

Levels of serum IgG subclasses in patients with liver disease: A retrospective study

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Abstract. Viral and alcoholic liver disease, drug induced liver disease (DILD), primary biliary cirrhosis (PBC) and autoimmune hepatitis (AIH) are among the most common liver diseases observed in clinical practice. These diseases lack unique clinical characteristics at the beginning of pathogenesis, which renders specific diagnosis difficult. Immunoglobulin G (IgG) subclasses are the main isoform of antibodies that can be found in the serum that serve important protective roles in immunity. The present study aimed to investigate the serum IgG subclass distribution in patients with the five common liver diseases aforementioned. The present study retrospectively recorded and analyzed the serum IgG subclass levels of different patients, who were grouped according to their clinical diagnosis. Serum IgG subclass levels were measured using immunonephelometric assays. IgG3 levels were found to be significantly increased whereas IgG4 levels were significantly decreased in patients with PBC. In patients with AIH, IgG1 levels were significantly increased. By contrast, IgG1/IgG level ratios in patients with viral liver disease were significantly increased. No clear pattern in the distribution characteristics of IgG subclasses could be observed in cohorts with alcoholic liver disease and DILD in the present study. Additionally, model for end-stage liver disease scores regarding IgG1 in patients with AIH shared a synergistic relationship. Anti-mitochondrial antibody subtype

M2 (AMA-M2) and IgG3 in patients with PBC demonstrated a synergistic relationship. These results suggested that IgG subclasses may be used as biomarkers to further the understanding of liver disease, which could allow for early diagnosis.

Introduction

Chronic liver disease and its associated complications are responsible for >2 million deaths per year worldwide (1-3). Viral and alcoholic liver disease, drug induced liver disease (DILD), primary biliary cirrhosis (PBC) and autoimmune hepatitis (AIH) are among the most common types of liver diseases observed in clinical practice (4,5). However, these diseases lack unique clinical characteristics, making their diagnoses difficult to distinguish. Generally, the causes of viral liver disease, alcoholic liver disease and DILD are clearly defined. However, PBC and AIH are autoimmune diseases that have no known clear causes. Certain autoimmune antibodies, including anti-mitochondrial antibodies (AMAs) and anti-nuclear antibodies (ANAs), have been reported to be important for the diagnosis of autoimmune diseases (6). A disrupted balance between pathogen recognition and the innate and adaptive immune systems is a common cause of liver disease progression (7).

Immunoglobulin G (IgG) is a major antibody isotype in the blood that protects the body against pathogenic infection. The IgG family is comprised of IgG1, IgG2, IgG3 and IgG4 (8). Differences in the structural composition of the IgG subclasses determines their function, which include antigen binding, immune complex formation, complement activation, triggering of effector cells, half-life and placental transport (9). IgG1 is the most abundant subclass in the serum, whilst IgG3 has the shortest half-life (7 days) (10). In addition, responses to different antigens will lead to marked skewing towards specific IgG antibody subclasses (11). Although deficiencies in selective subclasses are not usually harmful, they may lead to enhanced susceptibility towards specific classes of pathogens. However, this condition is rarely observed (12). IgG subclasses contribute to the immunopathogenesis of liver diseases by regulating immunoglobulin Fcγ receptor (FcγR) and complement interactions (13). Radioimmunoassay or ELISA assays can be used to measure the levels of specific serum IgG subclasses but are not particularly accurate or convenient (14).

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Abbreviations: DILD, drug-induced liver disease; PBC, primary biliary cirrhosis; AIH, autoimmune hepatitis; HCs, healthy controls; IgG, immunoglobulin G; IgG4-RD, IgG4-related disease; MELD, model for end-stage liver disease

Key words: IgG, viral liver disease, alcoholic liver disease, drug-induced liver disease, primary biliary cirrhosis, autoimmune hepatitis

Immunonephelometric assays are therefore the preferred method for this application (15).

IgG4-related disease (IgG4-RD) is a fibroinflammatory, immune system-mediated systemic disease that was reported for the first time in 2014 in Japan (16). IgG4-RD is described as a novel clinical entity of unknown origin that involves multiple organs (17). The current diagnostic approach for IgG4-RD includes serum IgG4 levels >135 mg/dl (18). Other diagnostic criteria include IgG4-related tests, imaging and typical histopathological examination (19). However, accurate diagnosis of IgG4-RD is challenging, since IgG4-RD can be easily misdiagnosed as autoimmune pancreatitis, inflammatory pseudotumour and Küttner's tumor (20). IgG4-related hepatobiliary diseases include IgG4-related sclerosing cholangitis and IgG4-related hepatopathy (21).

Previous studies have focused on the relationship between the IgG subclasses and diseases that affect the body's immunity, including the significant increase of IgG1 and IgG3 in patients with rheumatoid arthritis (22), IgG subclass determination in renal disease progression (23) and distinguishing patients with human immunodeficiency virus infections who exhibit varying degrees of infection (24). However, few prior studies have previously analyzed serum IgG subclass levels fully in clinically common liver diseases and limited data are available regarding IgG4 levels in larger patient cohorts for diseases other than IgG4-RD (25-27). Both liver diseases and IgG4-RD exhibit changes in IgG4 levels (28). Therefore, to further understanding, provide an early diagnosis strategy of different liver diseases and evaluation of IgG4 levels in patients with non-IgG4-RD, the present study analyzed serum IgG subclass levels in patients with five common liver diseases.

