

## Autoimmune hepatitis in patients with chronic HBV and HCV infections: patterns of clinical characteristics, disease progression and outcome

Eirini I. Rigopoulou,\* Kalliopi Zachou,\* Nikolaos Gatselis,\* George K. Koukoulis,\*\* George N. Dalekos\*

\* Department of Medicine and Research Laboratory of Internal Medicine, Medical School, University of Thessaly, Larissa, Thessaly, Greece.

\*\* Department of Pathology, Medical School, University of Thessaly, Larissa, Thessaly, Greece.

### ABSTRACT

We retrospectively investigated the characteristics, patterns of disease progression, outcome and difficulties in the management in 11 patients with concurrent autoimmune hepatitis (AIH) and HBV or HCV infections (5 HCV and 6 HBV including 2 with HDV co-infection) since there are scarce data on this issue. HCV or HBV diagnosis preceded that of AIH in all patients by many years. At initial clinical and histological assessment almost half of patients had cirrhosis (45.5%) with the group of AIH and HCV carrying the highest frequency (4/5; 80%). In two thirds of patients, mostly with HCV and HBV/HDV, AIH was assumed to be IFN-alpha-induced and experienced difficulties in achieving sustained virological response. On the contrary, the outcome of patients with HBV and AIH was better compared to those with AIH and HCV or HDV. In conclusion, chronic viral hepatitis infections concomitant with AIH are often very difficult to recognize and therefore, a significant delay in AIH diagnosis in this specific group of patients is usual. HBV patients with concomitant AIH seem to carry the most favorable outcome compared to those with HCV probably because of the use of nucleos(t)ide analogues which contrary to IFN-alpha can control HBV replication with no adjacent effect, related to exacerbation of autoimmune phenomena.

**Key words.** Autoimmune hepatitis. Hepatitis B. Hepatitis C. Hepatitis D.

### INTRODUCTION

Infections with hepatitis B (HBV) or hepatitis C (HCV) viruses are major public health problems worldwide.<sup>1,2</sup> In Thessaly region of central Greece, HBV prevalence in the general population is estimated at 4.26%, although clusters of higher HBV prevalence have also been reported in the same area.<sup>3</sup> In contrast, HCV prevalence is rather low in our region (0.34%) but similarly to HBV, HCV clusters of higher prevalence have been identified, as well.<sup>4</sup>

Clinical and laboratory features of HBV and HCV can sometimes be mistaken with those of autoimmune

hepatitis (AIH), a disease characterized by increased immunoglobulins, circulating autoantibodies and a favourable response to immunosuppression.<sup>5-7</sup> Indeed, coexistence of AIH with HCV or HBV should be considered especially in areas endemic for viral hepatitis, since viruses have long been associated with either the induction of autoimmune phenomena or the development of overt autoimmune diseases.<sup>5,7</sup>

Non-organ specific autoantibodies (NOSA) particularly antinuclear antibodies (ANA) and anti-smooth muscle antibodies (SMA) have been reported frequently in HBV- and HCV-infected patients.<sup>7-9</sup> In most of these cases NOSA are detected in lower titers compared to those found in AIH patients, usually lack F-actin specificity of SMA and do not affect the treatment outcome, disease severity or progression of chronic viral liver diseases.<sup>7-11</sup> In addition, antibodies against liver-kidney microsomes type-1 (anti-LKM-1), which are detected in AIH type-2 (AIH-2), are reported in 3-10% of HCV cases.<sup>5,7,8,10</sup> Although the presence of autoantibodies does not signify the existence of autoimmunity, the discrimination between AIH and viral liver disease

Correspondence and reprint requests: George N. Dalekos, M.D., Ph.D.

Professor of Medicine

Head, Department of Medicine and Research Laboratory of Internal Medicine  
University of Thessaly, School of Medicine Biopolis, Larissa, 41110 Larissa,  
Greece.

