Characterization of overlap syndrome between primary biliary cirrhosis and autoimmune hepatitis according to antimitochondrial antibodies status

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SUMMARY

Aims — Codification of variant forms between Primary Biliary Cirrhosis (PBC) and Autoimmune Hepatitis (AIH) has not been definitively standardized. The aim of this study was to compare among 102 consecutive patients, 2 subsets of overlap syndrome (OS, N=21) with and without antimitochondrial antibody (AMA) to two groups of patients with typical PBC (N=43) or AIH (N=38).

Methods — OS was defined by the presence in the same patient of at least 2 of 3 accepted criteria of PBC and AIH. Twelve patients with OS were AMA negative and 9 were AMA positive.

Results — A lower level of alanine transaminase (139±48 vs 269±154 IU/L, P<0.05) and a trend towards a higher level of alkaline phosphatase or gamma-glutamyl transpeptidase was observed in OS without AMA than in OS with AMA (693±200 vs 544±124 IU/L; 370±66 vs 241±77 IU/L, respectively). All AMA-negative patients with OS had antinuclear and/or antismooth muscle antibodies. OS without AMA differed from those with AMA in that they had more severe bile duct damage including destructive cholangitis (P<0.05), ductopenia (P<0.05), ductular hyperplasia (P<0.05) and a higher METAVIR fibrosis score (2.5±0.3 vs 1.3±0.3, P<0.05). The response to therapy was not different between PBC, AIH and OS.

Conclusions — According to the presence of AMA, 2 homogeneous subgroups of patients with overlap syndrome between PBC and AIH may be identified. AMA status affects clinical presentation and liver disease severity of OS.

RÉSUMÉ

Caractéristiques des formes frontières entre cirrhose biliaire primitive et hépatite auto-immune en fonction du statut pour les anticorps anti-mitochondries

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Objectifs — La codification des formes frontières entre cirrhose biliaire primitive (CBP) et hépatite auto-immune (HAI) n’est pas encore standardisée. Le but de cette étude était de comparer parmi 102 malades consécutifs, 2 sous-groupes de forme frontière (N = 21) avec et sans anticorps anti-mitochondries (AAM) avec 2 groupes de malades atteints d’une forme typique de CBP (N = 43) ou d’HAI (N = 38).

Méthode — Les formes frontières étaient définies par la présence chez le même malade d’au moins 2 des 3 critères classiques de CBP et d’HAI. Parmi les 21 malades ayant une forme frontière, 12 n’avaient pas d’AAM et 9 étaient porteurs d’AAM.

Résultats — Un taux plus faible d’ALAT (139±48 vs 269±154 UI/L, P < 0,05) et une tendance à une activité plus élevée de phosphatas- ses alcalines ou de gammaGT étaient observés pour les formes frontières sans AAM par rapport à celles avec AAM (693±200 vs 544±124 UI/L; 370±66 vs 241±77 UI/L, respectivement). Toutes les formes frontières sans AAM avaient des anticorps antinucléaires et/ ou des anticorps antimusculaires lisses. Les formes frontières sans AAM étaient différentes de celles avec AAM et présentaient des lésions biliaires plus sévères incluant une cholangite destructive (P < 0,05), une hyperplasie ductulaire (P < 0,05) et un score de fibrose plus élevé (2,5±0,3 vs 1,3±0,3, P < 0,05). La réponse au traitement n’était pas différente entre les malades avec une forme frontière et ceux ayant une CBP ou une HAI.

Conclusions — En fonction de la présence d’AAM, 2 sous-groupes homogènes de malades avec une forme frontière entre CBP et HAI peuvent être identifiés. Le statut en AAM modifie la présentation clinique et la sévérité de la maladie hépatique des malades ayant une forme frontière.

Introduction

The diagnostic criteria of primary biliary cirrhosis (PBC) and autoimmune hepatitis (AIH) have been codified on the basis of clinical laboratory and histological findings [1, 2]. However, some patients exhibit overlapping features between PBC and AIH [4-11]. The codification of these variant forms has not been definitively standardized.

