <0.001).

There was a significant progressive increase in the proportion of patients with raised serum IgA levels with increasing stages of fibrosis from 31% for subjects with stage 0 fibrosis to 80% with stage 4 fibrosis (p <0.001, Fig. 2A). If 1.5$\times$ ULN was used as the level for an elevated serum IgA then a similar relationship between serum IgA and fibrosis is seen (Fig. 2B). In addition, there was a significant positive association between serum IgA levels and the stage of fibrosis (p <0.001, see Fig. 3A). A similar relationship was observed between fibrosis and serum IgA/Total immunoglobulin levels (p <0.001, Fig. 3B).

Multivariate analysis of factors associated with the presence of advanced fibrosis

A comparison of the clinical and laboratory factors between subjects with no/mild fibrosis (Kleiner stage 0–2) and patients with advanced fibrosis (stage 3–4) is shown in Table 2. In order to determine which of these factors were independently associated with advanced fibrosis a logistic regression analysis was conducted adjusting for age, gender, BMI, presence of diabetes, gamma-glutamyl transferase, platelets, AST/ALT ratio, serum IgA, and total serum immunoglobulins. Age, platelets, AST/ALT ratio, BMI and serum IgA were all independently associated with advanced fibrosis (see Table 3). A model to predict the presence of advanced

Table 1. Demographic, clinical and biochemical data for 285 patients with NAFLD, and a comparison between subjects with normal serum IgA levels and patients with raised IgA levels.

Characteristic	All patients, n = 285	Normal IgA, n = 155	Elevated IgA, n = 130	*p* value**
Age (yr)	49.8 ± 12	48 ± 13	52 ± 12	0.01*
Sex (% male)	61%	63%	60%	0.66#
BMI (kg/m^2)	34.0 ± 5.3	34.0 ± 5.3	34.0 ± 5.3	0.98*
Diabetes	39%	32%	48%	0.006#
Waist circumference (cm)	108 ± 13	108 ± 15	109 ± 11	0.65*
ALT (IU/L)	80 ± 62	81 ± 56	79 ± 69	0.81*
AST (IU/L)	53 ± 38	51 ± 33	55 ± 43	0.35*
GGT (IU/L)	122 ± 166	119 ± 180	123 ± 147	0.83*
ALB (g/L)	45 ± 4	45 ± 3	44 ± 5	0.004*
Ferritin (μg/L)	199 ± 344	173 ± 140	230 ± 488	0.2*
Platelets (x10^9/L)	247 ± 72	253 ± 61	239 ± 84	0.12*
Cholesterol (mmol/L)	5.5 ± 1.3	5.6 ± 1.4	5.5 ± 1.3	0.4*
Trigycerides (mmol/L)	2.8 ± 2	2.8 ± 2.1	2.7 ± 1.3	0.77*
AST/ALT ratio	0.76 ± 0.32	0.71 ± 0.31	0.80 ± 0.34	0.02*
Serum IgA (g/L)	2.75 (1.83-4.14)			
Serum IgG (g/L)	11.4 (9.6-13.5)	10.4 (9.3-12.3)	12.5 (10.4-14.3)	<0.001^
Serum IgM (g/L)	1.03 (0.74-1.39)	1.05 (0.74-1.41)	1.01 (0.72-1.38)	0.94^
Total Igs (g/L)	15.6 (13-18.2)	13.9 (12.2-15.9)	18.0 (15.6-20.4)	<0.001^
IgA/total Igs	0.18 (0.13-0.23)	0.14 (0.11-0.17)	0.24 (0.20-0.29)	<0.001^
Fibrosis stage				<0.001#
0	105 (37%)	72 (46%)	33 (26%)	
1	89 (31%)	50 (32%)	39 (30%)	
2	36 (13%)	18 (12%)	18 (14%)	
3	30 (10%)	10 (7%)	20 (15%)	
4	25 (9%)	5 (3%)	20 (15%)	
Stage 3 or 4 fibrosis	55 (19%)	15 (10%)	50 (31%)	<0.001#
Simple steatosis/ NASH	110 (39%)/ 175 (61%)	76 (49%)/ 79 (51%)	34 (26%)/ 96 (74%)	<0.001#

Mean ± SD, median (IQR). Total Igs = IgA + IgG + IgM.
**Comparison between patients with normal and raised serum IgA.
*Student's *t* test.
#Chi squared test.
^Mann-Whitney U test.