Tel.: ++30-2410-682285. Fax: ++30-2410-671863

E-mail: dalekos@med.uth.gr

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with autoimmune features is mandatory, since these two conditions involve different disease strategies; viral hepatitis is often treated with interferon-alpha, which may unmask or provoke autoimmune hepatic reactions and even "true" AIH, whereas AIH requires immunosuppression, which could enhance viral replication in cases of viral hepatitis.<sup>5,7,12,13</sup>

Currently, there are scarce data to demonstrate the interaction between AIH and viral hepatitis. Therefore, in this study we present our experience by reporting the patients' characteristics, patterns of disease progression and outcome and also the difficulties in diagnosis and management of 11 patients with HBV or HCV infections and concurrent AIH.

## MATERIALS AND METHODS

During 1/2001-9/2009, we indentified 11 patients with concurrent viral hepatitis and AIH. The characteristics of the patients along with those of 98, 303, and 59 randomly selected patients from our records with HCV, HBV and AIH, respectively are shown only in a descriptive way in table 1 since these groups are not appropriate for statistical comparisons. The diagnosis of HCV or HBV was based on the EASL statements.<sup>1,2</sup> AIH diagnosis was based on the criteria of the International Autoimmune Hepatitis Group (IAIHG) which were modified in 2008.<sup>13,14</sup>

ANA, SMA, anti-LKM and liver cytosol type-1 (anti-LC1) antibodies were tested by indirect immunofluorescence (IIF) according to previous reports;<sup>7,8,12-14</sup> anti-LKM, anti-LC1 and antibodies against soluble liver antigen/liver pancreas (anti-SLA/LP) were also tested by ELISA. Western blot analysis was also used for the detection of anti-LKM-1, anti-LKM-type 3 (anti-LKM-3), anti-SLA/LP and anti-LC1.<sup>5,7,10,12,13</sup>

Treatment response in HBV and HCV was assessed according to internationally accepted guidelines,<sup>1,2</sup> while in AIH according to the IAIHG<sup>14,15</sup> and our recent report where mycophenolate mofetil (MMF) was used as first-line therapy.<sup>6</sup> All subjects consented to participate in the study at the time of the interview. Data are presented as mean  $\pm$  standard deviation (SD) or median (range) as appropriate.

## RESULTS

### Patients with AIH and HBV infection (n = 6, table 1)

All HBV patients were anti-HBe-positive/HBeAg-negative. HBV infection was active in 3 patients at

diagnosis of viral liver disease including 2 with low viral replication (2.269 and 3.481 IU/mL), and inactive in 3 patients, amongst whom 2 had also HDV co-infection as attested by the detection of HDV-RNA by a quantitative PCR.<sup>16</sup> HBV was diagnosed in all patients before their first visit (median: 35 months); HBV( $\pm$ HDV) diagnosis preceded that of AIH in all patients.

At first assessment, 1 patient had clinically evident cirrhosis with liver biopsy performed in all and in closest proximity to AIH diagnosis. At initial liver biopsy 1 patient had cirrhosis, 2 severe fibrosis, 2 moderate and 1 mild fibrosis. All but one had moderate portal inflammation, while 3 had moderate/severe interface hepatitis and 2 mild interface hepatitis. Of note, plasmacytosis, rosetting formation and emperipolysis was evident in all liver biopsies.

All patients received antiviral treatment during follow-up. In detail, one patient received combination treatment (Peg-IFN-alpha and lamivudine) for 19 months and after virological response remained on lamivudine monotherapy for 7 months. Due to virological breakthrough adefovir was added, which was later on switched to entecavir because of partial virological response. Both patients with HBV/HDV co-infection received IFN-alpha based regimens. Actually, the female patient received high dose of IFN-alpha (9MU three times/week) for 2 years with no virological response, followed by 48 weeks of adefovir (as part of a randomized trial)<sup>16</sup> with no virological response. Therefore, she was switched to combination of Peg-IFN-alpha for at least 36 months, receiving in parallel immunosuppression. Combination treatment with Peg-IFN-alpha and adefovir was also initiated in the second patient, a male with HBV/HDV co-infection,<sup>16</sup> though IFN-alpha had to be ceased one month later due to severe exacerbation of liver disease with sharp increase of transaminases,  $\gamma$ -globulins and anti-LKM-3 detection. In these 3 patients, the diagnosis of AIH was established because of worsening of transaminases and significant increase of IgG levels during IFN-alpha-based treatment, compatible features of AIH on liver biopsy and detectable ANA and/or SMA and anti-LKM-3 in one patient. At the time of AIH diagnosis, HBV-DNA was undetectable. In the remaining 3 treatment-naïve HBV patients with either no or low viral replication, AIH diagnosis was based on the presence of increased transaminases and IgG levels, compatible liver histology and detectable ANA and/or SMA and anti-LKM-1 in one patient. The latter 3 patients received lamiduvine preemptively due to initiation of immunosuppression for AIH.