Serum antimitochondrial antibody (AMA) positivity is the most useful diagnostic test for PBC. The specificity and the sensitivity of this marker are not absolute because it has been found in other auto-immune liver disease and 5 to 10% of patients with typical clinical and histological PBC are negative for AMA [2, 6]. To date the classification of AMA negative patients is still debated. Some authors [6, 10, 11], have used the term of autoimmune cholangitis for a variant form of PBC with clinical and pathological features of PBC but with negative AMA and positive antinuclear antibodies (ANA). Conversely, Micheletti et al. [4] as well as Czaia et al. [12] have described under the same term of autoimmune cholangitis, patients with negative serum AMA and auto-immune features overlapping between
PBC and AIH. On the other hand, Chazouilleres et al. [5] proposed to classify overlap syndrome (OS) as lying between PBC and AIH in patients with at least 2 of 3 accepted criteria for each disease [5]. Taking into account all these studies, overlap syndrome might therefore be a large entity including distinct subsets of patients not yet defined [13].

The aim of this study was to compare using the Chazouilleres et al. criteria [5], 2 subgroups of OS with and without AMA to two groups of patients with typical PBC or AIH. The response of the variant syndrome to immunosuppressive and ursodeoxycholic acid (UDCA) therapies was also evaluated.

Patients and methods

Study population

Between 1997 and 2003, a cohort of 102 consecutive patients referred for type 1 AIH, PBC or OS with a follow up for at least one year were studied. Patients with other causes of liver disease such as primary sclerosing cholangitis, viral hepatitis, exposure to hepatotoxic drugs or alcohol abuse were excluded. For each patient, standardized clinical and laboratory findings as well as liver histology were available at the time of initial evaluation. Ultrasonography of the liver and the biliary tract was normal in all patients.

The diagnostic criteria proposed by Chazouilleres et al. [5] were:

— Group 1: 43 patients had a diagnosis of PBC on the following criteria: 1) serum alkaline phosphatase (AP) levels at least two times the upper limit of normal values; 2) a positive test for AMA; and 3) a liver biopsy specimen showing florid bile duct lesions.

— Group 2: 38 patients had a diagnosis of type 1 AIH on the following criteria: 1) serum alanine transaminase (ALT) levels at least five times the upper limit of normal values; 2) serum immunoglobulin G (IgG) levels at least two times the upper limit of normal values or a positive test for anti-smooth muscle antibodies (ASMA); and 3) a liver biopsy showing moderate or severe periportal or perisepal lymphocytic piecemeal necrosis.

— Group 3: 21 patients were designed to have a PBC-AIH overlap syndrome. This diagnosis required the presence of at least two of three accepted criteria of PBC and AIH. Among them, 12 patients were AMA negative and 9 patients were AMA positive. Magnetic resonance cholangiography was performed in 20 patients. All of them had a normal cholangiography.

Immunoserological assessment

ASMA, ANA, AMA, ANCA were sought on murine section, Hep-2 cells and neutrophil spreading by indirect immunofluorescence. ASMA had specificity against smooth muscle actin confirmed with dot (DTek Diasorin, Stillwater USA). AMA negative samples were tested by western blotting against liver mitochondrial antigens as previously described [14]. No patient found to be negative at immunofluorescence was positive with Western blotting analysis.

HLA typing

Only 68 of the 102 patients were evaluated for class I (A1 and B8 loci) and class II (DR3 and DR4 loci) HLA using a standard microlymphocytotoxicity technique as previously described [15].

Histological assessment

All patients underwent an initial liver biopsy. A single pathologist with a special interest in liver disease read all the samples. Specimens were staged according to METAVIR score [16] and other international criteria used for AIH and PBC [1, 17].

Response to treatment

Because treatment strategy was standardized in our unit since 1999 only, the response of 33, 28 and 14 patients from group 1, group 2 and group 3 were analysed respectively:

— Group 1: PBC (N=33), patients received UDCA 15 mg/Kg/day.

— Group 2: AIH (N=28), all the patients were treated with an initial dose of 0.75 mg/Kg of prednisone daily during one month. When serum ALT was lower than twice the upper normal value, prednisone was progressively tapered by 2.5 mg per day every two weeks. Corticosteroid therapy was combined with azathioprine at a dose of 1 mg/Kg per day.

— Group 3: OS (N=14), all the 14 patients were treated by UDCA and prednisone as previously described above. Azathioprine at a dose of 1 mg/Kg/day was added to UDCA and corticosteroid therapy.

Patients with normal or a decrease ≥50% of ALT, GGT and AP level after one year of treatment were considered to have complete or partial response, respectively.

Statistical analysis

Results were expressed as mean ± SD. Chi 2 method with Yates’ correction and the Fisher’s exact test were used to compare dichotomous variables. Differences in the means of continuous variables were assessed by the Student’s paired T-test. The Mann-Whitney’s test was used to compare nonparametric variables in independent samples. A P corrected (Pc) with Bonferroni’s correction was used for HLA analysis [18].