Materials and methods

Patients. Clinical information from 32 patients with viral liver disease, 26 with alcoholic liver disease, 39 with DILD, 28 with PBC, 29 with AIH and 30 healthy controls (HCs) was recorded at the Clinical Laboratory Center, Beijing YouAn Hospital, Capital Medical University (Beijing, China) between May 2018 and March 2019. Patients with other types of liver or autoimmune diseases, including acute and chronic fatty liver disease, metabolic liver diseases, rheumatoid-immune diseases and systemic lupus erythematosus, were excluded from the present study. The patients recruited into the present study only had diseases affecting the liver. All patients with viral liver disease met the guidelines of Prevention and Treatment for Chronic hepatitis B (29) and C (30). Patients with alcoholic liver disease met the 2010 American Association for the Study of Liver Diseases diagnosis (AALSD) (31). Those with DILD met the diagnostic criteria of the Roussel Uclaf Causal Relationship Assessment Method (RUCAM), with a RUCAM causality scale of >6 points (32). Patients with PBC met the 2018 AALSD PBC diagnosis guidelines (33). Patients with AIH met the 2015 Chinese Consensus on the Diagnosis and Management of Autoimmune Hepatitis criteria (34). The laboratory test results, including alanine transaminase (ALT), aspartate aminotransferase (AST), γ -glutamyl transferase (GGT), alkaline phosphatase (ALP), bilirubin and albumin of HCs, were found to lie within the normal range. All baseline

information of the patients recruited into the present study, including age, sex distribution and laboratory parameters, are listed in Table I. The present study was approved by the Ethics Committee of Beijing YouAn Hospital, Capital Medical University (Beijing, China).

IgG and other indices measured. Baseline blood samples were collected from all participants and all samples were processed at the Clinical Laboratory Center of Beijing YouAn hospital, Capital Medical University. Serum IgG subclass levels were measured using immunonephelometric assays using molecular biology kits (N Latex IgG1, cat. no. OQXI; N Latex IgG2, cat. no. OQXK; N Latex IgG3, cat. no. OPAV; N Latex IgG4, cat. no. OPAU and N Supplementary Reagent/Precipitation; all Siemens Healthineers) in Siemens BNII automatic protein analyzer (Siemens Healthineers) according to the manufacturer's protocols. IgG levels were determined regardless of disease stage. The normal ranges of adult IgG subclasses were defined as follows: i) IgG1, 4.05-10.11 g/l; ii) IgG2, 1.69-7.86 g/l; iii) IgG3, 0.11-0.85 g/l; and iv) IgG4, 0.03-2.01 g/l (normal ranges derived from IgG subclass kit instructions). Serum IgG levels were defined as the sum of IgG1, IgG2, IgG3 and IgG4.

IgM (cat. no. OSAT) and IgA (cat. no. OSAR) levels were also determined by electrochemiluminescence using a Modular E170 analyzer (Roche Diagnostics). Anti-mitochondrial antibody (AMA), anti-nuclear antibody (ANA) and anti-smooth muscle antibody (ASMA) levels were measured using an indirect immunofluorescence assay (cat. no. FA1510-1; EuroImmuno AG; PerkinElmer, Inc.). AMA type 2 (AMA-M2) levels were measured using ELISA (cat. no. EA1590-9601-8G; EuroImmuno AG; PerkinElmer, Inc.). Autoantibodies titers $\geq 1:100$ were considered positive and an absorbance value of AMA-M2 ≥ 25 RU/ml was also considered positive.

The model for end-stage liver disease (MELD) score used in the present study was obtained from the United Network for Organ Sharing (UNOS) for the prioritization of transplant organs, which was calculated using the following formula (35): $MELD = 3.8 \times \{\ln [\text{serum bilirubin (mg/dl)}]\} + 11.2 \times \{\ln [\text{international normalized ratio (INR)}]\} + 9.6 \times \{\ln [\text{serum creatinine (mg/dl)}]\} + 6.4 \times (\text{constant for liver disease etiology})$. Ln was the natural logarithm with base e. If disease etiology was biliary or alcoholic, constant for liver disease etiology was considered to be 0 whereas other etiologies, such as viral hepatitis, were set to 1. A MELD score >6 was considered to be abnormal.

Statistical analysis. Normally distributed numerical variables were assessed using Q-Q plot graphical methods and were presented as the mean \pm SD. Data were instead presented as the median (interquartile range, 25th and 75th percentile) when numerical variables were not normally distributed. Categorical variables were presented as numbers and percentages. One-way ANOVA and Kruskal-Wallis tests were performed to compare IgG subclass levels. Multiple comparison between the groups was performed using Scheffe's post hoc test. Statistical differences between two groups were analyzed using Student's t-test or Mann-Whitney test. χ^2 test was applied to analyze the percentages of elevated IgG subclasses. Additionally, Pearson's correlation coefficient was used to evaluate the relationship between the levels of two subclasses of antibodies. $P < 0.05$

was considered to indicate a statistically significant difference. SPSS version 22.0 (IBM Corp.) and GraphPad Prism version 7.0 for Windows (GraphPad Software, Inc.) were used for data analysis. All experiments were repeated three times and there were three replicates for each experiment.