**Table 1.** Demographic, clinical, laboratory and histological characteristics of HCV or HBV patients with concurrent AIH and HCV, HBV and AIH patients.

	AIH+HCV (n = 5)	AIH+HBV (n = 6; 2/6 HBV+HDV)	HCV (n = 98)	HBV (n = 303)	AIH (n = 59)
Sex (male/female)	1/4	2/4	62/36	183/120	41/18
Age at diagnosis of viral infection (years)	45.4 ± 16	35 ± 14	44 ± 17	44.4 ± 15.6	NA
Age at AIH diagnosis (years)	52.8 ± 17	39.8 ± 12.5	NA	NA	50 ± 16
Age at last follow up (years)	53 ± 17	44 ± 12	50 ± 12	53 ± 18.3	58 ± 13.2
Diagnosis of viral hepatitis before AIH (no/yes)	0/5	0/6	NA	NA	NA
Interval between diagnosis of viral and AIH (months)	89 ± 80	58 ± 51	NA	NA	NA
Source of viral infection	3 unknown, 2 transfusion	2 unknown, 1 transfusion, 3 from country with high prevalence (2 from Albania and 1 from Romania)	33 unknown, 22 drug abuse, 32 transfusion, 4 multiple hospitalizations, 3 multiple sexual partners, 1 occupational exposure	9 unknown, 46 sexual, 9 transfusion, 103 vertical, 98 intrafamilial, 38 folk remedies	NA
Active viral infection at diagnosis (no/yes)	0/5	3/3 <sup>s</sup>	0/98	187/116	NA
HCV genotype	1b in all	NA	1a/1b in 50, 2a/c in 6, 3a in 27, 4 in 9, undefined in 6	NA	NA
Histology at initial diagnosis (no/yes)	0/5 (4 cirrhosis, 1 moderate fibrosis)	0/6 (1 cirrhosis, 2 pre-cirrhotic, 2 moderate fibrosis, 1 mild fibrosis)	25/73 (18 cirrhosis, 53 mild/ moderate fibrosis, 2 severe fibrosis)	202/101 (23 cirrhosis, 78 mild or moderate fibrosis)	5/54 (16 severe fibrosis or cirrhosis, 38 mild/ moderate fibrosis)
Clinically and/or histologically established cirrhosis at initial diagnosis (no /yes)	1/4	5/1	80/18	265/38	45/14

Development of cirrhosis during follow-up in non-cirrhotic patients (no/yes)	1/0	3/2	78/2	260/5	45/0
Antiviral treatment (no/yes)	0/5	0/6 (3 preemptive due to immunosuppression for AIH)	0/98	187/116	NA
Type of antiviral treatment	Peg-IFNa plus ribavirin	3 Nucleos(t)ide and 3 Peg-IFN-a ± nucleos(t)ide analogues	67 IFNa or Peg-IFNa plus ribavirin, 31 IFNa or Peg-IFNa monotherapy	50 IFNa or Peg-IFNa, 56 Nucleos(t)ide analogues	NA
Response to antiviral treatment (no/yes/NA)	3/1/1¶	2/4/0	39/59/0	45/71/0	NA
Treatment of AIH (no/yes)	0/5	0/6	NA	NA	0/59
Duration of autoimmune therapy (months)	28 ± 19.5	44 ± 21	NA	NA	26 (3-92)
Type of autoimmune therapy	Predni + MMF	Predni + MMF	NA	NA	Predni + MMF
Response to autoimmune therapy at last follow up (no/yes/NA)	(0/2/3*)	(2/4/0)	NA	NA	3/56/0
Total follow-up (months)	38 ± 33	77 ± 36	51 ± 28	75 ± 23	42 (3-117)
Liver related death (no/yes)	5/0	6/0	98/0	279/24	57/2

