*Hepatology Research* 2012; **42**: 428–432 doi: 10.1111/j.1872-034X.2011.00931.x

***Case Report***

# Antimitochondrial antibody -M2 positive autoimmune hepatitis during standard of care for chronic hepatitis C

Fabio Salvatore Macaluso,[1] Nicola Alessi[1] and Daniela Cabibi[2]

[1]*Biomedical Department of Internal and Specialist Medicine, Section of Gastroenterology, University of Palermo, Palermo, Italy and* [2]*Department of Human Pathology, University of Palermo, Palermo, Italy*

The current standard of care (SoC) for chronic hepatitis C, i.e. the combination of a pegylated-interferon (PEG-IFN) with ribavirin (RBV), may activate underlying autoimmune conditions. Particularly, interferon (IFN) has been known to induce or exacerbate autoimmune hepatitis (AIH) and primary biliary cirrhosis (PBC) in hepatitis C virus patients. We describe a severe, acute-onset antimitochondrial antibody (AMA)-M2 positive AIH appearing during the last weeks of SoC in a woman with chronic hepatitis C and no previous history of autoimmunity, and resolving on protracted steroids. In this context, the relevance of the characterization of the immunoglobulin isotype of portal plasma cells for a more appropriate diagnosis of autoimmune liver diseases can be emphasized.

## INTRODUCTION

THE CURRENT STANDARD of Care (SoC) for chronic hepatitis C, i.e. the combination of a pegylated-interferon (PEG-IFN) with ribavirin (RBV),[1] may activate underlying autoimmune conditions such as thyroiditis[2] or systemic lupus erythematosus.[3] Particularly, interferon (IFN) has been known to induce or exacerbate autoimmune hepatitis (AIH)[4–10] and primary biliary cirrhosis (PBC)[11,12] in hepatitis C virus (HCV) patients. We describe a severe, acute-onset antimitochondrial antibody (AMA)-M2 positive AIH appearing during the last weeks of SoC in a woman with chronic hepatitis C and no previous history of autoimmunity, and resolving on protracted steroids.

## CASE REPORT

AN ASYMPTOMATIC 20-year old Caucasian woman (height 160 cm, weight 57 kg, body mass index [BMI] 22.3 kg/m$^2$) with chronic hepatitis C (HCV genotype 1b, HCV RNA 1 170 000 IU/mL) underwent a liver biopsy in July 2005 as part of her diagnostic workup before receiving SoC. At the time of biopsy (see Table 1) alanine aminotransferase (ALT) was twice the upper limit of normal (u.l.n.) while γ-glutamiltransferase (GGT), alkalyne phosphatase (AP) and bilirubin were normal. Non-organ specific autoantibodies (NOSAs, tested by indirect immunofluorescence for antinuclear antibody (ANA), AMA, SMA and by immunoblotting for AMA type M2, liver kidney/microsome type 1 [LKM-1], LC1, soluble liver antigen/liver pancreas [SLA/LP]) were negative. At biopsy a pattern of mild chronic hepatitis (grade 2/3, stage 1/4 according to Scheuer's classification) and no sign of PBC or AIH were evidenced (Fig. 1a). Immunohistochemistry for cytokeratin 7 (CK7 antibody, clone OV-TL12/30, Dako; Dako A/S, Glostrup, Denmark) highlighted the presence of normal portal bile ducts, without reactive bile duct proliferation (Fig. 1b). The intrahepatic immunoglobulin (Ig) IgG/IgM plasma cells assessment (polyclonal anti-IgG and anti-IgM; Novocastra, Newcastle, United Kingdom) showed a low number of portal plasma cells with an IgM plasma cell number < 4/portal tract (Fig. 1c).

An intended course of 48 weeks of SoC (PEG-IFN α-2b 1.5 μg/kg per week; RBV 1000 mg/day) was started in September 2005. HCV-RNA was undetectable (<50 IU) at week 4 and week 12, and all liver tests were normal at week 12. At week 42 a slight rise of AST (2 x u.l.n.) and ALT (1.5 x u.l.n.) was noted. GGT, AP and bilirubin were normal, and HCV-RNA still negative.