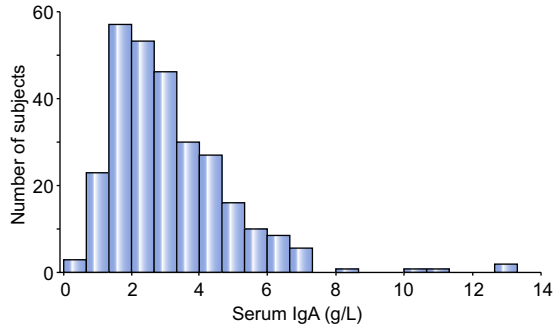

Fig. 1. Distribution of serum IgA levels in the cohort of subjects with NAFLD.

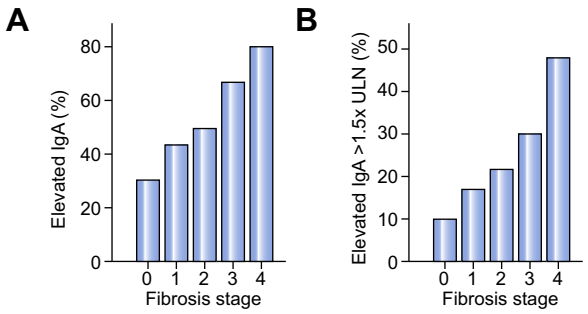

Fig. 2. Relationship between serum IgA and fibrosis. (A) Proportion of patients with elevated (>1× ULN) serum IgA levels by fibrosis stage. (B) Proportion of patients with elevated (>1.5× ULN) serum IgA levels by fibrosis stage.

fibrosis in patients with NAFLD was constructed from the independent predictors of advanced fibrosis: [−9.9 + (0.078 × age [yr]) + (−0.01 × platelets [×10^9]) + (1.962 × AST/ALT ratio) + (0.103 × BMI [m/kg^2]) + (0.35 × serum IgA [g/L])]. This model had an AUROC of 0.87 (95% CI 0.82–0.92)), and performed slightly better than the NAFLD fibrosis score and the FIB-4 score for the prediction of advanced fibrosis (Table 4). AUROCs for a diagnosis of other stages of fibrosis are shown in Table 5. The distribution of the BAAAP score according to fibrosis is shown in Fig. 5.

Relationship between IgA and the presence steatohepatitis

In order to determine whether the positive association between serum IgA levels and NASH was dependent entirely on fibrosis

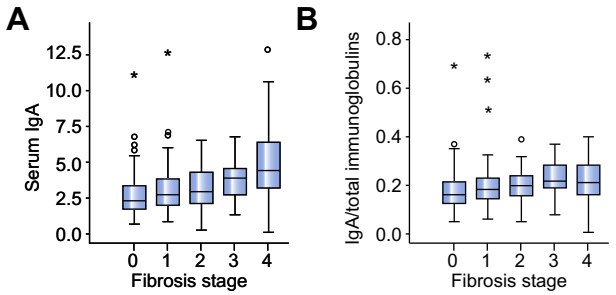

Fig. 3. Relationship between serum IgA and serum IgA/total immunoglobulins and fibrosis. (A) Relationship between serum IgA levels and stage of fibrosis. (B) Relationship between serum IgA/total immunoglobulin ratio and stage of fibrosis.

or whether steatohepatitis was independently associated, a separate analysis was conducted including only patients with stage 0 or 1 fibrosis (n = 194). In that cohort significantly more patients with histological evidence of NASH had elevated serum IgA levels than subjects with simple steatosis (46% *vs.* 30%, $p = 0.026$), suggesting that steatohepatitis is independently associated with raised serum IgA levels. Subjects with elevated serum IgA levels were more likely to have NASH than simple steatosis (74% *vs.* 26%, $p < 0.001$).