Abbreviations are same as in text. Results are expressed as mean ± SD, median and IQR in parenthesis, unless otherwise stated. NA: not applicable. Ys: years. Predni: prednisolone. MMF: mycophenolate mofetil. ¶ 1 patient has not completed antiviral treatment at last follow up visit, though had early virological response (HCV-RNA negative at 12 weeks of antiviral treatment). \* Two patients had discontinued treatment mainly because of side effects; in 1 patient duration of immunosuppressive treatment was short to assess response to regimen. § Two out of 3 inactive HBV carriers had active hepatitis D infection.

Even though viral replication was low, in one of them lamivudine had to be switched to adefovir due to the emergence of viral mutants with virological response later on (mean time of antiviral treatment:  $63 \pm 12$  months).

In terms of autoantibodies, all patients had at least two NOSA before the initiation of antiviral treatment, including SMA in all (median titer: 1/80; range: 1/80-1/320) and ANA in 5 patients (median titer: 1/80; range: 1/80-1/320) with fine-speckled pattern in all. At initial evaluation and before the initiation of antiviral treatment one patient with HBV and one with HBV/HDV co-infection had detectable anti-LKM antibodies.

At AIH diagnosis, all patients had at least 2 NOSA, including ANA with fine speckled pattern in 5 and homogeneous in 1 patient, and SMA in all (median titer: 1/80; range: 1/80-1/320 for both antibodies). LKM titers remained unchanged during follow-up. At AIH diagnosis the simplified score was 4 in 2 patients and 5 in 4. In 3 patients, who received IFN-alpha-based treatment (1 with HBV and 2 with HBV/HDV co-infection) AIH had been postulated to be IFN-alpha-induced. After AIH diagnosis, combination treatment with prednisolone and MMF was initiated.<sup>6</sup>

At last follow-up, 1 was on remission and off immunosuppression for 17 months, having received in total 39 months of prednisolone and MMF. Four patients were still under immunosuppression, including 1 on low dose combination of prednisolone (5 mg/day) and MMF (1 g/day), 2 under prednisolone only (10 mg/day) and 1 under MMF only (1 g/day). Three of these four patients were on remission, while the female patient with HBV/HDV co-infection had incomplete response. The remaining male patient with HBV/HDV co-infection died of non-liver related causes (traffic accident).

Overall, among 3 non-cirrhotics at initial evaluation, two with HBV/HDV co-infection developed cirrhosis during the follow-up period (Table 1). However, cirrhosis in these two patients was already present at the time of AIH diagnosis (after IFN-alpha-based treatment for viral liver disease and before the initiation of immunosuppression).

#### **Patients with AIH and HCV infection (n = 5, table 1)**

HCV infection (genotype 1b) was active in all at diagnosis (HCV-RNA range: 159.615 IU/mL to more than 700.000 IU/ml). HCV was diagnosed in all before the first visit (median: 77 months) and preceded that of AIH also in all ( $89 \pm 80$  months).

At first assessment 4/5 patients had clinically evident cirrhosis which was confirmed by liver biopsy in all in closest proximity to AIH diagnosis. All patients had features of moderate/severe interface hepatitis and other features of AIH, including plasmacytosis, rosetting formation and emperipolesis. Due to exacerbation of liver biochemistry together with increase of IgG levels during Peg-IFN-alpha treatment a repeat liver biopsy was performed in two (6 and 43 months succeeding the initial biopsy), which demonstrated similar features with worsening of interface hepatitis, plasmacytosis and emperipolesis.

All received combination antiviral treatment (Peg-IFN-alpha plus ribavirin) before AIH diagnosis (mean duration of treatment before AIH diagnosis:  $7.5 \pm 3.9$  months). In detail, one demonstrated sustained virological response to 48-weeks combination treatment, after having failed a first course of treatment with conventional IFN-alpha and ribavirin. Three patients were non-responders including one who had achieved early virological response and relapsed after having stopped treatment due to severe side effects and had never received treatment again especially due to the possibility of exacerbation of liver disease by IFN-alpha in the context of AIH. The remaining one patient was under treatment at the time of data collection with early virological response.