*Correspondence: Dr Fabio Salvatore Macaluso, Dipartimento Biomedico di Medicina Interna e Specialistica, Sezione di Gastroenterologia, University of Palermo, Palermo, Italy. Email: fsmacaluso@gmail.com*
*Received 11 June 2011; revision 11 October 2011; accepted 13 October 2011.*

**Table 1** Laboratory, virological and histological features before receiving standard of care (SoC) (July 2005)

| | |
|---|---|
| Aspartate aminotransferase (IU/L) | 48 (n.v. < 31) |
| Alanine aminotransferase (IU/L) | 73 (n.v. < 31) |
| Alkaline phosphatase (IU/L) | 54 (n.v. < 104) |
| γ-glutamil-transferase (IU/L) | 14 (n.v. < 36) |
| Total bilirubin (mg/dL) | 0.45 |
| Total immunoglobulins (g/dL) | 0.86 (n.v. < 1.7) |
| Anti-HCV (EIA) | Positive |
| Quantitative HCV-RNA (IU/mL) | 1 170 000 |
| ANA (IFI) | Negative |
| AMA (IFI) | Negative |
| SMA (IFI) | Negative |
| AMA type M2 (IB) | Negative |
| LKM-1 (IB) | Negative |
| LC-1 (IB) | Negative |
| SLA/LP (IB) | Negative |
| Histology | Mild chronic HCV-related hepatitis |
| Immunohistochemistry for CK7 | Normal portal bile ducts |
| Intrahepatic IgG/IgM plasma cells | Low number of portal plasma cells with an IgM plasma cells number <4/portal tract |

AMA, antimitochondrial antibody; ANA, antinuclear antibody; CK7, Cytokeratin 7; EIA, enzyme immunoassay; IB, immunoblotting; IFI, indirect immunofluorescence; IgG, immunoglobulins G; IgM, immunoglobulins M; IU, international units; LC-1, liver cytosol type 1; LKM-1, liver kidney/microsome type 1; n.v., normal values; SLA/LP, soluble liver antigen/liver pancreas; SMA, smooth muscle antibody.

Therapy was stopped at week 48 in August 2006. At the time of stopping, AST and ALT were nine and 11 times the u.l.n., GGT seven times the u.l.n., AP 2.5 times the u.l.n., total bilirubin 6.1 mg/dL and HCV RNA negative. The patient had no itching or any physical complaints. A detailed medical history ruled out a potential involvement of hepatotoxic drugs, herbal remedies, dietary supplements or alcohol consumption. Abdominal US showed normal gallbladder and bile ducts. Ten days after discontinuing SoC (see Table 2), AST and ALT were 14 and 13 times the u.l.n., GGT seven times the u.l.n., AP three times the u.l.n., and total bilirubin 6.5 mg/dL, with no reduction of serum albumin or INR. Hepatitis B surface antigen (HBsAg), IgM Anti-hepatitis A virus (HAV), IgM anti-viral capsid antigen (VCA), IgM anti-cytomegalovirus (CMV) were all negative, and HCV RNA once more undetectable. AMA type M2 had become positive (+++ by immunoblotting), while all other NOSAs were negative. IgM and IgG serum levels were 170 and 2150. The human leukocyte antigen (HLA) profile was: DRB1-11, DRB3, DQB1-03.[7]

A liver biopsy (August 2006) showed severe periportal lymphocytic piecemeal necrosis with abundant plasma cells in the portal and periportal infiltrate and pseudo-rosettes formed by hepatocytes, suggesting AIH. Severe hepatocellular damage with ballooning degeneration, apoptotic bodies, centro-zonal necrosis and small bile duct proliferation, as highlighted by CK7 antibody, were present, all of them characteristic of an acute – onset (Fig. 2a–d). On the other hand, despite the AMA-M2 positivity, no features of PBC such as small bile duct damage, portal peri-ductal granulomas and peri-portal orcein-positive granules, or markers of chronic chola-