Results

Clinical and laboratory findings at diagnosis

Of 102 patients, 21 (20.5%) could be classified as having OS. No difference was observed between the 3 groups for the age at diagnosis (table I). In patients with OS a significantly (P<0.05) higher prevalence of male sex (38.1%) was observed as compared to PBC (6.9%). The sex ratio was not different between OS and AIH groups. No difference was observed between the 3 groups for clinical symptoms or associated immune diseases. The most frequent associated autoimmune diseases were sicca syndrome (25%, 14%, and 23%), Raynaud phenomenon (20%, 7%, and 9%), systemic sclerosis (15%, 0%, 9%) and thyroiditis (18%, 17%, and 19%) observed in PBC, AIH and OS, respectively. A significantly greater serum AST and ALT levels (table I) was observed in OS than in patients with PBC (P<0.05). Patients with OS were undistinguishable from those with PBC with regard to serum AP, gamma glutamyl-transpeptidase (GGT), bilirubin or gammaglobulins values. Conversely, patients with OS (table I) differed from patients with type 1 AIH by lower serum ALT and higher serum levels of AP, GGT and IgM.
respectively), within patients with OS, no difference in response
(60.6%, 89.2% and 71.4% for PBC, AIH and OS patients,
respectively). Within patients with OS, no difference in response
was greater in AMA negative OS than in those with AIH (58.3%
(P<0.05), ductopenia (P<0.05) or ductular hyperplasia (P<0.05).
All of the AMA negative patients have either destructive cholangitis
as well as the presence of granulomas was similar in patients
with PBC as in AMA negative OS. In addition, in the group of OS
patients without AMA, fibrosis score was higher than in AMA
positive OS (2.58 ± 0.3, P<0.05).

Comparison of OS with and without AMA

All the patients with OS who were negative for AMA at
immunofluorescence technique tested negatively using Western
blot assay. All the positive tests were concordant using both tech-
niques. Patients without AMA tended to be older and more often
males than AMA positive ones. In addition, AMA-negative OS
patients without AMA differed from those with AMA (table III) by
the importance of biliary lesions including destructive cholangitis
(P<0.05), ductopenia (P<0.05) or ductular hyperplasia (P<0.05).
All of the AMA negative patients have either destructive cholangitis
(58.3%) or ductopenia (41.6%). The progressive biliary injury
as well as the presence of granulomas was similar in patients
with PBC as in AMA negative OS. In addition, in the group of OS
patients without AMA, fibrosis score was higher than in AMA
positive OS (2.58 ± 0.3 vs 1.33 ± 0.3, P<0.05). Moreover, the
number of patients with severe liver disease (F3/F4 in METAVIR)
was greater in AMA negative OS than in those with AIH (58.3%
vs 17.8%, P<0.05).

Response to therapy

Complete response to treatment was less frequent (P<0.05) in
patients with PBC (33.3%) than in those with AIH (60.7%) (table IV). In the 14 patients from OS group who had standard-
ized treatment, a complete response was achieved in 7 patients
(50%). Partial response, defined by a decrease of at least 50% of
biochemical liver function tests, was similar in the 3 groups
(60.6%, 89.2% and 71.4% for PBC, AIH and OS patients,
respectively). Within patients with OS, no difference in response
to treatment was observed according to AMA status.

Discussion

The term of variant syndrome or OS, actually covers a heter-
ogeneous group of patients with mixed features of PBC and AIH.
Diagnostic criteria for OS are still unclear [13] particularly for
AMA negative patients. Contrary to PBC or AIH, the classification

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### Table I. – Patients characteristics.

