

Liver, Pancreas and Biliary Tract

Intrahepatic IgG/IgM plasma cells ratio helps in classifying autoimmune liver diseases

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ABSTRACT

Background/Aim: Plasma cells infiltrate in the liver is a prototype lesion of autoimmune liver diseases. The possible role of plasma cells isotyping (IgM and IgG) in the liver in the diagnostic definition of autoimmune liver disease, and particularly in variant syndromes such as autoimmune cholangitis and the primary biliary cirrhosis/autoimmune hepatitis overlap syndrome, is less defined.

Methods: We analysed the clinical, serological and histological features of 83 patients with autoimmune liver disease (40 primary biliary cirrhosis, 20 autoimmune hepatitis, 13 primary sclerosing cholangitis, 4 autoimmune cholangitis and 6 overlap syndrome) compared to 34 patients with chronic hepatitis C and evaluated the expression of IgM and IgG plasma cells in their liver by immunostaining.

Results: By Spearman's correlation, the mean-counts of IgM plasma cells in portal tracts were significantly correlated with female gender, serum alkaline phosphatase, gamma-glutamyl transferase and IgM values, positivity for anti-mitochondrial antibody-M2 and, on liver biopsy, with bile duct changes, orcein-positive granules and granulomas. Whereas IgG plasma cells resulted more correlated with alanine aminotransferase levels. IgG/IgM ratio lower than 1 was found not only in primary biliary cirrhosis but also in all patients with autoimmune cholangitis. Conversely, all patients with overlap syndrome showed IgG/IgM ratio higher than 1.

Conclusion: Immunostaining for IgM and IgG plasma cells on liver tissue can be a valuable parameter for better diagnosis of autoimmune liver disease and also for variant or mixed syndromes.

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1. Introduction

Primary biliary cirrhosis (PBC), autoimmune hepatitis (AIH) and primary sclerosing cholangitis (PSC) cover 90% of the spectrum of autoimmune liver diseases. Diagnosis is based on clinical, biochemical, serological and histological findings [1]. Approximately 10% of patients with PBC or AIH are affected by variant syndromes (VS), presenting simultaneous serological and/or histological features of both diseases. The diagnosis of VS can be difficult in clinical practice because there are no standardized definitions. Biochemical and serological criteria are used to characterize the overlap syndrome (OS), and a liver biopsy that reveals biliary duct injuries and moderate to severe interface hepatitis simultaneously [2–4] helps confirming the diagnosis of OS. Likewise, another variant syn-

drome, called autoimmune cholangitis (AIC) or anti-mitochondrial antibody negative PBC [5], can also be missed on biopsy, particularly when histology lacks florid bile duct lesions and/or other typical lesions that contribute to the histological diagnosis of PBC [6,7].

Plasma cells infiltrate is a histological feature frequently seen in the liver tissue of patients with autoimmune liver disease. The presence of plasma cells in the portal and peri-portal inflammation infiltrate is a well-known feature of PBC and of AIH, and a predominance of immunoglobulin M (IgM) in PBC and of immunoglobulin G (IgG) in AIH has been reported [8,9]. In patients with PBC, owing to an innate immunity-induced hyper-responsive state the serum levels of IgM are elevated [10,11], though the specificity of this test is low; an increase in IgM serum levels is also reported in patients with PSC [12].

Using immuno-histochemical techniques, we analysed the plasma cells infiltrate in liver biopsies of a cohort of patients affected by autoimmune chronic liver disease, evaluating the amount of cells expressing immunoglobulin isotypes M and G (IgM, IgG). The aim of the study was to investigate the relation between plasma cells infiltrate and other clinical and histological features

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of autoimmune liver disease, as well as the possible role of plasma cells infiltrate in the diagnosis of VS.

2. Patients and methods

2.1. Patients

We prospectively analysed the records of 83 consecutive, untreated patients with clinical, serological and histological features of immune-mediated liver disease referred at our Liver Unit for the liver biopsy between 2004 and 2008, comparing them with 34 consecutive patients with chronic hepatitis C (CHC) evaluated and underwent liver biopsy between September and December 2008. The diagnosis of PBC was based on the presence of elevated serum values of alkaline phosphatase (AP) and/or gamma-glutamyl transferase (GGT), anti-mitochondrial antibody (AMA) detectable in the serum, and on liver biopsy showing such suggestive features as bile duct loss, chronic cholangitis, epithelioid granulomas, orcein-positive granules, interface and lobular hepatitis and fibrosis [1,6,7,13,14].

The diagnosis of AIH was made according to the revised criteria of the International Autoimmune Hepatitis Group (IAHG) [15]. Only patients with a score of >15, and without previous immunosuppressive therapy, were included in the study. A diagnosis of PSC was made in the presence of characteristic changes in the intrahepatic and extrahepatic biliary tree, seen with direct cholangiographic imaging, and in the presence of compatible histological features [16]. Some patients were considered affected by VS. In particular, a diagnosis of OS was formulated for patients who fulfilled at least two of the three criteria for the diagnosis of PBC, and had clinical and histological features of AIH based on an aggregate revised IAHG score of at least 10 points in the absence of immune-suppressive therapy [17]. A diagnosis of AIC was formulated for patients who showed a predominant biochemical cholestatic expression, and fulfilled at least 2 criteria for diagnosis of PBC, but with an aggregated revised IAHG score ≤ 10 . None of the patients had a previous or current history of alcohol or drug abuse.