In summary, 1 patient achieved sustained response, 3 were non-responders and 1 had early virological response at the time of this writing. In terms of autoantibodies, all patients had at least two NOSA before the initiation of antiviral treatment, including ANA in all (median: 1/80; range: 1/80-1/640 with fine speckled pattern in 2, homogeneous in 2 and nucleolar in 1 patient), SMA in 4/5 (median: 1/120; range: 1/80-1/160) and anti-LC1 in one.

At AIH diagnosis all patients had at least two NOSA, including ANA (median: 1/80; range: 1/80-640) with fine speckled pattern in 3 and homogeneous in 2 patients, SMA in all (median: 1/160; range: 1/80-1/320) and anti-LC1 in one. In another patient, AIH diagnosis was based on the presence of at least 2 NOSA, in addition to anti-SLA/LP identified after IFN-alpha treatment. Retrospective evaluation of sera before IFN-alpha administration did not reveal anti-SLA/LP in this patient. At AIH diagnosis the simplified score was 4 in 1 patient, 5 in 2 and 6 in 2. AIH had been postulated IFN-alpha-induced in all patients. After AIH diagnosis, combination treatment with prednisolone and MMF was initiated in all as already described.<sup>6</sup>



At last follow-up, two patients have stopped treatment (one due to possible development of hepatocellular carcinoma during regular screening and was lost to follow-up thereafter and one due to side effects), two were on remission, including one receiving low dose of prednisolone and MMF and one on MMF maintenance monotherapy. The remaining one patient had recently started combination of prednisolone and MMF with no sufficient time to access response to treatment. At the time of this writing all patients are alive. The only one non-cirrhotic patient did not develop cirrhosis during follow-up.

## DISCUSSION

We describe the characteristics of 11 patients with concurrent viral hepatitis and AIH alongside with clues to diagnosis, patterns of disease progression and outcome as well as difficulties in decision management. In all patients the diagnosis of viral hepatitis preceded that of AIH by many years. This could be elucidated by various scenarios. It is reported that AIH can remain silent for variable period of time before developing symptoms.<sup>5,6,13,14</sup> Of relevance, viral infections can operate as triggers for the development of autoimmunity. HCV infection in particular has been implicated in the activation of latent AIH either by the virus itself or through IFN- $\alpha$ .<sup>5,7,12,13,17</sup> However, in real life it is often difficult to distinguish between these scenarios (Table 2).

All our HBV and HCV patients with concurrent AIH were characterised by the presence of at least two NOSA at first assessment. In terms of autoantibody specificity, the vast majority of patients had SMA with F-actin specificity and ANA with homogenous or speckled pattern, suggesting that strong au-

toimmune propensity might have existed beforehand.<sup>5,7</sup> Indeed, most commonly, a homogenous (34-58%) or speckled (21-34%) pattern of ANA by IIF is demonstrable in cases with AIH-1.<sup>18-20</sup> So far however, neither a liver-specific nuclear antigen nor a liver-disease specific ANA has been identified. For this reason, subtyping of ANA in cases of AIH-1 seems to have limited clinical implication and diagnostic relevance in routine clinical practice<sup>7,13,18-21</sup> leading the committee for autoimmune serology of the IAIHG to suggest ANA testing at the screening stage for AIH diagnosis preferably on freshly rodent multi-organ substrate panel that should include kidney, liver and stomach than on HEp2 cells.<sup>22</sup>

We and others have published previously in several reports the detection of NOSA during the course of chronic HBV and HCV infections which is not a surprising event.<sup>7-9,11,18,23-25</sup> In fact, it usually represents a part of the natural course of these infections.<sup>7-9,11,18,23-25</sup> In this context, according to our records the prevalence of NOSA in our HBV and HCV series (in total almost 800 and 600 HBV and HCV patients, respectively) ranges between 10-30% for each specific NOSA (ANA, SMA, ANCA, anti-cardiolipin, etc.) in HBV and 25-80% for each specific NOSA in HCV patients, with up to 55% for ANA and 80% for SMA positivity in the latter group of HCV-infected patients.<sup>6-8,18,26,27</sup> However, these antibodies in most of our cases of chronic viral hepatitis infections are detected in low titers (1/40-1/160), usually lack F-actin reactivity and have not an homogenous or speckled ANA pattern by IIF compared with those found in our AIH series of patients (n = 180, with almost half having simultaneous ANA and SMA detection, 35% only SMA and 15% ANA alone with titers above 1/160 in almost 85%