Results

Basic characteristics of study participants. Blood samples from patients with five types of liver disease were analyzed. The viral liver disease cohort included a total of 32 patients (13 females and 19 males; age, 18-75 years) who were infected with the hepatitis virus, mainly hepatitis B and hepatitis C virus. In the DILD cohort, there were a total of 39 patients (23 females and 16 males; age, 12-83 years) who had liver damage caused by adverse drug reactions. These drug reactions included those caused by herbals and antimicrobials such as amoxicillin-clavulanate, nitrofurantoin and sulfamethoxazole-trimethoprim, which of which can cause liver diseases (36,37). Additionally, 26 patients, 28 patients and 29 patients were respectively included into the alcoholic liver disease cohort (1 female and 25 males; age, 36-77 years), the PBC cohort (25 females and 3 males; age, 20-78 years) and the AIH cohort (23 females and 6 males; age, 16-76 years) and 30 patients comprised the HC cohort (13 females and 17 males; age, 28-75 years).

The baseline characteristics of the participants in the present study are presented in Table I. Among the five cohorts, there were significant differences in sex, with more males exhibiting alcoholic liver disease and more females exhibiting PBC compared with those in the HC group. Laboratory parameters also significantly differed among the six cohorts. All patients with liver diseases exhibited higher AST, GGT and ALP levels but lower albumin levels compared those in the HC group. In the DILD group, patients exhibited higher ALT and bilirubin levels compared with those in the HC group. Data were presented as the median (interquartile range, 25th and 75th percentile). Bilirubin levels in the alcoholic liver disease group were demonstrated to be ~7X higher compared with those in the HC group.

Serum IgG subclass levels among patients with different liver diseases. Serum concentrations of IgG subclasses are presented in Table II and Fig. 1. Levels of each IgG subclass and ratio of the IgG subclasses to total IgG significantly differed among the six cohorts in the present study. However, this was not observed for IgG2. IgG1 levels in patients with AIH and the IgG1/IgG ratio in patients with viral liver disease were significantly higher compared with those in the HC group. Additionally, higher levels of IgG3 and IgG3/IgG, and lower levels of IgG4 and IgG4/IgG were detected in patients with PBC compared with those in the HC group. No notable in the distribution characteristics of IgG subclasses could be observed in the alcoholic liver disease and DILD cohorts in the present study. IgG1 levels were also significantly correlated with those of serum albumin in patients with AIH (r=0.488), PBC (r=0.709), DILD (r=0.578), alcoholic liver disease (r=0.644) and viral liver disease (r=0.534). The results also revealed that ALT, AST, GGT, ALP and bilirubin levels were not significantly associated with the levels of any of the IgG subclasses.

Table I. Baseline characteristics of participants in the five liver disease cohorts.

Variables	HC (n=30)	V-LD (n=32)	LD (n=26)	DILD (n=39)	PBC (n=28)	AIH (n=29)	Statistics	P-value
Age, years	53.8±8.8	50.2±15.4	57.0±10.7	50.1±16.1	56.9±12.3	54.3±14.2	F=1.58	0.167
Female/male	13/17	13/19	1/25 ^a	23/16	25/3 ^a	23/6	χ ² =51.58	<0.001
Laboratory parameters (normal range)								
ALT (9-50 U/l)	23.5 (16.8, 29.2)	40.6 (17.9, 74.4)	29.4 (16.2, 105.5)	66.9 (32.7, 206.1) ^b	42.8 (16.8, 74.1)	40.1 (17.4, 136.5)	H=21.85	0.001
AST (15-40 U/l)	23.5 (20.8, 27.2)	60.6 (30.6, 99.0) ^b	58.8 (37.9, 109.9) ^b	96.8 (42.1, 168.1) ^b	62.9 (28.1, 133.4) ^b	82.1 (28.0, 144.0) ^b	H=45.7	<0.001
GGT (10-60 U/l)	17.0 (12.0, 22.0)	74.1 (38.7, 164.2) ^a	105.1 (38.0, 723.2) ^b	147.6 (93.9, 359.8) ^b	131.5 (35.5, 258.9) ^b	99.2 (46.6, 206.6) ^b	H=36.90	<0.001
ALP (45-125 U/l)	51.0 (41.0, 59.5)	113.0 (83.0, 182.0) ^b	167.0 (95.2, 315.3) ^b	146.0 (109.0, 424.0) ^b	215.7 (117.0, 424.0) ^b	120.6 (78.6, 181.8) ^b	H=42.15	<0.001
Bilirubin (5-21 μmol/l)	18.3 (14.0, 20.0)	28.5 (14.8, 96.6)	122.3 (22.3, 209.7) ^a	42.5 (25.3, 221.4) ^a	33.8 (17.1, 118.6)	31.2 (17.3, 80.4)	H=17.74	0.003
Albumin (40-55 g/l)	44.8±2.3	32.9±6.5 ^b	32.9±4.8 ^b	35.7±5.0 ^a	33.7±5.7 ^b	33.5±6.2 ^b	F=10.63	<0.001

Data are presented as mean ± standard deviation or median (25, 75% percentile). ^aP<0.001 vs. HC. ^bP<0.05 vs. HC. V-LD, viral liver disease; A-LD, alcoholic liver disease; DILD, drug-induced liver disease; PBC, primary biliary cirrhosis; AIH, autoimmune hepatitis; AST aspartate aminotransferase; ALT alanine aminotransferase; GGT γ-glutamyl transpeptidase; ALP alkaline phosphatase.