HBV chronic hepatitis and genetic liver diseases (Wilson's disease, $\alpha 1$ -antitrypsin deficit and hereditary haemochromatosis) in the cases were excluded by means of current virological, serological and clinical tests. The study was performed in accordance with the principles of Good Clinical Practice, the principles of the Declaration of Helsinki and its appendices, and local and national laws.

2.2. Laboratory assessment

Routine clinical examinations and serological studies were performed for all patients at the time of first admission to our Liver Unit and were available in the hospital files. All patients were tested for antinuclear antibody (ANA), smooth muscle antibody (SMA), liver–kidney microsomal type 1 antibody (LKM1), and AMA, with indirect immunofluorescence method using mouse stomach tissue and kidney tissue section as substrates (Alifax, Padua, Italy). A serum titre of 1:80 or higher was considered positive for ANA and SMA, and a titre of 1:40 or higher was considered positive for anti-LKM1 and AMA. E2 subunit of pyruvate dehydrogenase mitochondrial auto-antigen (M2) was detected by enzyme-linked immunosorbent assay (immuno-blotting) against pyruvate dehydrogenase complex (Euroimmun version 2006, Lubeck, Deutschland). Serum IgM levels were measured with standard techniques.

2.3. Histological evaluation

Percutaneous liver biopsies, performed with a 16-gauge Menghini needle, were 10% formalin fixed and paraffin embedded. We

used 5 μ m thick serial sections that were rewashed, rehydrated and stained with haematoxylin–eosin, Shikata's orcein, PAS diastase, and Gomori stain for reticular fibres, and also with Masson's trichromic stain. Slides of liver specimens were coded and re-read by a pathologist (D.C.), who was unaware of patient's identity and clinical or serological features. Only biopsies at least 15 mm long and containing more than eight portal tracts were evaluated.

As mentioned above we investigated the histological features associated with PBC: chronic non-suppurative destructive cholangitis involving interlobular bile ducts, with typical duct lesions consisting of lymphocytic infiltration, irregularity and eosinophilic changes of the epithelium; and bile duct loss and/or atypical ductular proliferation, which we generally coded as "ductular changes." In addition, we considered other features that helped confirm histological diagnosis, such as portal epithelioid granulomas, which were identified as the presence of small aggregates of histiocytoid cells in the portal tract, often surrounding a biliary duct, and chronic cholestasis, as evidenced by the presence of peri-portal orcein-positive granules [6].

We also investigated the typical histological findings of AIH, such as mononuclear and plasma cells infiltration of the portal spaces expanding into the liver lobule; destruction of the hepatocytes at the periphery of the lobule, with erosion of the limiting plate (interface hepatitis); connective tissue collapse from the portal area into the lobule (bridging collapse); and rosette formation due to hepatic regeneration [15]. Characteristic histological features of PSC were evaluated, such as degeneration and proliferation of epithelial cells of bile ducts, infiltration of the bile ducts by lymphocytes and neutrophils, and the formation of concentric layers of connective tissue surrounding bile ducts (onionskin lesion) [16]. Finally, the grade of interface hepatitis and the stage of fibrosis were evaluated according to the histological scoring system proposed by Hiramatsu et al. [6].

Immunohistochemical staining was performed using the streptavidin–biotin–complex technique. The primary antibodies used in this study were polyclonal antibodies anti-IgG and anti-IgM, both diluted 1/2000 (Novocastra, Newcastle, United Kingdom). Before incubation with anti-IgG and anti-IgM antibodies, a pre-treatment with pepsin was done to improve the immunostaining. The endogenous peroxidase activity was blocked by incubation of liver sections in a solution of 0.3% hydrogen peroxidase and absolute methanol. The non-specific antigenic sites were blocked using a protein block solution included in the kit. After incubation for 15 min at room temperature, the sections were incubated with the secondary antibody, which was followed by streptavidin–biotin–complex. Amino-ethyl-carbazole was used as chromogen. Slides counterstained with Mayer's haematoxylin were then routinely mounted. For immunohistochemistry evaluation, we considered as positive internal control the presence of at least two positive staining plasma cells for each antibody in at least two portal tracts. We counted the number of plasma cells containing IgG and IgM in the three most representative portal tracts for each specimen, calculated the mean values of the number of plasma cells present in the three most representative portal tracts, and evaluated the ratio between mean IgG and IgM plasma cells.

2.4. Statistical methods

Continuous variables were summarized as mean \pm SD, or median and range when appropriate, and categorical variables as frequency and percentage. Significant differences were calculated using the ANOVA or Kruskal–Wallis test for continuous variables, while a χ^2 test was used for categorical variables. Correlation coefficients were calculated by Spearman's test. Multiple linear regression analysis was performed through SPSS software (SPSS Inc., Chicago, IL, USA).