**Table 2.** Laboratory values at the time of viral hepatitis and autoimmune hepatitis diagnosis in the individual groups of patients with viral hepatitis and autoimmune hepatitis.

Laboratory tests	AIH+HCV (at viral hepatitis diagnosis, n = 5)	AIH+HCV (at diagnosis of AIH, n = 5)	AIH+HBV(±HDV) (at viral hepatitis diagnosis, n = 6)	AIH+HBV (±HDV) (at diagnosis of AIH, n = 6)
Bilirubin (mg/dL)	0.7 ± 0.1	0.7 ± 0.1	0.7 ± 0.3	1.2 ± 0.7
Albumin (g/dL)	4.2 ± 0.4	4.1 ± 0.3	4.3 ± 0.3	3.7 ± 0.5
PT (sec)	11.7 ± 0.6	10 ± 5.1	12.6 ± 0.8	11 ± 4.9
ALT (IU/L)	176 ± 99	228 ± 194	99 ± 44	100 ± 49
AST (IU/L)	113 ± 68	86 ± 97	75 ± 21	46 ± 34
γ GT (IU/L)	89 ± 61	77 ± 61	76 ± 73	84 ± 81
ALP (IU/L)	93 ± 31	95 ± 33	95 ± 34	88 ± 51
IgG (mg/dL)	2036 ± 631	2170 ± 534	2410 ± 891	2178 ± 699
IgM (mg/dL)	102 ± 46	102 ± 48	281 ± 316	280 ± 251

Abbreviations are same as in text.

of cases). The frequent detection of NOSA in patients with viral liver diseases perhaps may explain why in all of our present cases with concurrent AIH and viral liver diseases the diagnosis of AIH followed that of chronic viral hepatitis by many months to years considering also that the isolated detection of NOSA in HBV and HCV patients is not by definition a synonym of AIH.

The existence of NOSA has occasionally been associated with more severe liver disease in patients with chronic viral hepatitis B and C infections,<sup>28</sup> although response to IFN- $\alpha$  therapy was usually not impaired.<sup>7,8,10,18,23,29</sup> Particularly for HCV infection however, we have shown by the investigation at three time-points (baseline, end of treatment, end of follow-up) of a large number of age, sex, HCV-genotype and HCV-viral load-matched patients, that the absence of ANA and the decrease of SMA titers during treatment were the only independent predictors for achieving better treatment response.<sup>8</sup> Nevertheless, from our records the induction and/or unmasking of sub-clinical AIH in these published series of our patients was extremely rare although some of them suffered from ALT flares.<sup>8,26</sup> Our findings are in accordance with other two studies in adults HCV-infected patients, where NOSA-positive/HCV-positive patients achieved lower benefit from therapy than those without NOSA in the first study,<sup>30</sup> while in the second, the anti-viral treatment was effective in NOSA-positive HCV patients but sustained response was less likely in those infected with HCV-genotype 1.<sup>10</sup>

In this context, HCV/anti-LKM positive patients are reported to have similar response rates to IFN- $\alpha$ , though they represent a subgroup of patients with greater propensity to develop hepatic flares during treatment.<sup>6,12,17</sup> However, mild elevations of transaminases are not uncommon during IFN- $\alpha$ -based treatments<sup>31,32</sup> and therefore, one should be skeptical and cautious in an attempt to distinguish between simple and relatively innocent hepatic flares during treatment and the possible induction of latent AIH due to IFN- $\alpha$ . In such cases of viral hepatitis infections other parameters like the presence of hypergammaglobulinemia, liver histology with features of AIH and autoantibody detection and absence of viral replication could be helpful in order to establish a concurrent diagnosis of AIH. Indeed, in two third of our patients, mostly with HCV and HBV/HDV co-infection, AIH was assumed to be IFN- $\alpha$ -induced since laboratory and clinical features suggestive of AIH were more pronounced during or after IFN- $\alpha$  treatment whereas, at the time of AIH diagnosis, all patients had inactive re-