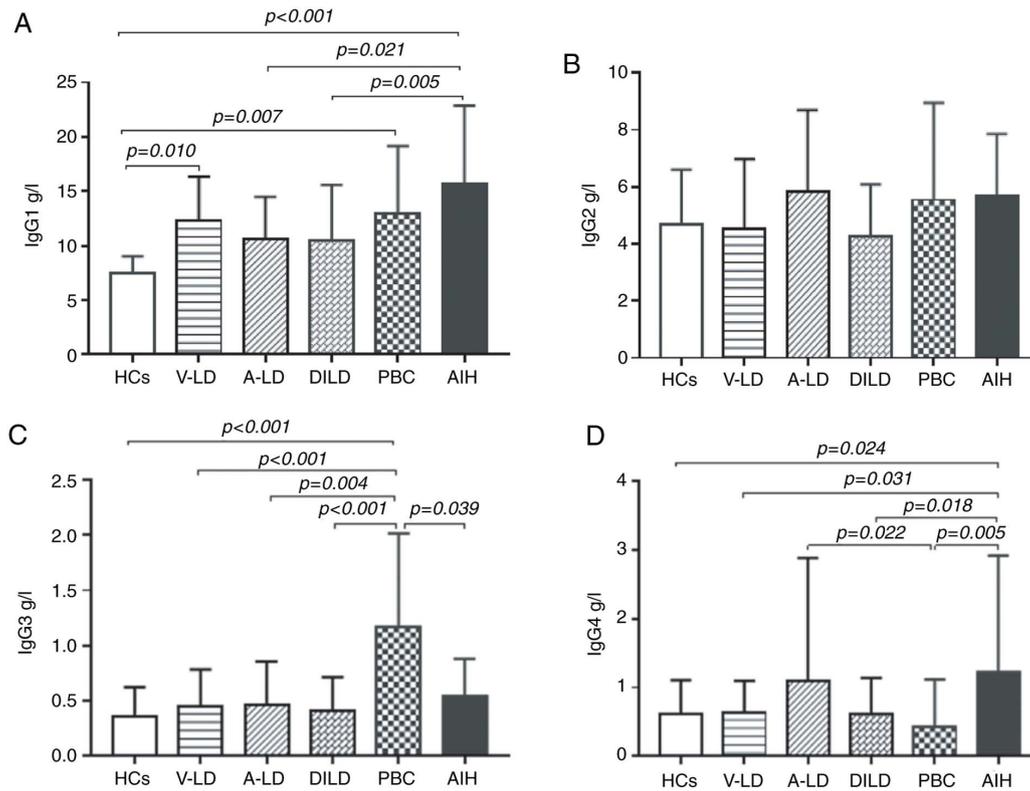


Figure 1. Serum IgG subclass levels among patients with different liver diseases. (A) IgG1 levels. Data are presented as the mean \pm standard deviation from three independent experiments. (B) IgG2, (C), IgG3 and (D) IgG4 levels. Data are presented as the median and 25th-75th percentiles from three independent experiments. Multiple comparison between the groups was performed using Scheffe post hoc test following Kruskal-Wallis tests. HCs, healthy controls; V-LD, viral liver disease; A-LD, alcoholic liver disease; DILD, drug-induced liver disease; PBC, primary biliary cirrhosis; AIH, autoimmune hepatitis.

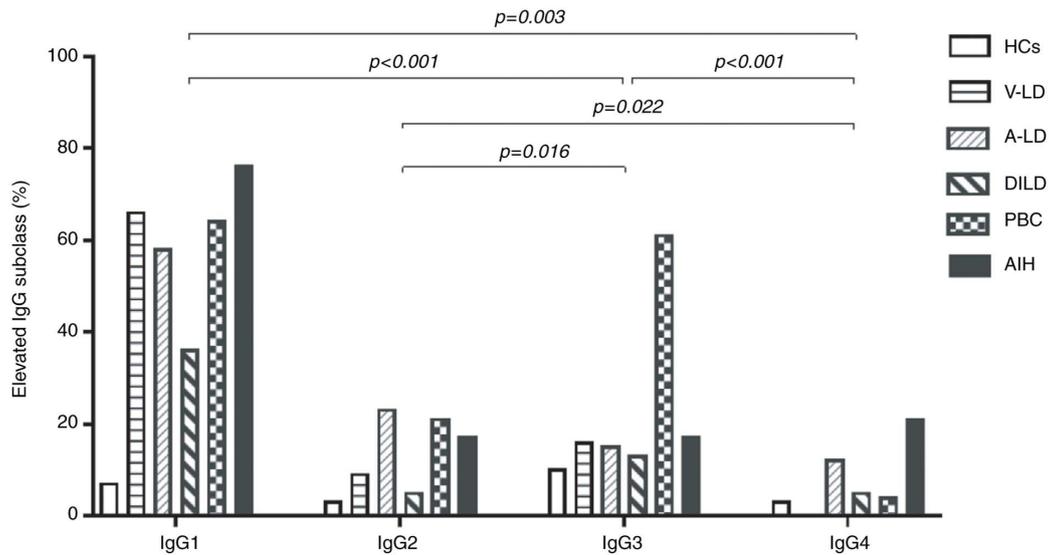


Figure 2. Elevated percentage of each IgG subclass among the different cohorts. All the statistical tests were performed using χ^2 test. HCs, healthy controls; V-LD, viral liver disease; A-LD, alcoholic liver disease; DILD, drug-induced liver disease; PBC, primary biliary cirrhosis; AIH, autoimmune hepatitis.