<table>
<thead>
<tr>
<th>Group 1</th>
<th>Group 2</th>
<th>Group 3</th>
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<tbody>
<tr>
<td></td>
<td>PBC N=43 (%)</td>
<td>AIH N=38 (%)</td>
</tr>
<tr>
<td>Age</td>
<td>54.1±1.8</td>
<td>45.8±3.7</td>
</tr>
<tr>
<td>Male/female</td>
<td>3 (6.9)/40 (93)</td>
<td>10 (26.3)/28 (73.6)</td>
</tr>
<tr>
<td>AST (N=30 IU/L)</td>
<td>68.9±8.5</td>
<td>423±102</td>
</tr>
<tr>
<td>ALT (N=37 IU/L)</td>
<td>73.7±9.6</td>
<td>443±92</td>
</tr>
<tr>
<td>GGT (N=35 IU/L)</td>
<td>377±83.1</td>
<td>184±38</td>
</tr>
<tr>
<td>ALk Phos (N=280 IU/L)</td>
<td>525±70</td>
<td>355±62</td>
</tr>
<tr>
<td>Bilirubin (N=174 μL/L)</td>
<td>13.9±2.4</td>
<td>85±24</td>
</tr>
<tr>
<td>Gamma-globulins (N&lt;16.3 g/L)</td>
<td>18.5±1</td>
<td>21.5±1.8</td>
</tr>
<tr>
<td>IgG (N&lt;12.1 g/L)</td>
<td>16.3±1.5</td>
<td>21.7±2.2</td>
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<tr>
<td>IgM (N&lt;1.6 g/L)</td>
<td>6±1</td>
<td>1.5±0.7</td>
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<tr>
<td>Auto-antibodies</td>
<td></td>
<td></td>
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<tr>
<td>ANA&gt;1:80</td>
<td>20 (46.5)</td>
<td>30 (78.9)</td>
</tr>
<tr>
<td>ASMA&gt;1:80</td>
<td>2 (4.6)</td>
<td>26 (68.4)</td>
</tr>
<tr>
<td>AMA&gt;1:80</td>
<td>43 (100)</td>
<td>0</td>
</tr>
<tr>
<td>pANCA&gt;1/100</td>
<td>0</td>
<td>5 (13.1)</td>
</tr>
<tr>
<td>HLA</td>
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<td></td>
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<tr>
<td>A1-B8-DR3</td>
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</tr>
<tr>
<td>A1</td>
<td>2/26² (7.7)</td>
<td>6/28² (21.4)</td>
</tr>
<tr>
<td>B8</td>
<td>2/26² (7.6)</td>
<td>11/28⁰ (39.2)</td>
</tr>
<tr>
<td>DR3-DR4</td>
<td>8/26² (30.7)</td>
<td>10/28² (35.7)</td>
</tr>
<tr>
<td>DR3</td>
<td>4/26² (15.4)</td>
<td>0/28⁰</td>
</tr>
<tr>
<td>DR4</td>
<td>14/26² (53.8)</td>
<td>10/28² (35.7)</td>
</tr>
<tr>
<td></td>
<td>4/26² (15.4)</td>
<td>12/28² (42.9)</td>
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</table>

* number of patients tested
+ value±SE (%)
- P<0.05, group 3 vs group 1
- P<0.05, group 3 vs group 2

(P<0.05). The rate of ANA and ANCA positivity was similar in
the 3 groups. ASMA positivity was much more frequent in
patients with OS than in those with PBC (P<0.01). No patient
with AIH had AMA whereas these antibodies were found in 9 of
21 (42.8%) patients with OS (P<0.01). The only significant dif-
ference in HLA typing was a higher HLA-A1 frequency found in
OS patients as compared to patients with PBC (50% vs 7.6%,
respectively: P<0.05).

Comparison of OS with and without AMA

All the patients with OS who were negative for AMA at
immunofluorescence technique tested negatively using Western
blot assay. All the positive tests were concordant using both tech-
niques. Patients without AMA tended to be older and more often
males than AMA positive ones. In addition, AMA-negative OS
patients had lower ALT level (P<0.05) and a trend towards
higher GGT and AP values than AMA positive ones. Further-
more, AMA and/or ASMA were found in all the AMA negative
patients (table II).

Comparison of histologic patterns between
the different groups

Comparison between OS and PBC or AIH groups

Patients with OS (table III) had less severe destructive cholan-
gitis, ductopenia or ductular hyperplasia (P<0.05) than those
with PBC. Conversely, all of these biliary lesions were more
frequent in patients with OS than in those with AIH (P<0.05).
Moderate to severe piecmeal necrosis as well as portal lympho-
plasmocytic infiltrates were observed less frequently in the OS
group than in patients with AIH. No difference was observed
between the 3 groups for the severity of liver injury graded using
METAVIR activity or fibrosis score.
of this entity lacks official established criteria. For example, for some authors, autoimmune cholangitis is a subset of AMA-negative PBC [6, 10, 11] whereas the same term was used [4, 12, 13] to describe an independent entity overlapping between PBC and AIH. Chazouillères et al. [5] proposed OS to lie between PBC and AIH in the presence of at least 2 of 3 accepted criteria for each disease. In the present work, we have compared using the Chazouillères et al. criteria [5], patients with OS subdivided into AMA positive and negative sub-groups to patients with typical PBC or AIH.