Following multivariate analysis (correcting for age, BMI, ALT/AST ratio, platelets, presence of diabetes, GGT, serum IgA, serum total immunoglobulins, and ferritin), AST/ALT (OR 4.8), BMI (OR 1.1), serum IgA (OR 1.4), and platelets (OR 0.99) were independent predictors of steatohepatitis on biopsy, but the model $[-2.721 + (1.572 \times \text{AST/ALT ratio}) + (0.374 \times \text{serum IgA [g/L]}) + (0.084 \times \text{BMI [m/kg}^2\text{]}) + (0.007 \times \text{platelets [}\times 10^9\text{]})]$ only predicted steatohepatitis with modest accuracy (AUROC 0.74).

Table 3. Multivariate analysis of factors associated with advanced fibrosis (stage 3–4) compared with stage 0–2 fibrosis.

Factor	Odds ratio	95% CI	*p* value
Age	1.08	1.04-1.13	<0.001
Platelets	0.99	0.98-0.995	<0.001
AST/ALT ratio	7.1	2.1-24	0.002
BMI	1.1	1.03-1.19	0.004
Serum IgA	1.4	1.17-1.71	<0.001

IgG, IgM, and total immunoglobulins

Serum IgG and total immunoglobulins levels were significantly higher in patients with cirrhosis (stage 4) compared with subjects with stage 0–3 fibrosis ($p < 0.001$ for both, Fig. 4A and B). However, there was no significant relationship between serum IgG and total Immunoglobulin levels and fibrosis for patients with stage 0–3 fibrosis ($p = 0.07$ and $p = 0.59$ respectively). There was no relationship between IgM levels and fibrosis.

Relationship between serum immunoglobulins and diabetes

In line with previous reports [28,29], serum IgA levels were significantly higher in patients with diabetes compared to those without (3.26 [2.15–4.57] *vs.* 2.45 [1.17–3.51], $p < 0.001$). This relationship persisted when only patients with stage 0–1 fibrosis were considered (diabetics 2.69 [1.96–4.23] *vs.* non-diabetics 2.34 [1.70–3.26]; $p = 0.04$), suggesting this relationship was independent of fibrosis. When patients with simple steatosis were considered separately there was a trend towards higher serum IgA levels in diabetics compared with non-diabetics (2.49 [1.94–4.27] *vs.* 2.21

Table 2. Clinical and biochemical characteristics of subjects with stage 0–2 fibrosis and patients with advanced fibrosis.

Characteristic	Stage 0-2, n = 230	Stage 3-4, n = 55	*p* value
Age (yr)	48 ± 13	59 ± 9	<0.001*
Sex (% male)	67%	38%	<0.001#
BMI (kg/m^2)	33.7 ± 5.2	35.3 ± 5.4	0.04*
Diabetes	34%	58%	0.001#
Waist circumference (cm)	108 ± 14	112 ± 12	0.06*
ALT (IU/L)	84 ± 66	65 ± 43	0.05*
AST (IU/L)	51 ± 38	58 ± 35	0.18*
GGT (IU/L)	103 ± 100	196 ± 306	0.03*
ALB (g/L)	45 ± 3	43 ± 7	0.09*
Ferritin	206 ± 375	170 ± 170	0.49*
Platelets (x10^9/L)	258 ± 67	200 ± 78	<0.001*
Cholesterol (mmol/L)	5.7 ± 1.3	5.0 ± 1.3	0.001*
Trigycerides (mmol/L)	2.8 ± 2.1	2.5 ± 1.7	0.24*
AST/ALT ratio	0.69 ± 0.26	1.02 ± 0.41	<0.001*
IgA	2.46 (1.76-3.62)	4.15 (2.82-5.08)	<0.001^
IgG	11.0 (9.4-13.0)	12.9 (11.2-15.3)	<0.001^
IgM	1.0 (0.73-1.38)	1.1 (0.8-1.44)	0.2^
Total Igs	15.0 (12.7-17.6)	18.2 (15.3-22.3)	<0.001^
IgA/total Igs	0.17 (0.13-0.23)	0.21 (0.17-0.28)	<0.001^

*Student's t test.
#Chi squared test.
^Mann-Whitney U test.

Table 4. Assessment of diagnostic yield of serum immunoglobulins and other non-invasive scores for advanced fibrosis/cirrhosis.