IgG subclass levels in each patient cohort. The percentage of patients with PBC exhibiting elevated IgG3 levels was four times higher compared with that in the other groups (Fig. 2). The frequency of elevated IgG1 in patients with AIH was the highest of all cohorts, whilst the percentage of elevated IgG1 in patients with DILD was the lowest compared with that in the five disease groups. Compared with other IgG subclasses,

the percentages of IgG1 and IgG4 elevations were the highest and lowest, respectively. In addition, the percentage of IgG1 elevation was markedly higher in all five cohorts compared with the other IgG subclasses.

Frequency of elevated IgG subclasses in each group and the merged cohort. Overall, 68.2% (105/154) participants in

Table II. Serum IgG subclass levels.

A, IgG subclass measurements										
IgG subclass	HCs (n=30)	V-LD (n=32)	A-LD (n=26)	DILD (n=39)	PBC (n=28)	AIH (n=29)	Statistic	P-value		
IgG1, g/l	7.49±1.53	12.62±4.52 ^a	10.57±3.93	10.45±5.16	12.96±6.20 ^a	15.64±7.25 ^b	F=8.826	<0.001		
IgG2, g/l	4.65 (3.63, 5.46)	4.12 (2.53, 5.66)	5.08 (3.49, 7.91)	3.89 (3.00, 5.23)	4.46 (3.01, 7.53)	5.22 (3.72, 7.93)	H=10.581	0.060		
IgG3, g/l	0.26 (0.15, 0.51)	0.35 (0.20, 0.56)	0.34 (0.23, 0.65)	0.30 (0.20, 0.55)	1.11 (0.60, 2.49) ^b	0.43 (0.28, 0.73)	H=34.486	<0.001		
IgG4, g/l	0.51 (0.26, 0.68)	0.67 (0.20, 0.93)	0.41 (0.23, 0.84)	0.46 (0.25, 0.77)	0.22 (0.05, 0.59) ^a	0.56 (0.20, 1.53)	H=10.824	0.028		
IgG, g/l	13.14±2.88	18.25±6.29 ^a	18.17±6.36 ^a	15.72±5.62	21.11±10.21 ^a	23.07±7.92 ^b	F=8.325	<0.001		

B, Ratios of each IgG subclass with total IgG										
IgG subclass	HCs (n=30)	V-LD (n=32)	A-LD (n=26)	DILD (n=39)	PBC (n=28)	AIH (n=29)	Statistic	P-value		
IgG1/IgG, %	58.01±9.47	69.69±9.64 ^a	59.26±12.86	64.82±10.89	63.23±10.81	66.49±12.99	F=4.712	<0.001		
IgG2/IgG, %	34.75±9.44	24.15±8.09 ^a	32.16±9.57	28.50±10.20	26.10±8.39 ^a	26.03±9.87 ^a	F=5.658	<0.001		
IgG3/IgG, %	1.72 (2.89, 3.72)	2.15 (1.14, 3.56)	1.95 (1.60, 3.35)	2.27 (1.28, 3.04)	5.84 (3.55, 13.77) ^b	2.22 (0.94, 3.46)	H=31.794	<0.001		
IgG4/IgG, %	3.82 (2.06, 5.94)	3.29 (1.73, 5.16)	2.50 (1.21, 6.90)	3.10 (2.06, 4.80)	1.40 (0.50, 2.20) ^b	3.10 (1.00, 5.61)	H=21.888	0.001		

Data are presented as mean ± standard deviation or median (25, 75% percentile). Statistical differences between two groups were analyzed using Student's t-test or Mann-Whitney test. ^aP<0.05 vs. HC; ^bP<0.001 vs. HC. HCs, healthy controls; V-LD, viral liver disease; A-LD, alcoholic liver disease; DILD, drug-induced liver disease; PBC, primary biliary liver disease; AIH, autoimmune hepatitis.

Table III. Frequency of elevated IgG subclasses in each group and all five cohorts combined.