Table 1
Clinical, biochemical and histological features of patients.

Variables	PBC (n = 40)	AIH (n = 20)	PSC (n = 13)	Variant syndromes (n = 10)	CHC (n = 34)	p
Mean age (years)	49.53 ± 11.89	47.53 ± 19.21	48.85 ± 21.43	52.10 ± 14.27	51.45 ± 11.73	0.9
Female gender	37 (92.5)	15 (75)	4 (31)	10 (100)	15 (44)	<0.001
ALT (IU/ml, UNL)	2.04 ± 1.55	8.72 ± 10.88	1.97 ± 1.56	3.63 ± 2.79	2.22 ± 2.41	<0.001
GGT (IU/ml, UNL)	8.01 ± 12.04	3.92 ± 4.18	4.30 ± 5.03	6.45 ± 4.63	0.71 ± 0.66	0.003
AP (IU/ml, UNL)	3.24 ± 3.81	1.15 ± 0.90	1.67 ± 1.05	2.41 ± 2.61	0.53 ± 0.41	<0.001
• Autoantibodies no AMA overall	14 (35)	20 (100)	8 (66.7)	9 (90)	13 (38.2)	0.1
• Anti nuclear	14 (35)	18 (90)	5 (38.5)	6 (60)	8 (23.5)	
• Anti smooth muscle	1 (2.5)	8 (40)	5 (38.5)	7 (70)	2 (5.9)	
Anti liver/kidney microsomal	0	3 (15)	0	0	4 (11.7)	
Anti-mitochondrial Ab/M2	38 (95)	0	0	3 (30)	1 (2.9)	<0.001
Median serum IgM (mg/dl) ^a	395.00 (167–3576)	257.00 (100–366)	111.0 (80–220)	348.00 (130–560)	109.0 (78–200)	0.001
Histological features						
Interface hepatitis > 1 ^b	12 (30)	13 (65)	2 (15.4)	7 (70)	10 (29.4)	0.42
Fibrosis > 1 ^b	8 (20)	5 (25)	0	2 (20)	8 (23.5)	0.5
Ductular changes	37 (92.5)	10 (50)	9 (69.2)	8 (80)	7 (20.6)	<0.001
Orcein-positive granules	21 (52.5)	0	3 (23.1)	4 (40)	5 (14.7)	<0.001
Granulomas	23 (57.5)	4 (20)	0	5 (50)	0	<0.001

Continuous variables: mean ± standard deviation or median (min-max); categorical variables: absolute value (%).

Abbreviations: UNL, upper normal limit; ALT, alanine aminotransferase; GGT, gamma-glutamyl transferase; AP, alkaline phosphatase; M2, E2 subunit of pyruvate dehydrogenase mitochondrial auto-antigen.

^a Normal range: 40–240 mg/dl.

^b According to histological grading and staging system explained in ref. [6].

The ROC curve of the IgM plasma cells counts was performed using MedCalc[®] software (version 8.0; Mariakerke, Belgium). The box plots were generated through GraphPad Prism[®] software (version 4.02; San Diego, CA, U.S.A.). Differences were considered significant for *p* values of <0.05.

3. Results

3.1. Clinical and histological features of patients

Demographic, biochemical, serological and histological features of patients at baseline are shown in Table 1. Forty patients who satisfied the diagnosis of PBC, 20 patients with diagnosis of AIH, 13 patients with a diagnosis of PSC and 10 patients with VS were compared to 34 patients with a diagnosis of CHC, according to the criteria mentioned above. Female gender was prevalent in PBC and in VS compared to other groups (*p* < 0.001). ALT levels were significantly higher in patients with AIH compared to those of other groups (AIH: 8.72 UNL; PBC: 2.04 UNL; PSC: 1.97 UNL; VS: 3.63 UNL and CHC: 2.22 UNL IU/ml; *p* < 0.001). Conversely, GGT levels were higher in PBC and in VS patients compared to the other two groups (PBC: 8.01 UNL; VS: 6.45 UNL; AIH: 3.92 UNL; PSC: 4.30 UNL and CHC: 0.71 UNL IU/ml; *p* < 0.003). Likewise, AP levels were significantly more elevated in PBC and in VS patients (PBC: 3.24 UNL; VS:

2.41 UNL; AIH: 1.15 UNL; PSC: 1.67 UNL and CHC: 0.53 UNL IU/ml; *p* < 0.001).

Autoantibodies were present in all patients with AIH, in 90% of patients with VS and in 67% of those with PSC. AMA-M2 was found in patients with PBC (95%), in VS patients (30%) and in only one patient with CHC (3%) (*p* < 0.001). We found the positivity of antinuclear antibody (ANA) with indirect immunofluorescence method in 14 of our PBC patients: a centromer pattern in 7 patients, a nucleolar pattern in 3 patients, a membranous in 3 and a multi-nuclear dots pattern in one patient.

More elevated median serum IgM levels were found in PBC and in VS patients (395 mg/dl and 348 mg/dl, respectively) compared to those observed in AIH (257 mg/dl), while in PSC and CHC patients we observed normal levels of serum IgM (*p* = 0.001).