Diagnosis of advanced fibrosis (stage 3-4)							
Test	AUROC	95% CI	Cut-off	Sens	Spec	PPV	NPV
IgA	0.72	0.64-0.79	2.74	80%	56%	29%	93%
NAFLD fibrosis score	0.81	0.74-0.87	-1.455 0.676	73% 31%	67% 98%	33% 77%	92% 87%
FIB-4 score	0.85	0.80-0.90	1.30 3.25	80% 24%	74% 99%	40% 84%	94% 86%
BAAAP score	0.87	0.82-0.92	-1.75 0.25	82% 40%	70% 97%	38% 75%	95% 88%

Table 5. Accuracy of the BAAAP score for the diagnosis of specific stages of fibrosis.

Diagnostic accuracy of the BAAAP score in comparing stages of fibrosis							
Stage of fibrosis	AUROC	95% CI	Cut-off	Sens	Spec	PPV	NPV
1-4 *vs.* 0 (63% prevalence)	0.71	0.65-0.77	-3.0 -1.75	79% 51%	66% 79%	80% 81%	56% 49%
2-4 *vs* 0-1 (31% prevalence)	0.78	0.72-0.84	-1.75 -0.5	70% 27%	74% 96%	52% 75%	85% 75%
3-4 *vs* 0-2 (19% prevalence)	0.87	0.82-0.92	-1.75 0.25	82% 40%	70% 97%	38% 75%	95% 88%
4 *vs* 0-3 (9% prevalence)	0.92	0.87-0.96	-1.75 0.25	100% 56%	34% 94%	13% 48%	100% 96%

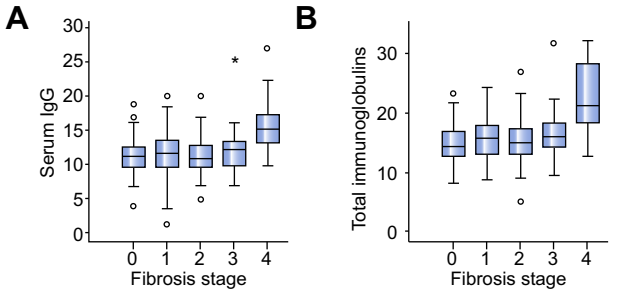

Fig. 4. Relationship between serum IgG and serum total immunoglobulins and fibrosis. (A) Relationship between the serum IgG levels and fibrosis stage. (B) Relationship between the total immunoglobulin levels and fibrosis stage.

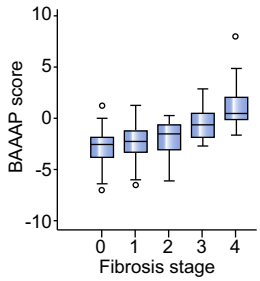

Fig. 5. Box-plot showing the distribution of the BAAAP score according to stage of fibrosis.

[1.58–3.07] $p = 0.07$). There was no relationship between serum IgG levels or total Immunoglobulin levels and diabetes in patients with NAFLD ($p = 0.51$ and 0.19 respectively).

Serum IgA levels and fibrosis progression

Eighty-one patients had a follow-up liver biopsy after a median of 6.4 [3.5–8.9] years. Of these, 29 (36%) progressed by 1 Kleiner stage of fibrosis or more. In 40 (49%) patients there was no change in fibrosis stage and 12 (15%) patients had regression of fibrosis ($\geqslant$1 Kleiner stage) between the biopsies. Overall, 27 (33%) patients with follow up liver biopsies had raised serum IgA levels at the time of the original biopsy. There was a trend towards higher serum IgA levels in the progressors compared with non-progressors (2.75 g/L [1.94–3.93] *vs.* 2.22 [1.59–3.12], $p = 0.08$). Unfortunately few patients had serum IgA levels determined at the follow up biopsy so we were unable to assess whether serum IgA levels changed with fibrosis progression or regression.

Discussion

It is accepted that serum immunoglobulin levels are frequently increased in patients with cirrhosis and specific elevations of serum immunoglobulins are seen in some liver diseases such as PBC (IgM), autoimmune hepatitis (IgG) and alcoholic liver disease (IgA) [23–25]. In the present study, we evaluated serum immunoglobulin levels in a large cohort of patients with histologically characterised NAFLD of varying severity. The key findings of the study were that serum IgA levels were elevated in almost half the NAFLD cohort and there was a significant positive relationship between serum IgA levels and fibrosis. Moreover, the serum IgA level was an independent predictor of advanced fibrosis and was included in a model that accurately predicted advanced fibrosis (Kleiner Stage 3–4) in this cohort of patients with NAFLD.