Patient group	One type of increased IgG subclass ^a (n, %)	Two types of increased IgG subclasses ^b (n, %)	Three types of increased IgG subclasses ^c (n, %)	IgG1, IgG2, IgG3 and IgG4 increased ^d (n, %)	Only IgG4 increased (n, %)
V-LD (n=32)	17 (53.1)	3 (9.4)	2 (6.2)	0 (0)	0 (0)
A-LD (n=26)	12 (46.2)	3 (11.5)	2 (7.7)	1 (3.8)	3 (11.5)
DILD (n=39)	11 (28.2)	6 (15.4)	0 (0)	0 (0)	2 (5.1)
PBC (n=28)	8 (28.6)	9 (32.1)	4 (14.3)	1 (3.6)	1 (3.6)
AIH (n=29)	18 (62.1)	5 (17.2)	2 (6.9)	1 (3.4)	6 (20.7)
All five cohorts combined (n=154)	66 (42.8)	26 (16.9)	10 (6.5)	3 (1.9)	12 (7.8)

^aNumber of patients whose IgG1, IgG2, IgG3 or IgG4 was elevated. ^bNumber of patients who exhibited two types of elevated IgG subclasses, specifically IgG1 and IgG2; IgG1 and IgG3; IgG1 and IgG4; IgG2 and IgG3; IgG2 and IgG4; or IgG3 and IgG4. ^cNumber of patients who exhibited three types of elevated IgG subclasses, specifically IgG1, IgG2 and IgG3; IgG1, IgG2 and IgG4; IgG1, IgG3 and IgG4; or IgG2, IgG3 and IgG4. ^dAll four types of IgG subclasses were elevated. The normal range for IgG levels in adults used in this analysis were: i) IgG1, 4.05-10.11 g/l; ii) IgG2, 1.69-7.86 g/l; iii) IgG3, 0.11-0.85 g/l; and iv) IgG4, 0.03-2.01 g/l. HCs, healthy controls; V-LD, viral liver disease; A-LD, alcoholic liver disease; DILD, drug-induced liver disease; PBC, primary biliary cirrhosis; AIH, autoimmune hepatitis.

Table IV. The titer of ANA and ASMA between PBC and AIH patients.

Group	ANA titer				ASMA titer				Positive rates (% , n)	
	Negative	1:100	1:320	1:1,000	Negative	1:100	1:320	1:1,000	ANA	ASMA
PBC (n=26) ^a	5	2	1	18	26	0	0	0	80.8% (21/26)	0% (0/26)
AIH (n=28) ^b	3	3	6	16	19	3	4	2	89.3% (25/28)	32.1% (9/28)

^aTwo patients with PBC lacking ANA and SAMA test data. ^bOne patient with AIH lacking ANA and SAMA test data. V-LD, viral liver disease; A-LD, alcoholic liver disease; DILD, drug-induced liver disease; PBC, primary biliary cirrhosis; AIH, autoimmune hepatitis.

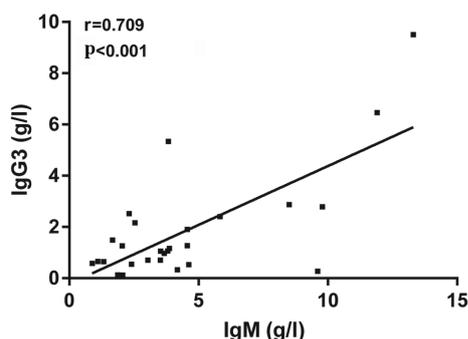


Figure 3. Correlation between IgG3 and IgM concentrations in patients with PBC.

the merged cohort (five cohorts combined) had \geq one type of elevated IgG subclass (Table III). The most frequently observed increase was one type of increased IgG subclass, whilst four types of elevated IgG subclasses was the least common. In 25.3% (39/154) patients, \geq two IgG subclasses were simultaneously increased.

Relationship between IgG3 and IgM concentrations in patients with PBC. IgA levels in patients with PBC were found

to be 3.65 (1.78, 5.82) g/l and 3.06 (1.53, 6.30) g/l in patients with AIH. Data were presented as the median (interquartile range, 25 and 75th percentile). IgA did not significantly differ between patients with PBC and AIH. IgM levels in patients with PBC were 3.60 (2.12, 4.60) g/l and 1.80 (1.0, 3.0) g/l in patients with AIH. IgM levels in patients with PBC were significantly higher compared with that in patients with AIH. Pearson's correlation coefficient analysis was subsequently used to evaluate the relationship between IgG3 and IgM concentrations in patients with PBC. The results revealed that IgG3 levels were significantly correlated with those of serum IgM in patients with PBC (Fig. 3). These results suggested that IgG3 and IgM may serve synergistic effects in patients with PBC.

Relationship between concentrations of IgG3 and AMA-M2 in patients with PBC. PBC is an autoimmune disease of the liver characterized by the presence of AMA in 90-95% of patients (38). Serum AMA, particularly that of the AMA-M2 subtype, is regarded to be one of the most specific and acceptable diagnostic indicators of PBC (39). In the present study, IgG3 levels were not found to be significantly associated with AMA-M2 titers in patients with PBC (Fig. 4). A total of 94.1% patients with PBC demonstrated medium to

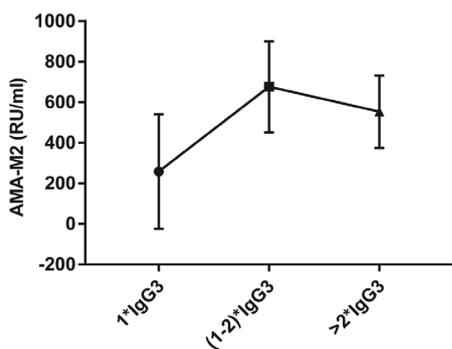


Figure 4. Relationship between concentrations of IgG3 and AMA-M2 in patients with PBC. Each datapoint was presented as the mean \pm standard deviation. AMA-M2, anti-mitochondrial M2; 1*IgG3, normal reference range values of IgG3; (1-2)*IgG3, (1-2) times of reference range of IgG3; >2*IgG3, IgG3 levels >2X upper limit of normal range.