A moderate–severe grade of interface hepatitis in liver biopsy was more frequently observed in VS and in AIH patients (70% and 65%, respectively) compared to PBC (30%), to CHC (29%) and PSC patients (15%), though with no significant differences (*p* = 0.42). We found a similar rate of moderate–severe stage of fibrosis in PBC, AIH, VS and CHC patients (from 20 to 25% of the patients), but in none of the PSC patients (*p* = 0.5). Ductular changes were prevalent in PBC and in VS patients (92.5% and 80%, respectively) compared to AIH (50%) and PSC (69%); we found this feature only in 21% of CHC patients (*p* < 0.001). Orcein-positive granules were observed

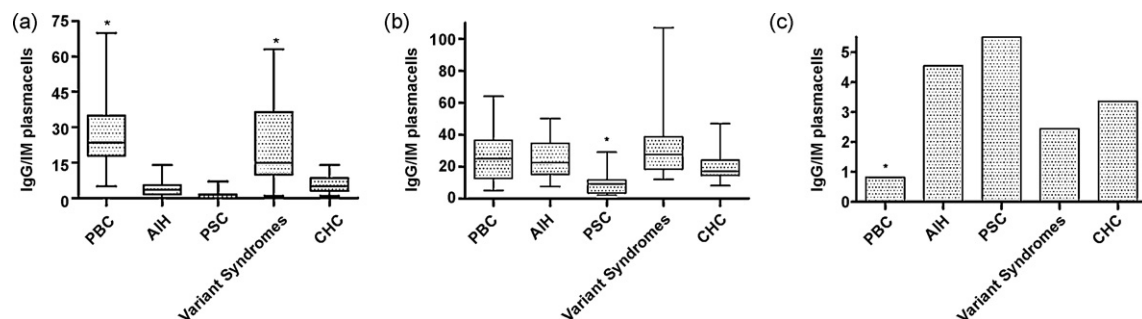


Fig. 1. (a) Box plots of IgG plasma cells counts in patients affected by different autoimmune liver diseases. (b) Box plots of IgM plasma cells counts in patients affected by different autoimmune liver diseases. (c) Histograms of ratio of IgG/IgM plasma cells counts in patients affected by different autoimmune liver diseases. (*) Significant difference in the comparison with the other groups.

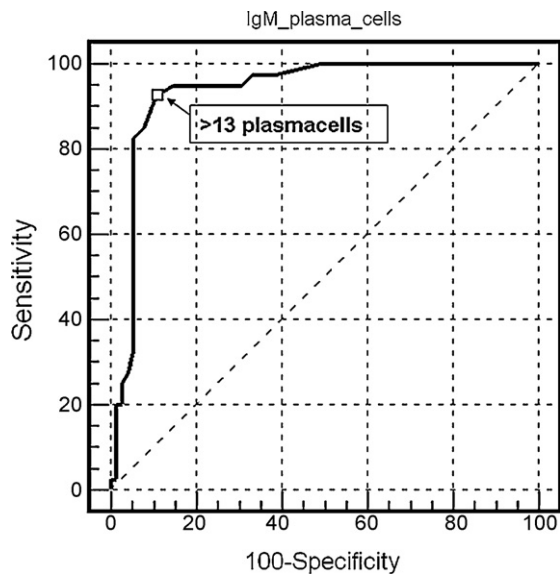


Fig. 2. ROC curve of IgM plasma cells counts according to clinical/histological diagnosis of PBC. Cut-off point: >13 mean IgM plasma cells in the portal tract of liver biopsy (sensitivity 92.5%; specificity 89.3%). Area under the ROC curve: 0.94 (95% confidence interval 0.87–0.97; $p < 0.001$).

in 52.5% of the patients with PBC, in 40% of those with VS, but in only 23% of the patients with PSC and in 15% of those with CHC, ($p < 0.001$). No patients with AIH showed this feature in liver biopsy. Likewise, epithelioid granulomas were found more frequently in PBC and in VS patients as compared to others (PBC: 57.5%; VS: 50%; AIH: 20%; PSC and CHC: 0%) ($p < 0.001$).

3.2. Analysis of IgM and IgG plasma cells counts in different autoimmune liver diseases

The median value of IgG plasma cells mean-counts observed in the portal spaces of liver biopsies was significantly lower in the PSC group (9 cells/portal tract) as compared to the PBC group (25 cells/portal tract), to AIH (24.5 cells/portal tract), to VS (27.5 cells/portal tract) and to CHC patients (17 cells/portal tract) respectively ($p < 0.001$) (Fig. 1a). The median value of IgM

Table 2

Spearman's rho correlation analysis of IgM and IgG plasma cells scores and clinical, serological and histological features of patients.