With increasing rates of obesity worldwide, NAFLD has become very common, affecting 20–30% of the population in many developed countries [6]. It is important to identify subjects with advanced fibrosis from this large proportion of the population as they are at the highest risk of liver-related complications [32]. Many studies have looked at factors associated with fibrosis in NAFLD and repeatedly age, AST/ALT ratio, platelet count, the presence of diabetes and BMI and albumin have been shown to be associated with advanced fibrosis [16,17,20–22]. Some of these factors are included in various models to predict advanced fibrosis such as the BARD score, the FIB-4 score and the NAFLD fibrosis score, which can be used clinically to exclude or diagnose advanced fibrosis with reasonable accuracy [16,17,20–22]. Our results show that, in addition to these known factors, serum IgA levels were independently associated with advanced fibrosis. A statistical model (BAAAP score), incorporating BMI, serum IgA, age, AST/ALT ratio, and platelet count showed good diagnostic accuracy for advanced fibrosis (AUROC 0.87). However, it needs to be validated in an external cohort before it can be recommended for clinical use.

In the present study 46% of subjects with NAFLD had an elevated IgA (>1× ULN), whereas 10% had an elevated IgG and 8% an elevated IgM. Previous studies have shown that elevation of serum IgA levels was uncommon in other liver diseases such as hepatitis C, PBC and autoimmune liver disease [25,33]. It is well known that serum IgA levels are elevated in patients with alcoholic liver disease [26,27,29], but results of the present study suggest that the IgA is also specifically elevated in NAFLD. Therefore, when investigating patients with suspected liver disease, the finding of an elevated IgA should prompt clinicians to consider NAFLD as a diagnosis where there is no history of excessive alcohol consumption.

Approximately 10–30% of patients with NAFLD have NASH, which can progress to cirrhosis [34,35]. Currently the only effective method of diagnosing NASH is by liver biopsy, but this is invasive and therefore impractical for widespread use. In the present study we found that significantly more patients with an elevated serum IgA level had NASH than simple steatosis (74% *vs.* 26%) and serum IgA was an independent predictor of the presence of steatohepatitis on liver biopsy. Therefore, assessment of serum IgA levels along with other clinical factors might help clinicians to determine which patients need a biopsy to confirm NASH. The search for an accurate blood test to non-invasively diagnose NASH is an active area of clinical research.

Eighty one of the patients had follow up liver biopsies in the study and approximately a third of the patients had progression of fibrosis over a median follow up of 6 years. This rate of progression was similar to previous studies [11,36]. There was a trend to higher serum IgA levels in the patients who had fibrosis progression compared with the non-progressors. As we have demonstrated an association between higher IgA levels and fibrosis and steatohepatitis, it is likely that high IgA levels reflect more severe baseline disease that is more likely to progress. However, the number of patients with follow up liver biopsies was relatively small and so this association should be assessed in a larger cohort of patients with NAFLD.

The cause of raised IgA in patients with NAFLD is unknown. As well as being present in serum, IgA is secreted at high concentrations on mucosal surfaces that protect against external pathogens [37]. A major source of IgA is the gut, where it plays a role in the mucosal immune response against intestinal bacteria [37]. A previous study demonstrated that the intestinal microflora was different in subjects with obesity compared to lean controls, and the alterations were abolished after diet induced weight loss [38]. In addition, structural changes in the intestinal mucosa occur in patients with chronic liver disease that allows translocation of bacteria and bacterial products, such as LPS, to the circulation [39]. Toll-like receptors recognise bacterial products and evoke intense inflammatory reactions that might contribute to the pathogenesis of NASH [15,40]. Therefore, it is possible that Toll-like receptor stimulation of B cells in response to changes in bacterial microflora and bacterial translocation is responsible for high IgA levels seen in NAFLD. A potential mechanism involving TLR9 has been suggested to cause raised IgA levels in patients with ALD [41]. Further evaluation of the underlying mechanism in NAFLD is warranted.