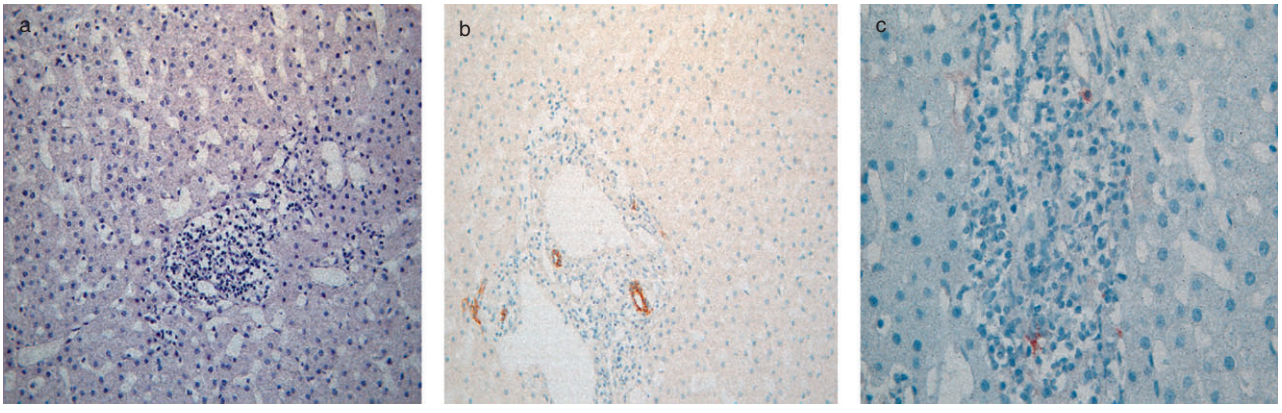


**Figure 1** Hepatitis C virus (HCV). (a) Portal lymphocytic infiltrate with focal piecemal necrosis (Hematoxylin–Eosin staining; 200×). (b) CK7 immunostaining evidences the presence of normal portal bile ducts (CK7 immunostaining, 200×). (c) Immunoglobulin M (IgM) immunostaining highlights very few IgM plasma cells (IgM immunostaining 400×).

**Table 2** Laboratory, virological and histological features 10 days after discontinuing standard of care (SoC) (August 2006)

| | |
|---|---|
| Aspartate aminotransferase (IU/L) | 441 (n.v. < 31) |
| Alanine aminotansferase (IU/L) | 408 (n.v. < 31) |
| Alkaline phosphatase (IU/L) | 333 (n.v. < 104) |
| γ-glutamil-transferase (IU/L) | 248 (n.v. < 36) |
| Total bilirubin (mg/dL) | 6,5 |
| IgM (mg/dL) | 170 (n.v. < 230) |
| IgG (mg/dL) | 2150 (n.v. < 1600) |
| Qualitative HCV-RNA | Negative |
| HBsAg | Negative |
| IgM anti-HAV | Negative |
| IgM anti-VCA | Negative |
| IgM anti-CMV | Negative |
| ANA (IFI) | Negative |
| AMA (IFI) | Negative |
| SMA (IFI) | Negative |
| AMA type M2 (IB) | Positive (+++) |
| LKM-1 (IB) | Negative |
| LC-1 (IB) | Negative |
| SLA/LP (IB) | Negative |
| Histology | Features of an acute-onset AIH without signs of CNSDC or granulomas |
| Immunohistochemistry for CK7 | Reactive bile duct proliferation |
| Intrahepatic IgG/IgM plasma cells | More than 25 IgG plasma cells/portal tract and an less than 4 IgM plasma cells/portal tract with an IgG/IgM ratio >1 |

AMA, antimitochondrial antibody; ANA, antinuclear antibody; CK7, Cytokeratin 7; CNSDC, chronic non-suppurative destructive cholangitis; IB, immunoblotting; IFI, indirect immunofluorescence; IgG, immunoglobulins G; IgM, immunoglobulins M; IU, international units; LC-1, liver cytosol type 1; LKM-1, liver kidney/microsome type 1; n.v., normal values; SLA/LP, soluble liver antigen/liver pancreas; SMA, smooth muscle antibody.

tostasis were evident. Immunohistochemistry for intrahepatic IgG/IgM plasma cells showed a large number of IgG plasma cells (more than 25 cells/portal tract) and an inconspicuous presence of IgM plasma cells (less than four cells/portal tract) in the portal tracts with an IgG/IgM ratio >1 (Fig. 2e,f).

Assuming a diagnosis of AMA-M2 positive AIH, treatment with prednisone (1 mg/kg per day) was started on 19 August 2006. Bilirubin initially peaked at 18 mg/dL at 1 week of steroid and then decreased. Upon discharge, after 2 weeks of steroid, bilirubin was 10.2 mg/dL, AST and ALT 3.5 and two times the u.l.n., GGT 2.5 times the u.l.n. and AP 1.5 times the u.l.n. Steroid dosage was tapered until discontinuation in March 2007. Meanwhile, AST/ALT and markers of cholestasis had become normal by November 2006 and has stayed normal since then. At last follow up visit (May 2008) AMA type M2 was still positive (++ by immunoblotting) and HCV RNA undetectable. All liver function tests were normal.