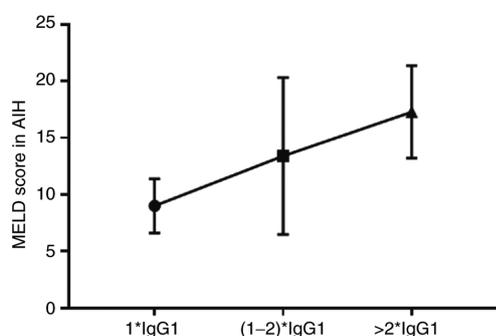


Figure 5. Association between IgG1 subclass concentration and MELD liver damage score in patients with AIH. Each datapoint was presented as the mean \pm standard deviation. MELD, model for end-stage liver disease; AIH, autoimmune hepatitis; 1*IgG1, normal reference range values of IgG1; (1-2)*IgG1, 1-2 times of reference ranges of IgG1; >2*IgG1, IgG1 levels >2X upper limit of normal.

high AMA-M2 titers (AMA-M2 \geq 200 RU/ml) when IgG3 was elevated. When elevated IgG3 was 1-2X higher than the reference interval, AMA-M2 titers were also higher compared with two groups of 1X and >2X reference interval of IgG3 (Fig. 4). The positive rate of the ANA autoantibody in patients with PBC was 80.8% (21/26) and 89.3% (25/28) in patients with AIH (Table IV), due to two patients with PBC and one patient with AIH lacking ANA test data. ANA titers did not significantly differ between patients with PBC and AIH. ASMA could not be detected in patients in the PBC group whereas the positive rate of ASMA in patients with AIH was 32.1% (9/28) (Table IV). The incidence of ASMA positivity in patients with AIH were significantly higher compared with those in the PBC cohort ($\chi^2=10.03$; $P=0.002$).

Association between IgG subclass concentration and liver damage in patients with AIH. MELD scores are used to calculate the degree of liver damage, such that the higher the MELD score, the greater the liver damage (40). Patients with AIH demonstrated higher MELD scores (13.28 \pm 6.10) compared with those in the PBC cohort (12.63 \pm 5.27), but statistical significance was not observed. In patients with AIH, IgG1 levels did not significantly associate with the MELD

Table V. Frequency of elevated serum IgG4 levels in patients.

Groups	Cases	N (IgG4 >135 mg/dl, %)	P-value ^a
V-LD	32	3 (9.4)	-
A-LD	26	4 (15.4)	0.222
DILD	39	3 (7.7)	0.809
PBC	28	1 (3.6)	0.165
AIH	29	6 (20.7)	0.079

^aP-value vs. V-LD. V-LD, viral liver disease; A-LD, alcoholic liver disease; DILD, drug-induced liver disease; PBC, primary biliary cirrhosis; AIH, autoimmune hepatitis.

scores. However, within the same cohort, MELD scores were the highest in the cohort exhibiting IgG1 levels > two times higher than the reference intervals (Fig. 5). IgG1 levels and MELD scores may therefore share a synergistic relationship.

Frequency of elevated serum IgG4 levels in patients. Serum IgG4 levels >135 mg/dl are considered to be diagnostic criteria for IgG4-RD (18). The number and frequency of patients with serum IgG4 levels >135 mg/dl were therefore calculated among the five cohorts. Among the 154 patients, only 17 (11.0%) had serum IgG4 levels >135 mg/dl (Table V). In addition, no significant difference in the frequency of elevated serum IgG4 levels was identified between patients with viral liver disease and patients without non-viral liver disease (Table V).

Discussion

Liver diseases can be caused by a variety of factors, including genetic predisposition, infection, autoimmunity or metabolism, which renders diagnosis challenging (4). In the present retrospective study, serum IgG subclass levels were analyzed in patients with five common liver diseases to further their understanding and the development of efficient novel early diagnosis strategies. The results revealed that certain serum IgG subclass levels were selectively increased or decreased depending of the type of disease.

Previous studies have demonstrated that serum IgG3 levels are elevated patients with PBC (41,42). However, little is known about IgG subclasses in patients with AIH. The results of the present study suggested that there were higher levels of IgG3 and IgG3/IgG but lower levels of IgG4 and IgG4/IgG in patients with PBC compared with the HC group, further validating the results of aforementioned studies. Additionally, compared with those in the HC group, IgG1 levels were significantly higher in patients with AIH. PBC and AIH are autoimmune diseases. The results of the present study indicated that IgG subclasses may serve a role in the differential diagnosis of patients with PBC and AIH. The four subclasses of IgG differ in their constant regions, particularly in their hinges and CH2 domains. IgG1 has the highest Fc γ R binding affinity, followed by IgG3, IgG2 and IgG4 in that order (43). When antigens enter the body, their chemical compositions stimulate an immune reaction, resulting in differential patterns of class switching. For different antigens, such as adhesion protein desmoglein 3,

interleukin (IL)-4 and IL-10, class switching tends to occur from IgG1 or IgG3 to IgG4 (44,45).