Variables	IgM plasma cells		IgG plasma cells	
	Spearman's rho	<i>p</i>	Spearman's rho	<i>p</i>
Age	-0.04	0.64	0.04	0.68
Gender	0.38	<0.001	0.14	0.16
ALT UNL	-0.05	0.56	0.25	0.01
GGT UNL	0.35	0.001	0.19	0.05
AP UNL	0.36	0.001	0.11	0.26
Autoantibodies	-0.11	0.27	0.22	0.03
AMA-M2	0.66	<0.001	0.14	0.24
Serum IgM ^a	0.58	<0.001	0.22	0.19
Interface hepatitis >1 ^b	0.03	0.76	0.28	0.005
Fibrosis >1 ^b	0.08	0.43	0.25	0.02
Ductular changes	0.45	<0.001	0.18	0.09
Orcein granules	0.51	<0.001	0.23	0.04
Granulomas	0.45	<0.001	0.22	0.06

Abbreviations: UNL, upper normal limit; ALT, alanine aminotransferase; GGT, gamma-glutamyl transferase; AP, alkaline phosphatase; AMA, anti-mitochondrial antibody; M2, E2 subunit of pyruvate dehydrogenase mitochondrial auto-antigen.

^a Normal range: 40–240 mg/dl.

^b According to histological grading and staging system explained in ref. [6].

Table 3 Clinical and histological features of the 4 patients with diagnosis of autoimmune cholangitis (or AMA-M2 negative PBC).

Patients	Age	Gender	ALT UNL	AP UNL	ANA/ASMA/anti-LKM	Typical histologic features	Serum IgM UNL	IAGH score	Mean IgM plasma cells	Mean IgG plasma cells	IgG/IgM
1 C-A	70	F	1.3	1.54	-/+/-	Duct changes, granulomas, orcein granules/A3/F2	1.45	4	63	17	0.27
2 G-DG	68	F	4.29	8.66	+/+/-	Duct changes, granulomas, orcein granules/A1/F1	0.54	5	34	31	0.91
3 S-I	57	F	2.87	5.66	-/+/-	Duct changes, orcein granules/A1/F1	1.06	5	32	20	0.62
4 L-P	53	F	1.0	1.13	+/-/-	Granulomas/A1/F1	1.08	9	15	12	0.82

Abbreviations: UNL, upper normal limit; ALT, alanine aminotransferase; GGT, gamma-glutamyl transferase; AP, alkaline phosphatase; ANA, antinuclear antibody; anti-LKM, liver kidney microsomal antibody; A (n.), activity of necro-inflammation (grade of interface hepatitis); F (n.), stage of fibrosis (according to histological grading and staging system explained in ref. [6]); IAGH, International Autoimmune Hepatitis Group.

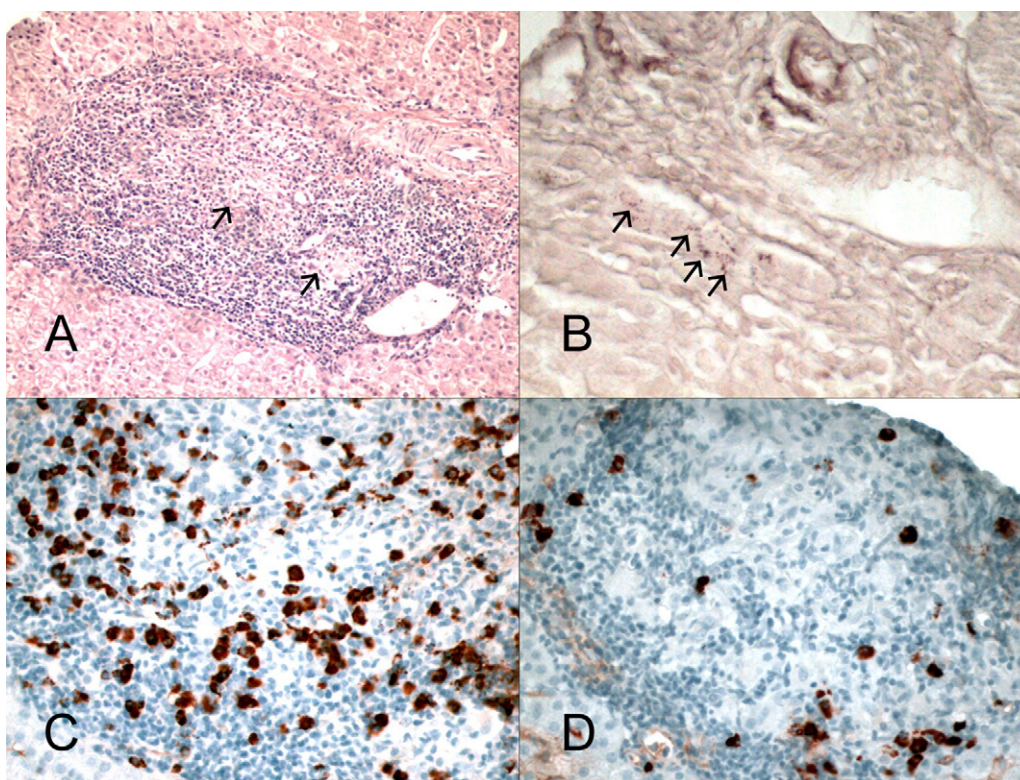


Fig. 3. Histological features and immunostaining for IgM and IgG plasma cells in liver biopsy of patients with autoimmune cholangitis. (A) Portal tract with mild interface hepatitis and portal lymphoplasmacytic infiltrate including aggregates of histiocytoid cells (arrows); haematoxylin–eosin: 100 \times . (B) Chronic cholestasis evidenced by the presence of peri-portal orcein-positive granules (arrows); Shikata's orcein: 400 \times . (C) Portal tract with numerous IgM positive staining plasma cells; whereas in (D) there are a few IgG positive staining plasma cells; immunostaining 250 \times .