## DISCUSSION

INDUCTION OR EXACERBATION of AIH and PBC is a known complication of SoC for chronic hepatitis C. In our case, a definite diagnosis was not initially easy to reach. The scoring system formulated in 1999 by the International Autoimmune Hepatitis Group (IAIHG) is extremely useful for the diagnosis of AIH.[13] Regarding hepatitis viral markers as negative (and this was probably correct, since HCV RNA was persistently undetectable), the score defined the autoimmune disease in our patient as "probable" AIH. Nevertheless, its applicability remains unclear in a PBC/AIH Overlap Syndrome context, since a score of "definite" AIH can be very rarely observed in patients with characteristic overlap syndrome, whereas nearly 20% of PBC subjects could be classified with "probable" AIH overlap.[14–16] Moreover, the simplified diagnostic score recently proposed by the IAIHG has not been validated in patients with suspected PBC–AIH Overlap Syndrome.[17]

Using the simple diagnostic criteria proposed by Chazouilleres *et al.*[18] and recently highlighted by the European Association for the Study of the Liver (EASL),[19] our patient could also be classified in the context of a PBC/AIH Overlap Syndrome, as we suspected before the liver biopsy just regarding the laboratory findings and the autoantibodies profile. Nevertheless, liver biopsy showed only the histological features of an acute-onset AIH (severe periportal lymphocytic piecemeal necrosis with many plasma cells in the portal and periportal infiltrate, presence of hepatocytic rosettes, severe hepatocellular damage with ballooning degeneration, apoptotic bodies and centrozonal necrosis) without alterations typical of PBC (bile duct damage, portal peri-ductal granulomas and chronic cholatostasis, as evidenced by peri-portal orcein-positive granules).

Furthermore, the presence of numerous IgM plasma cells in the portal tracts has been reported in PBC.[20] We