plication of HCV. In the remaining one third of patients, including treatment-naïve inactive HBV carriers, the diagnosis of AIH probably had been underestimated since there was biochemical activity as attested by increased transaminases and IgG levels, compatible liver histology, NOSA detection (including anti-LKM-1 in 1 patient), whereas there was no or low levels of HBV viremia. Of relevance, IFN- $\alpha$  treatment was associated with the development of AIH-specific anti-SLA/LP antibodies in a previously anti-SLA/LP negative patient.

As we have recently reported<sup>33,34</sup> the application of the simplified score in our patients with concurrent viral hepatitis and AIH was not efficient to render the diagnosis of AIH (probable AIH in only two patients). As highlighted from these studies,<sup>33,34</sup> the role of liver biopsy was unequivocal, since it was the factor that contributed most to AIH diagnosis. It is of interest that moderate/severe interface hepatitis and plasma cell infiltration were prominent in all our patients before receiving IFN- $\alpha$ , suggesting that AIH might be present from the outset.

Treating patients with combined features of AIH and HCV infection is an important therapeutic dilemma. Few reports of AIH/HCV overlap syndrome exist and standard guidelines for treating this condition have not been formulated.<sup>35</sup> In cases with predominant features of AIH, administration of immunosuppression is commonly recommended, considering that IFN- $\alpha$  could have more deleterious consequences in deteriorating AIH compared to the possible enhancement of HCV replication due to immunosuppression.

All our HCV patients received initially antiviral treatment according to internationally accepted guidelines mostly because features suggestive of AIH remained unrecognized upon deterioration of liver disease with antiviral treatment. All patients diagnosed with AIH during follow-up received immunosuppressive treatment with prednisolone and MMF according to our recent report<sup>6</sup> demonstrating remission mostly under treatment.

Patients with HCV and HBV/HDV co-infection necessitating treatment with IFN- $\alpha$  experience difficulties in achieving sustained virological response especially when receiving in parallel immunosuppression. All 4 AIH/HBV patients were on remission of both diseases at last follow-up suggesting a better outcome in these cases compared to those with AIH/HCV or AIH/HDV infections.

In conclusion, our data clearly show that chronic viral hepatitis infections concomitant with AIH are often very difficult to be recognized given the hete-

roogeneity of liver diseases, the absence of specific markers for the diagnosis and the shortfall of many centers outside reference centers to use reliable test for the detection of autoantibodies and enough expertise, including collaboration with histopathologists, for the interpretation of the laboratory results. Therefore, high clinical suspicion of concurrent AIH should be raised in HBV or HCV cases when an otherwise unexplained increase of transaminases and IgG levels (particularly in those received previously IFN-alpha) occurs, liver histology is compatible for AIH, and positivity for NOSA or other more AIH-specific antibodies like SLA/LP, LKM-1, LKM-3 or LC1 are detected and at the same time low or no viremia is recorded.

In addition, we showed that the outcome of HBV or HCV patients seems to be affected negatively by the concurrent AIH. Although the number of patients in this case study is relatively small, the patients with HBV and concomitant AIH had better outcome compared to those with HCV and AIH probably because of the use of nucleos(t)ide analogues which contrary to IFN-alpha can control HBV replication with no adjacent effect, related to exacerbation of autoimmune phenomena.

## AUTHORS' CONTRIBUTIONS

EIR, KZ and GND had the original idea for the study, designed the study protocol and wrote the paper. KZ and NG did the immune serology work-up and along with EIR and GND assessed the patients from the immunological and virological points of view. GKK did the interpretation of the histological data of the patients. KZ, NG and GND collected the whole data, treated the patient in collaboration with EIR and performed the follow-up assessment while contributed to the final version of the paper. GKK and GND wrote the final version of the paper. All authors have seen and approved the final draft of the paper.

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