plasma cells mean-counts was significantly higher in PBC (about 23.5 cells/portal tract) and in VS group (15 cells/portal tract), as compared to those found in AIH (4 cells/portal tract), in PSC (2 cells/portal tract) and in CHC group (5 cells/portal tract) ($p < 0.001$), with no significant differences between PBC and VS patients ($p = 0.2$) (Fig. 1b). The ratio of the median values of IgG/IgM plasma cells mean-counts was below 1 only in the PBC group (Fig. 1c) ($p < 0.001$).

The predictive strength of the IgM plasma cells mean-count for the diagnosis of PBC was evaluated by ROC curve, showing that the best cut-off to discriminate the diagnosis of PBC was above 13 IgM plasma cells, with an area under the curve of 0.94 (95% C.I.: 0.87–0.97; $p < 0.001$) (Fig. 2). The sensitivity at this cut-off was 92.5% (95% C.I.: 79.6–98.3) and the specificity 89.3% (95% C.I.: 80.1–95.3), with positive and negative predictive values of 82.2% (95% C.I.: 67.4–91.5) and 95.7% (95% C.I.: 87.2–98.9), respectively.

The correlations between IgM and IgG plasma cells mean-counts in the portal infiltrate of the liver tissue with biochemical, serological and histological variables is shown in Table 2. By Spearman's rho coefficient of correlation, the mean count of IgM plasma cells in portal tracts was significantly correlated with female gender (rho 0.38; $p < 0.001$), serum GGT values (rho 0.35; $p = 0.001$), AP levels (rho 0.36; $p = 0.001$), presence of AMA-M2 (rho 0.66; $p < 0.001$) and serum IgM levels (rho 0.58; $p < 0.001$). Moreover the presence of IgM plasma cells in portal tracts correlated with bile duct changes, (rho 0.45; $p < 0.001$), orcein-positive granules (rho 0.51; $p < 0.001$) and granulomas (rho 0.45; $p < 0.001$) on liver biopsy. By multiple linear regression analysis the natural logarithm of IgM plasma cells mean-counts resulted independently associated only with serum GGT values ($p < 0.001$).

Conversely, IgG plasma cells mean-counts were correlated with high levels of ALT (rho 0.25; $p = 0.01$), with GGT values (rho

0.19; $p = 0.05$), presence of autoantibodies (rho 0.22; $p = 0.03$) and with the following features on liver biopsy: presence of a moderate–severe interface hepatitis, (rho 0.28; $p = 0.005$), and fibrosis (rho 0.25; $p = 0.02$), and finally with presence of orcein granules (rho 0.23; $p = 0.04$) but only with ALT levels by multivariate analysis ($p = 0.02$).

4. IgM and IgG plasma cells count in liver biopsy of patients with variant syndromes

4.1. Patients with autoimmune cholangitis

Clinical and histological features, including IgM and IgG plasma cells mean-counts of the inflammatory infiltrate in liver biopsies of 4 patients with a diagnosis of AIC are reported in Table 3. These patients had elevated values of GGT and AP, met at least 2 criteria for diagnosis of PBC, but had negative values for AMA-M2, and had a IAHG score for diagnosis of AIH < 10 . We found a count of IgM plasma cells higher than 13 in all patients (Fig. 3). It is worth noting that IgG plasma cells were prevalent in patients with a more marked cytolytic activity, though the ratio of IgG/IgM plasma cells mean-counts was below 1 for all these patients.

4.2. Patients with overlap syndrome

Clinical and histological features of the 6 patients with overlap syndrome are summarized in Table 4. These patients met at least two of the three criteria for diagnosis of PBC, and had an IAHG score of at least 10 points, compatible with a probable/definitive diagnosis of AIH. Two of them resulted negative for AMA-M2, but showed histological features suggestive of PBC, such as presence of florid bile duct lesions with duct proliferation, and a lymphoplasmacytic

Table 4
Clinical and histological features of the 6 patients with diagnosis of overlap syndrome (PBC/AIH or AIC/AIH).