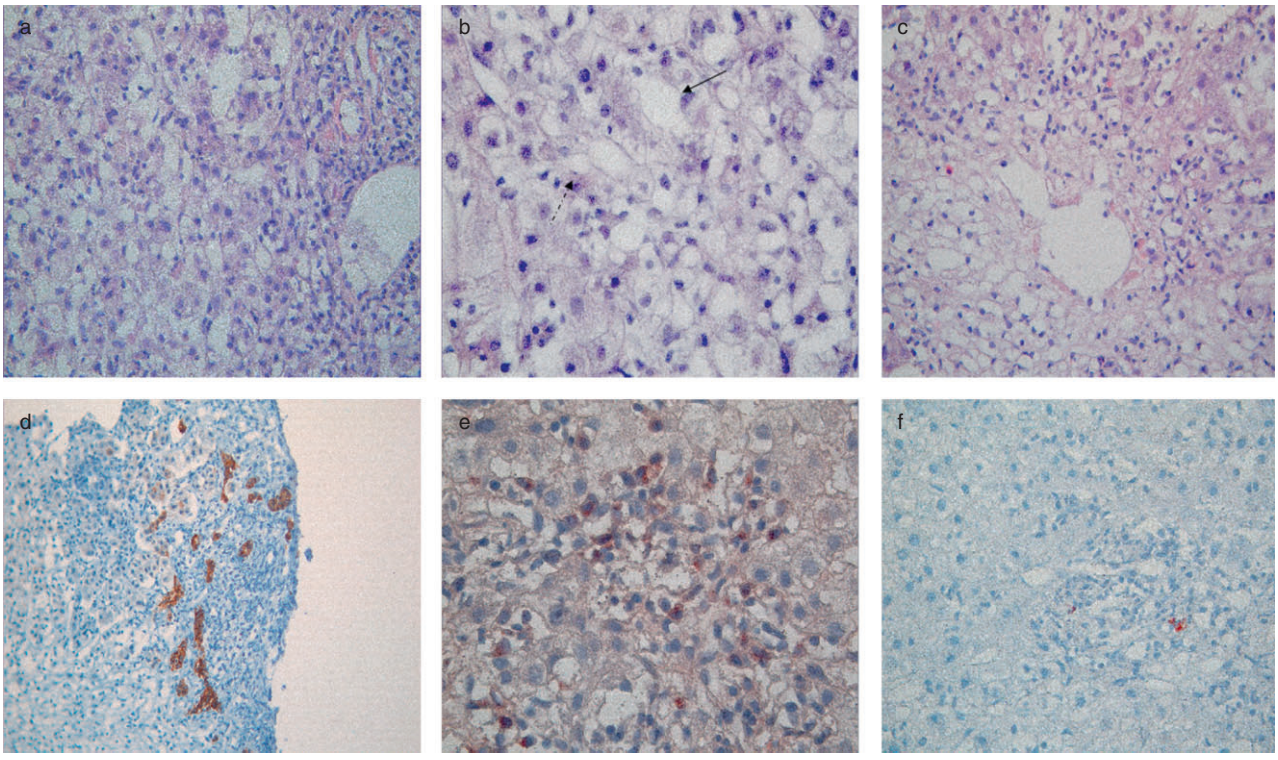


**Figure 2** Acute-onset autoimmune hepatitis. (a) Severe periportal lymphocytic piecemeal necrosis (Hematoxylin–Eosin staining; 400×). (b) Hepatocellular damage and ballooning degeneration of liver cells. The arrows show rosettes of liver cells (Hematoxylin–Eosin staining; 630×). (c) Centro-zonal necrosis, characteristic of an acute-onset (Hematoxylin–Eosin staining; 400×). (d) CK7 highlights small bile duct proliferation in the portal-periportal areas (CK7 immunostaining, 200×). (e,f) IgG plasma cells (e) are much more numerous than IgM plasma cells (f) in the portal tracts (E: IgG immunostaining 630×; F: IgM immunostaining 400×).

recently reported the increase of portal IgM plasma cells (13 IgM plasma cells/portal tract was the cut-off value) with an IgG/IgM plasma cells ratio <1 as specific of both PBC and "autoimmune cholangitis" patients (AIC),[21] a firmly accepted variant of PBC (AMA-negative PBC).[22] Ursodeoxycholic acid (UDCA) treatment should be preferred for these patients, independently of the presence or absence of AMA. On the other hand, all patients with AIH, PBC/AIH or AIC/AIH overlap syndromes showed an IgG/IgM ratio >1 and less than 13 IgM plasma cells/portal tract, so a therapeutic approach with steroids or steroids and UDCA should be preferred for these cases.[21]

In our patient, even if suggested by the AMA type M2 positivity and by the elevation of cholestatic liver enzymes, a diagnosis of PBC was unlikely for two reasons. First, AMA negativity by conventional indirect immunofluorescence at the initial diagnosis and, second, the absence of chronic non-suppurative destructive cholangitis (CNSDC) or granulomas by histopathological examinations. Moreover, the very low IgM plasma cells number (less than four cells/portal tract) with an IgG/IgM ratio >1 made a PBC or AIC diagnosis increasingly unlikely. Thus, our case is devoid of any histological and immunohistochemical features of PBC and the rapid elevation of cholestasis markers could be more easily explained by considering the acute setting of the disease.

On the other hand, AMA type M2 positivity is not an exclusion criterion for "simple" AIH: a recent study[23] has reported some cases of AMA positive AIH with no clinical or histologic evidence of PBC despite the continued detection of AMAs over a follow-up of up to 27 years. Other authors observed that clinical and histological features of AIH were not influenced by the AMA status.[24]

AMA-M2 positive AIH has been reported in the literature and, to our knowledge, this is the first case in which an acute cholestatic onset and the role of IFN as trigger has been shown. In this setting, the relevance of the characterization of the immunoglobulin isotype of portal plasma cells for a more appropriate diagnosis of autoimmune liver diseases can be emphasized.