Previous studies have found high serum IgA levels in subjects with type 2 diabetes and the metabolic syndrome [28,29]. As insulin resistance is a key feature of NAFLD, and the majority of subjects with the metabolic syndrome and type 2 diabetes have steatosis [42], it is not surprising that there is a shared association with elevated serum IgA levels in those conditions. In the present study we found that higher IgA levels were seen in diabetics than non-diabetics after correcting for fibrosis. Diabetes is associated with a low grade chronic inflammatory state, with increased levels of circulating proinflammatory cytokines such as TNFα and IL-6 [15,43,44]. Higher serum IgA levels in diabetics might therefore be a marker of higher levels of systemic inflammatory activity. Further studies evaluating serum cytokines and IgA levels in NAFLD might help elucidate the underlying mechanisms and determine the cause-effect relationship.

Serum IgA is composed of IgA1 and IgA2 subclasses, as well secretory IgA (a dimer of IgA). Previous studies have found elevations in all these IgA types as well as other qualitative changes in IgA, such as glycosylated IgA and IgA complexes, in patients with alcoholic cirrhosis [45]. It is therefore possible that similar changes could occur in NAFLD, (particularly glycosylation) and as such these could be biomarkers of fibrosis in NAFLD, but this needs to be formally assessed.

In conclusion, results from the present study show that serum IgA levels were frequently elevated in patients with NAFLD, with higher levels in subjects with NASH than simple steatosis. In addition, serum IgA levels were an independent predictor of advanced fibrosis in patients with NAFLD. Therefore, along with other clinical features and biochemical results, assessment of the serum IgA might help stage disease in subjects with suspected NAFLD.

Conflict of interest

The authors who have taken part in this study declared that they do not have anything to disclose regarding funding or conflict of interest with respect to this manuscript.

Authors' contributions

SM, Study concept and design, drafting of the paper, statistical analysis, and approval of the final draft submitted.

QMA, study concept and design, critical revision of the paper for important intellectual content, and approval of the final draft submitted.

CPD, study concept and design, critical revision of the paper for important intellectual content, and approval of the final draft submitted.

ADB, study concept and design, review of histology, critical revision of the paper for important and approval of the final draft submitted.

EH, acquisition of data, data management, and approval of the final draft submitted.

Acknowledgements

QMA is the recipient of a Clinical Senior Lectureship Award from the Higher Education Funding Council for England (HEFCE). The authors are members of the FLIP Fatty Liver – Inhibition of Progression research consortium funded by the European Union Seventh Framework Program (FP7/2007-2013) under grant agreement Health-F2-2009-241762.