Patients	Age	Gender	ALT UNL	GGT UNL	AP UNL	AMA-M2/ANA/ASMA/LKM	AMA-M2/ANA/ASMA/LKM	Typical histologic features	Serum IgM UNL	IAGH score	IgM plasma cells	IgG plasma cells	IgG/IgM
1 G-C	54	F	4.84	6.67	1.44	+/-	+/-	Duct changes, granulomas, orcein granules/A2/F1	2.33	10	15	45	3.0
2 RC-K	47	F	3.16	3.50	1.17	-/+/-	-/+/-	Duct changes, granulomas/A3/F1	0.98	15	12	32	2.67
3 V-L	36	F	13.0	1.00	1.00	+/-/-	+/-/-	Duct changes, rosette formation, eosinophils/A3/F1	0.84	11	13	38	2.92
4 R-S	63	F	4.19	9.22	1.16	+/-/+/-	+/-/+/-	Mild duct changes, eosinophils/A3/F1	1.02	12	8	20	2.44
5 V-S	51	F	10.00	3.00	1.00	-/+/-	-/+/-	Duct changes/A3/F1	1.23	11	39	107	2.74
6 R-G	23	F	4.67	4.58	1.35	+/-/-	+/-/-	A3/F2	1.70	11	1	24	24

Abbreviations: UNL, upper normal limit; ALT, alanine aminotransferase; GGT, gamma-glutamyl transferase; AP, alkaline phosphatase; AMA, anti-mitochondrial antibody; M2, E2 subunit of pyruvate dehydrogenase mitochondrial auto-antigen; ANA, antinuclear antibody; SMA, smooth muscle antibody; anti-LKM, liver kidney microsomal antibody; A (n.), activity of necro-inflammation (grade of interface hepatitis); F (n.), stage of fibrosis (according to histological grading and staging system explained in ref. [6]); IAGH, International Autoimmune Hepatitis Group.

infiltrate associated with severe peri-portal and lobular necro-inflammation. Moreover they simultaneously showed a chronic elevation of ALT and GGT, and the presence of ANA and ASMA, reaching an aggregate IAHG score compatible with a definitive diagnosis of AIH (revised IAHG score of 15) and a probable diagnosis of AIH (revised IAHG score of 11). This justified the diagnosis of OS (autoimmune cholangitis/AIH) for these two patients. The other 4 patients had a typical pattern of PBC/AIH overlap, simultaneously showing features of both diseases.

Regarding the type of plasma cells observed in the portal spaces of the liver biopsy, a ratio of IgG/IgM plasma cells mean-counts above 1 was found in all patients (Fig. 4).

5. Discussion

This study confirms the relevance of characterization of the immunoglobulin isotype of plasma cells infiltrating the liver parenchyma of patients with autoimmune liver diseases. We found, specifically in liver biopsy of patients with a definite diagnosis of PBC, a marked amount of IgM plasma cells in the portal and peri-portal tracts compared to patients with AIH and PSC such as to patients with CHC, so showed that this finding significantly correlated with every typical clinical and histological feature of PBC. Conversely, IgG plasma cells were observed mostly in cases characterized by a more pronounced inflammatory activity, for example those with a diagnosis of AIH.

These findings are in line with the evidence documented nearly 20 years ago by Milne and Horne [8], and with that of a recent study by Daniels et al. [9], which demonstrates that immunostaining for IgM and IgG plasma cells in liver biopsy can be helpful in differentiating PBC from AIH. In our study of a larger cohort of patients with autoimmune liver diseases, we aimed to explore the usefulness of this finding in the more difficult-to-define cases of VS, where a remarkable difference was found in the relative expression of IgM and IgG plasma cells (IgG/IgM ratio) between cases of AIC and those with OS of PBC/AIH.

When suspecting an autoimmune liver disease, biopsy is an important tool not only for the staging but also for differential diagnosis, particularly in cases in which clinical and laboratory features are ambiguous. However, some histological findings, such as plasma cells infiltration and piecemeal necrosis are seen in several diseases, for example in AIH and chronic hepatitis C (CHC) [14]. Similarly, lesions considered as representative of PBC, such as bile duct changes and epithelioid granulomas, have modest disease specificity. In fact, bile duct injuries are also reported in about 30% of AIH patients [18] and frequently in CHC [14], and granulomas are found in other conditions, such as sarcoidosis, drug-induced liver injury and, occasionally, in CHC [19,20]. In our study we observed ductular changes in about 92% of PBC patients, but in 50% of AIH in 69% of PSC and also in 21% of CHC patients, as well. In addition, granulomas, observed in about 57% of PBC patients, were also found in 20% of AIH patients. Orcein-positive granules were found in only 52% of PBC patients, in 23% of patients with PSC and in 15% of those with CHC.

In a small proportion of patients (5–10%) who show features of the whole suggestive of PBC, but without detectable serum AMA and/or M2 (AIC), the diagnosis can be difficult to make, particularly when the histological features are ambiguous. Moreover, in the cases characterized by mixed clinical and histological features of two diseases, such as OS, a histological misunderstanding may be generated, with relevant consequences for therapeutic decisions. Thus, an additional histological element able to discriminate among these different autoimmune liver diseases, principally in the doubtful cases, is desirable.