## REFERENCES


1 Ghany MG, Strader DB, Thomas DL, Seeff LB, American Association for the Study of Liver Diseases. Diagnosis, management, and treatment of hepatitis C: an update. *Hepatology* 2009; **49:** 1335–74.
2 Mandac JC, Chaudhry S, Sherman KE, Tomer Y. The clinical and physiological spectrum of interferon-alpha induced thyroiditis: toward a new classification. *Hepatology* 2006; **43:** 661–72.
3 Wilson LE, Widman D, Dikman SH, Gorevic PD. Autoimmune disease complicating antiviral therapy for hepatitis C virus infection. *Semin Arthritis Rheum* 2002; **32:** 163–73.
4 Shindo M, Di Bisceglie AM, Hoofnagle JH. Acute exacerbation of liver disease during interferon alfa therapy for chronic hepatitis C. *Gastroenterology* 1992; **102:** 1406–8.
5 Papo T, Marcellin P, Bernuau J, Durand F, Poynard T, Benhamou JP. Autoimmune chronic hepatitis exacerbated by alpha-interferon. *Ann Intern Med* 1992; **116:** 51–3.
6 Garcia-Buey L, Garcia-Monzon C, Rodriguez S *et al*. Latent autoimmune hepatitis triggered during interferon therapy in patients with chronic hepatitis C. *Gastroenterology* 1995; **108:** 1770–7.
7 Vispo E, Maida I, Moreno A, Barreiro P, Soriano V. Autoimmune hepatitis induced by pegylated interferon in an HIV-infected patient with chronic hepatitis C. *J Antimicrob Chemother* 2008; **62:** 1470–2.
8 Coriat R, Podevin P. Fulminant autoimmune hepatitis after successful interferon treatment in an HIV-HCV co-infected patient. *Int J STD AIDS* 2008; **19:** 208–10.
9 Kogure T, Ueno Y, Fukushima K *et al*. Fulminant hepatic failure in a case of autoimmune hepatitis in hepatitis C during peg-interferon-alpha 2b plus ribavirin treatment. *World J Gastroenterol* 2007; **13:** 4394–7.
10 Sezaki H, Arase Y, Tsubota A *et al*. Type C-chronic hepatitis patients who had autoimmune phenomenon and developed jaundice during interferon therapy. *J Gastroenterol* 2003; **38:** 493–500.
11 D'Amico E, Paroli M, Fratelli V *et al*. Primary biliary cirrhosis induced by interferon-alpha therapy for hepatitis C virus infection. *Dig Dis Sci* 1995; **40:** 2113–16.
12 Maeda T, Onishi S, Miura T *et al*. Exacerbation of primary biliary cirrhosis during interferon-alpha 2b therapy for chronic active hepatitis C. *Dig Dis Sci* 1995; **40:** 1226–30.
13 Alvarez F, Berg PA, Bianchi FB *et al*. International Autoimmune Hepatitis Group Report: review of criteria for diagnosis of autoimmune hepatitis. *J Hepatol* 1999; **31:** 929–38.
14 Heathcote EJ. Overlap of autoimmune hepatitis and primary biliary cirrhosis: an evaluation of a modified scoring system. *Am J Gastroenterol* 2002; **97:** 1090–2.
15 Talwalkar JA, Keach JC, Angulo P, Lindor KD. Overlap of autoimmune hepatitis and primary biliary cirrhosis: an evaluation of a modified scoring system. *Am J Gastroenterol* 2002; **97:** 1191–7.
16 Chazouilleres O, Wendum D, Serfaty L, Rosmorduc O, Poupon R. Long term outcome and response to therapy of primary biliary cirrhosis–autoimmune hepatitis overlap syndrome. *J Hepatol* 2006; **44:** 400–6.
17 Hennes EM, Zeniya M, Czaja AJ *et al*. Simplified criteria for the diagnosis of autoimmune hepatitis. *Hepatology* 2008; **48:** 169–76.
18 Chazouilleres O, Wendum D, Serfaty L, Montembault S, Rosmorduc O, Poupon R. Primary biliary cirrhosis–autoimmune hepatitis overlap syndrome: clinical features and response to therapy. *Hepatology* 1998; **28:** 296–301.
19 European Association for the Study of the Liver. EASL Clinical Practice Guidelines: management of cholestatic liver diseases. *J Hepatol* 2009; **51:** 237–67.
20 Daniels JA, Torbenson M, Anders RA *et al*. Immunostaining of plasma cells in primary biliary cirrhosis. *Am J Clin Pathol* 2009; **131:** 243–9.
21 Cabibi D, Tarantino G, Barbaria F, Campione M, Craxì A, Di Marco V. Intrahepatic IgG/IgM plasma cells ratio helps in classifying autoimmune liver diseases. *Dig Liver Dis* 2010; **42:** 585–92.
22 Hirshfield GM, Heathcote EJ. Antimitochondrial antibody-negative primary biliary cirrhosis. *Clin Liver Dis* 2008; **12:** 323–31.
23 O'Brien C, Joshi S, Feld JJ, Guindi M, Dienes HP, Heathcote EJ. Long-term follow-up of antimitochondrial antibody-positive autoimmune hepatitis. *Hepatology* 2008; **48:** 550–6.
24 Mishima S, Omagari K, Ohba K *et al*. Clinical implications of antimitochondrial antibodies in type 1 autoimmune hepatitis: a longitudinal study. *Hepatogastroenterology* 2008; **55:** 221–7.