References

[1] de Alwis NM, Day CP. Non-alcoholic fatty liver disease: the mist gradually clears. J Hepatol 2008;48:S104–S112.
[2] Anstee QM, Daly AK, Day CP. Genetics of alcoholic and nonalcoholic fatty liver disease. Semin Liver Dis 2011;31:128–146.
[3] Fan J-G, Zhu J, Li X-J, Chen L, Li L, Dai F, et al. Prevalence of and risk factors for fatty liver in a general population of Shanghai, China. J Hepatol 2005;43:508–514.
[4] Bellentani S, Saccoccio G, Masutti F, Croce LS, Brandi G, Sasso F, et al. Prevalence of and risk factors for hepatic steatosis in Northern Italy. Ann Intern Med 2000;132:112–117.
[5] Browning JD, Szczepaniak LS, Dobbins R, Nuremberg P, Horton JD, Cohen JC, et al. Prevalence of hepatic steatosis in an urban population in the United States: impact of ethnicity. Hepatology 2004;40:1387–1395.
[6] Chalasani N, Younossi Z, Lavine JE, Diehl AM, Brunt EM, Cusi K, et al. The diagnosis and management of non-alcoholic fatty liver disease: practice guideline by the American Gastroenterological Association, American Association for the Study of Liver Diseases, and American College of Gastroenterology. Gastroenterology 2012;142:1592–1609.
[7] Matteoni CA, Younossi ZM, Gramlich T, Boparai N, Liu YC, Mc-Cullough AJ. Non alcoholic fatty liver disease: a spectrum of clinical and pathological severity. Gastroenterology 1999;116:1413–1419.
[8] Adams LA, Lymp JF, St Sauver J, Sanderson SO, Lindor KD, Feldstein A, et al. The natural history of nonalcoholic fatty liver disease: a population-based cohort study. Gastroenterology 2005;129:113–121.
[9] Teli MR, James OF, Burt AD, Bennett MK, Day CP. The natural history of non alcoholic fatty liver: a follow up study. Hepatology 1995;22:1714–1719.
[10] Ekstedt M, Franzen LE, Mathiesen UL, Thorelius L, Holmqvist M, Bodemar G, et al. Longterm follow-up of patients with NAFLD and elevated liver enzymes. Hepatology 2006;44:865–873.
[11] Argo CK, Northup PG, Al-Osaimi AM, Caldwell SH. Systematic review of risk factors for fibrosis progression in non-alcoholic steatohepatitis. J Hepatol 2009;51:371–379.
[12] Bhala N, Angulo P, van der Poorten D, Lee E, Hui JM, Saracco G, et al. The natural history of nonalcoholic fatty liver disease with advanced fibrosis or cirrhosis: an international collaborative study. Hepatology 2011;54:1208–1216.
[13] Hui JM, Kench JG, Chitturi S, Sud A, Farrell GC, Byth K, et al. Long-term outcomes of cirrhosis in nonalcoholic steatohepatitis compared with hepatitis C. Hepatology 2003;38:420–427.
[14] Sanyal AJ, Banas C, Sargeant C, Luketic VA, Sterling RK, Stravitz RT, et al. Similarities and differences in outcomes of cirrhosis due to nonalcoholic steatohepatitis and hepatitis C. Hepatology 2006;43:682–689.
[15] Anstee QM, Targher G, Day CP. Progression of non-alcoholic fatty liver disease to diabetes, cardiovascular disease or cirrhosis. Nat Rev Gastroenterol Hepatol 2013;66:1033–1045.
[16] Harrison SA, Oliver D, Arnold HL, Gogia S, Neuschwander-Tetri BA. Development and validation of a simple NAFLD clinical scoring system for identifying patients without advanced disease. Gut 2008;57:1441–1447.
[17] Angulo P, Hui J, Marchesini G, Bugianesi E, George J, Farrell GC, et al. The NAFLD fibrosis score: a noninvasive system that identifies liver fibrosis in patients with NAFLD. Hepatology 2007;45:847–854.
[18] Kowdley KV, Belt P, Wilson LA, Yeh MM, Neuschwander-Tetri BA, Chalasani N, et al. Serum ferritin is an independent predictor of histologic severity and advanced fibrosis in patients with nonalcoholic fatty liver disease. Hepatology 2012;55:77–85.
[19] Sterling RK, Lissen E, Clumeck N, Sola R, Correa MC, Montaner J, et al. Development of a simple noninvasive index to predict significant fibrosis in patients with HIV/HCV coinfection. Hepatology 2006;43:1317–1325.
[20] Shah AG, Lydecker A, Murray K, Tetri BN, Contos MJ, Sanyal AJ. Comparison of noninvasive markers of fibrosis in patients with nonalcoholic fatty liver disease. Clin Gastroenterol Hepatol 2009;7:1104–1112.
[21] McPherson S, Stewart SF, Henderson E, Burt AD, Day CP. Simple non-invasive fibrosis scoring systems can reliably exclude advanced fibrosis in patients with non-alcoholic fatty liver disease. Gut 2010;59:1265–1269.
