

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

Stuart McPherson^{1,2}, Elsbeth Henderson¹, 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

Corresponding Author:

Dr Stuart McPherson

Liver Unit,

Level 6,

Freeman Hospital,

Freeman Road,

Newcastle upon Tyne

NE7 7DN

United Kingdom.

Phone: 44 (0) 191 233 6161

E mail: stuart.mcpherson@nuth.nhs.uk

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Abstract

Background: 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: 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 1999-2009 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 (>1x 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.

Key words:

NASH; IgA; IgG; fibrosis; steatohepatitis; fatty liver; immunoglobulins; diabetes.

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 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 [$>30/20\text{g/day}$ ethanol] respectively were excluded), as were any individuals with chronic viral hepatitis (hepatitis B and hepatitis C), autoimmune liver diseases, hereditary hemochromatosis, α 1-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: $\text{weight (kg) /height (m)}^2$. 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 >1x 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 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 \pm standard deviation (SD). Categorical variables were represented as percentages and non-normal variables were summarised as median and interquartile range (IQR). Chi squared 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 tests.

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. The accuracy of this score 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) respectively. 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 Figure 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 versus 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 ($p < 0.001$, Figure 2A). If 1.5x ULN was used as the level for an elevated serum IgA then a similar relationship between serum IgA and fibrosis is seen (Figure 2B). In addition, there was a significant positive association between serum IgA levels and the stage of fibrosis ($p < 0.001$, See Figure 3A). A similar relationship was observed between fibrosis and serum IgA/Total immunoglobulin levels ($p < 0.001$, Figure 3B).

In order to determine whether the positive association between serum IgA levels and NASH was dependent entirely on 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% $p < 0.001$).

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, Figure 4A and 4B). 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.

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 fibrosis in patients with NAFLD was constructed from the independent predictors of advanced fibrosis $[-9.9+(0.078 \times \text{age [yrs]})+(-0.01 \times \text{platelets [x10}^9\text{)})+(1.962 \times \text{AST/ALT ratio})+ (0.103 \times \text{BMI m/kg}^2) + (0.35 \times \text{serum IgA [g/L]})]$. 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).

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 [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 (≥ 1 Kleiner stage) between the biopsies. Overall, 27 (33%) patients with follow up liver biopsies had a 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] $p=0.08$).

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 (>1x 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%). Therefore, assessment of serum IgA levels along with other clinical factors might help clinicians 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 of 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 TNF α 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.

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.

Competing interests: None

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.

Tables

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 (years)	49.8 ± 12	48 ± 13	52 ± 12	0.01*
Gender (%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 Circ (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*
Triglycerides (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^
Totlgs (g/L)	15.6 (13-18.2)	13.9 (12.2-15.9)	18.0 (15.6-20.4)	<0.001^
IgA/totlgs	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). Totlgs = IgA+IgG+IgM. ** Comparison between patients with normal and raised serum IgA. *Student's t test, # Chi squared test, ^ Mann-Whitney U test.

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 (years)	48 ± 13	59 ± 9	<0.001*
Gender (%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 Circ (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*
Triglycerides (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^
Totlgs	15.0 (12.7-17.6)	18.2 (15.3-22.3)	<0.001^
IgA/totlgs	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 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

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. Diagnostic 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%

Figure legends

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

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

Figure 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/totalimmunoglobulin ratio and stage of fibrosis.

Figure 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.

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