A previous study revealed that alcoholic liver disease was associated with low serum concentrations of IgG and reduced IgG following alcohol administration in mice (46). However, the results of the present study demonstrated that IgG levels were elevated, which was not consistent with observations from this previous study. This may be due to different effects exerted by alcohol in murine and human systems. Alonso *et al* (46) previously injected alcohol into mice and measured IgG concentrations after 4 weeks and found that alcohol administration appeared to decrease IgG subclass concentrations. Patients with alcoholic liver disease frequently have a long history of drinking (47). It therefore may take some time for the IgG subclasses to be produced in patients with alcoholic liver disease. Additionally, Alonso's research objects were mice, the physiological functions of which were different from those of humans. As a result, further research is required to assess these effects in humans.

Previous studies have demonstrated that when compared with HCs, serum IgG1 and IgG3 levels were higher in virus-infected individuals (48). In the present study, IgG1/IgG levels were significantly increased in viral liver disease group when compared with HCs. IgG3 was also higher compared with that of HCs, but without significance. IgG serves a pivotal role in viral neutralization (49). Antigens can trigger B-cells directly, leading to a plethora of secondary signals that regulates cell differentiation and subsequent antibody production (50,51). The induction of specific IgG1 and IgG3 antibodies is therefore more likely to achieve the clearance of HBV and HCV (49).

The results of the present study revealed that the expression of IgG3 in patients with PBC was significantly correlated with the levels of serum IgM. Furthermore, 94.1% patients with PBC demonstrated medium to high AMA-M2 titers (AMA-M2 \geq 200 RU/ml) when IgG3 was elevated. Serum IgM and AMA-M2 levels are two of the most important and specific diagnostic indicators of PBC (52). IgG3, IgM and AMA-M2 may have synergistic effects in PBC, which may be involved in the pathogenesis of PBC. In addition, since AMA-M2 is primarily of the IgG-AMA subclass (52) and that currently available commercial kits are customized for IgG-AMA detection, measurement of AMA and IgG3 levels may be more accurate than radioimmunoassay in diagnosing PBC.

The MELD score was designed to calculate the degree of liver damage, where higher MELD score implies greater damage to the liver (40). In the present study, it was demonstrated that IgG1 levels and MELD scores may have a synergistic relationship in patients with AIH. Different antigens can stimulate an IgG1 response (51). Therefore, excessive IgG1 levels are more likely to harm the liver and cause autoimmune liver disease (53,54).

IgG4-RD is a systemic disease involving a number of organs, including the pancreas, lacrimal glands, lungs, liver and kidney (55). Two types of liver disease involvement have been reported in relation to IgG4-RD: IgG4-related AIH and IgG4-hepatopathy (55). Certain common liver diseases have been reported to share similarities with IgG4-RD. IgG4-related AIH is clinicopathologically similar to that of AIH, except for the elevated serum IgG4 levels and heavy infiltration of

IgG4-positive plasma cells in the liver tissue (56). In addition, IgG4-related AIH can be hard to diagnose as well-known IgG4-RD(s) (55). Yamamoto *et al* (28) revealed that except for IgG4-RD, increased IgG4 levels were observed several clinical cases, such as Churg-Strauss syndrome, multicentric Castleman's disease, eosinophilic disorders, and some patients with rheumatoid arthritis, systemic sclerosis, chronic hepatitis, and liver cirrhosis. Additionally, Ebbo *et al* (57) reported that 13.6% patients whose serum IgG4 levels were $>$ the cutoff value of 135 mg/dl were diagnosed with IgG4-RD. In the present study, only 11% patients exhibited serum IgG4 levels $>$ 135 mg/dl, which was lower compared with that of previous studies. The results suggested that serum IgG4 levels in the five patient cohorts of the present study were different from those with IgG4-RD in the previous studies aforementioned. These data may prove to be useful for differential diagnosis between IgG4-RD and five liver disease cohorts, although they share similarities of elevated serum IgG4 levels in pathogenic processes. Additionally, the present study suggested that IgG4 and other IgG subclasses may be used to distinguish liver diseases from IgG4-RD.

The present study has several limitations. Firstly, since this was an observational study, the effect of IgG4 on the clinical manifestation of patients was not determined. Therefore, a follow-up process should be performed in future studies. Secondly, different laboratories could have slightly different definitions for elevated IgG4. Finally, the number of samples in the present study was relatively small and the present study lacked patients at different stages of liver disease.

In conclusion, different liver diseases were found to be associated with different serum IgG subclass distributions. The present study suggested that IgG subclasses may serve as biomarkers for the early diagnosis of liver diseases. However, further studies on serum IgG subclass distributions may further elucidate the immunopathogenesis of liver disease.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

WZ drafted the manuscript. WZ and FJ collected the data. JS, YW, YJ, QG and JL performed the experiments. WZ and YZ

performed the statistical analysis and participated in the study design. WZ and YZ participated in the acquisition, analysis or interpretation of the data. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The present study was approved by the Ethics Committee of Beijing YouAn Hospital, Capital Medical University (Beijing, China).

Patient consent for publication

Not applicable.

Competing interests

All authors declare that they have no competing interests.

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