[22] McPherson S, Anstee QM, Henderson E, Day CP, Burt AD. Are simple noninvasive scoring systems for fibrosis reliable in patients with NAFLD and normal ALT levels? Eur J Gastroenterol Hepatol 2013;25:652–658.
[23] Tomasi TB, Tisdale WA. Serum gamma-globulins in acute and chronic liver diseases. Nature 1964;201:834–835.
[24] Husby G, Skrede S, Blomhoff JP, Jacobsen CD, Berg K, Gjone E. Serum immunoglobulins and organ non-specific antibodies in diseases of the liver. Scand J Gastroenterol 1977;12:297–304.
[25] Martin DM, Vroon DH, Nasrallah SM. Value of serum immunoglobulins in the diagnosis of liver disease. Liver 1984;3:214–218.
[26] Iturriaga H, Pereda T, Estévez A, Ugarte G. Serum immunoglobulin A changes in alcoholic patients. Ann Clin Res 1977;9:39–43.
[27] van de Wiel A, van Hattum J, Schuurman HJ, Kater L. Immunoglobulin A in the diagnosis of alcoholic liver disease. Gastroenterology 1988;94:457–462.
[28] Rodríguez-Segade S, Camiña MF, Paz JM, Del Río R. Abnormal serum immunoglobulin concentrations in patients with diabetes mellitus. Clin Chim Acta 1991;203:135–142.
[29] Gonzalez-Quintela A, Alende R, Gude F, Campos J, Rey J, Meijide LM, et al. Serum levels of immunoglobulins (IgG, IgA, IgM) in a general adult population and their relationship with alcohol consumption, smoking and common metabolic abnormalities. Clin Exp Immunol 2008;151:42–50.
[30] ADA. Diagnosis and classification of diabetes mellitus. Diabetes Care 2004;27:S5–S10.
[31] Kleiner DE, Brunt EM, Van Natta M, Behling C, Contos MJ, Cummings OW, et al. Design and validation of a histological scoring system for nonalcoholic fatty liver disease. Hepatology 2005;41:1313–1321.
[32] Anstee QM, McPherson S, Day CP. How big a problem is non-alcoholic fatty liver disease? BMJ 2011;343:d3897.
[33] Gonzàlez-Quintela A, Alende MR, Gamallo R, Gonzàlez-Gil P, López-Ben S, Tomé S, et al. Serum immunoglobulins (IgG, IgA, IgM) in chronic hepatitis C. A comparison with non-cirrhotic alcoholic liver disease. Hepatogastroenterology 2003;54:2121–2126.
[34] Wanless IR, Lentz JS. Fatty liver hepatitis (steatohepatitis) and obesity: an autopsy study with analysis of risk factors. Hepatology 1990;12:1106–1110.
[35] Williams CD, Stengel J, Asike MI, Torres DM, Shaw J, Contreras M, et al. Prevalence of nonalcoholic fatty liver disease and nonalcoholic steatohepatitis among a largely middle-aged population utilizing ultrasound and liver biopsy: a prospective study. Gastroenterology 2011;140:124–131.
[36] Ekstedt M, Franzen LE, Mathiesen UL, Thorelius L, Holmqvist M, Bodemar G, et al. Long-term follow-up of patients with NAFLD and elevated liver enzymes. Hepatology 2006;44:865–873.
[37] Wood JM, Kerr MA. The function of IgA in immunity. J Pathol 2006;208:270–282.
[38] Ley RE, Turnbaugh PJ, Klein S, Gordon JI. Microbial ecology: human gut microbes associated with obesity. Nature 2006;444:1022–1023.
[39] Weist R, Garcia-Tsao G. Bacterial translocation in cirrhosis. Hepatology 2005;41:422–433.
[40] Mencin A, Kluwe J, Schwabe RF. Toll-like receptors as targets in chronic liver diseases. Gut 2009;58:704–720.
[41] Massonnet B, Delwail A, Ayrault JM, Chagneau-Derrode C, Lecron JC, Silvain C. Increased immunoglobulin A in alcoholic liver cirrhosis: exploring the response of B cells to Toll-like receptor 9 activation. Clin Exp Immunol 2009;158:115–124.
[42] Bugianesi E, McCullough A, Marchesini G. Insulin resistance: a metabolic pathway to chronic disease. Hepatology 2005;42:987–1000.

[43] Pickup JC, Mattock MB, Chusney GD, Burt D. NIDDM as a disease of the innate immune system: association of acute-phase reactants and interleukin-6 with metabolic syndrome X. Diabetologia 1997;40:1286–1292.
[44] Arkan MC, Hevener AL, Greten FR, Maeda S, Li Z-W, Long JM, et al. IKK-[beta] links inflammation to obesity-induced insulin resistance. Nat Med 2005;11:191–198.
[45] Tissandie E, Morelle W, Berthalot L, Vrtovsnik F, Daugas E, Walker F, et al. Both IgA nephropathy and alcoholic cirrhosis feature abnormally glycosylated IgA1 and soluble CD89-IgA and IgG-IgA complexes: common mechanisms for distinct diseases. Kidney Int 2011;80:1352–1363.