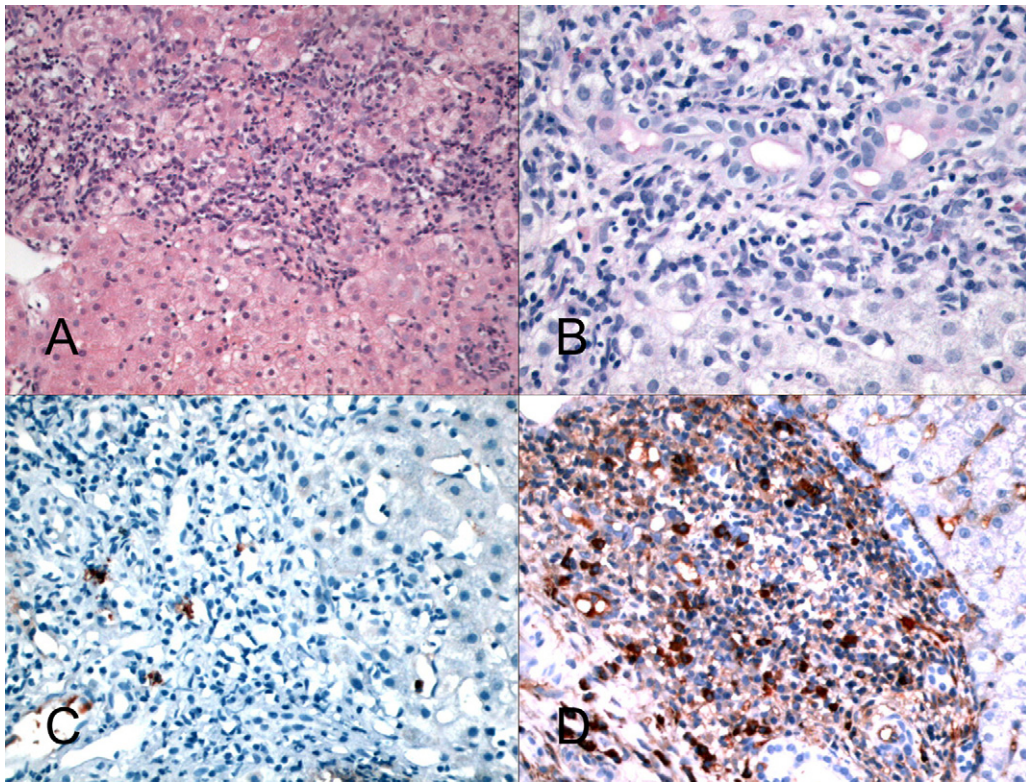


Fig. 4. Histological features and immunostaining for IgM and IgG plasma cells in liver biopsy of patients with overlap syndrome (PBC/AIH) (revised score IAHG: 11). (A) Portal tract with severe interface hepatitis; haematoxylin–eosin: 100 \times . (B) Portal tract with “chronic non-suppurative destructive cholangitis”; PAS diastase: 400 \times . (C) Portal tract with a few IgM positive staining plasma cells while in (D) IgG positive staining plasma cells are numerous; immunostaining 250 \times .

In this study a more conspicuous IgM plasma cells infiltrate was specific for PBC, and a ratio of IgG/IgM plasma cells mean-counts of less than 1 was found only in this group of patients and in the subgroup of patients with a diagnosis of AIC. This pathological entity, which the older literature considered distinct from PBC, is now firmly accepted as a variant of PBC [5]. This is in line with our observation of the analogous behaviour of plasma cells immunoglobulin expression. Therefore, independently of the presence of autoantibodies and ALT levels, we prefer to treat these patients solely with UDCA, as is done with AMA-M2 positive PBC patients.

In the subgroup of patients with a diagnosis of overlap PBC/AIH or AIC/AIH, we found a value of the IgG/IgM ratio higher than 1 in all of them because they showed a more pronounced inflammatory activity, explained by biochemical and histological features, evidently related to the predominance of IgG plasma cells on liver biopsy. For these patients we prefer a combined therapeutic approach with steroids and UDCA.

Elevated serum IgM levels have been well described as a typical clinical finding of PBC [11], and in our cohort, patients with PBC showed higher serum IgM values with respect to the others. However, this parameter in our cohort was unable to discriminate PBC from AIH and/or the different subgroups of VS.

Our study thus provides further evidence of the efficacy of this additional histological evaluation, specifically for ambiguous cases of autoimmune liver diseases, such as the variant and mixed syndromes.

Contributors

D. Cabibi designed the study and contributed to data acquisition. G. Tarantino was responsible for writing the manuscript and for statistical analyses. V. Di Marco and A. Craxì (Director of the GI & Liver Unit) were responsible for writing the manuscript, and

have seen and approved the final version. F. Barbaria and M. Campione performed laboratory procedures. All authors have seen and approved the final version of the manuscript.

Conflict of interest statement

None declared.

List of abbreviations

AIC, autoimmune cholangitis; AIH, autoimmune hepatitis; AILD, autoimmune liver disease; AMA, anti-mitochondrial antibody; ANA, antinuclear antibody; AP, alkaline phosphatase; GGT, gamma-glutamyl transferase; LKM, liver kidney microsomal type 1 antibody; M2, E2 subunit of pyruvate dehydrogenase mitochondrial auto-antigen; OS, overlap syndrome; PBC, primary biliary cirrhosis; PSC, primary sclerosing cholangitis; SMA, smooth muscle antibody; VS, variant syndrome.

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