Among our cohort of 102 consecutive patients, OS represented 20% of the overall population with autoimmune liver disease. A high prevalence of OS had already been reported [5, 8] though diagnostic criteria were not strictly similar to ours. Retrospective studies showed that variant syndrome occurs in 5% of AIH [7] and in 9% to 19% of PBC [5, 7]. Detection depends on the diagnostic criteria used and large collaborative studies are required to separate and codify these different entities. Indeed, in a first study [7], a different algorithm was used by Czaja et al., to designate OS patients who had sufficient criteria of AIH to describe an independent entity overlapping between PBC and AIH. Chazouillères et al. [5] proposed OS to lie between PBC and AIH in the presence of at least 2 of 3 accepted criteria for each disease. In the present work, we have compared using the Chazouillères et al. criteria [5], patients with OS subdivided into AMA positive and negative sub-groups to patients with typical PBC or AIH.

Interestingly, using different diagnostic criteria, our subgroup of AMA-negative OS had characteristics very similar to those described by Czaja et al. [12] or Michieletti et al. [4] under the term of autoimmune cholangitis.

As compared to PBC, AIH or AMA-positive OS, a trend towards a decrease of female sex was observed in our AMA-negative OS group. AMA negative OS were cytolytic but the level of serum ALT was lower than in OS with AMA (P<0.05). The same tendency was reported in previous studies [4, 12]. To rule out a false negativity of AMA related to immunofluorescence technique [14], all our negative samples were also tested using a Western blot assay (all were negative). Interestingly, all the AMA-negative OS had either ANA or ASMA associated to a high level of gammaglobulins suggesting that this homogeneous liver disease was related to an autoimmune mechanism. In addition, AMA negative OS had aggressive chronic cholestatic liver injury. Indeed, as it was usual in PBC, our AMA-negative OS had a trend towards a higher level of γ-glutamyl transpeptidase and alkaline phosphatase serum concentration than AMA-positive OS, but the difference was not statistically significant. In addition, significant florid bile duct lesions were observed on histological examination in our variant syndrome without AMA, as compared to AMA-positive ones (P<0.05). Biliary lesions were very similar in AMA-negative OS as those found in PBC.
particularly for destructive cholangitis, ductopenia, ductular hyperplasia and even for the presence of granuloma. All the AMA-negative OS had typical biliary lesions with either destructive cholangitis (58.3%) or ductopenia (41.6%). Therefore, PBC and AMA-negative OS could not be differentiated morphologically. Moreover, as compared to the other 3 groups, AMA-negative OS had a greater METAVIR fibrosis score and seven of the twelve patients (58%) had severe liver lesions (F3/F4 in META-VIR). Chronic biliary lesions have also been reported in other clinical settings such as drug-induced ductopenia or primary sclerosing cholangitis. To limit the risk of confusion with atypical primary sclerosing cholangitis, all patients underwent ultrasonography of the liver and the biliary tract and the twenty patients with OS studied by magnetic resonance cholangiography had no biliary lesions. In addition, none of our patients had received drugs before the onset of liver disease. In the absence of AMA, the mechanism of biliary lesions remains speculative. The constant finding of ANA and/or ASMA associated to a high serum level of gammaglobulins could suggest a destruction of bile ducts related to an auto-immune response leading to chronic cholestasis and liver fibrosis.

Taking all these data into account, the identification of patients with liver disease overlapping between PBC and AIH who respond favourably to UDCA associated to corticosteroids and azathioprine is the gold standard for AIH. Some recent studies have suggested a favorable response to UDCA alone or associated with prednisone in patients with OS who were treated with standardized treatment by UDCA associated to prednisone with azathioprine, a complete biochemical response was observed in 50% of the cases and a partial response with a decrease of at least a half of biochemical values was achieved in 71.4% of patients. Within the OS group, the response to treatment was not related to AMA status, and results appeared close to those obtained in PBC or AIH. Unfortunately, histological evaluation on a second liver biopsy was not performed and our biochemical responses need to be confirmed. Conversely, Czaja et al. [12] have shown that variant syndromes did not respond well to corticosteroids or UDCA. However, in their study [11], combination therapy was not tested and the duration of the follow up was short. As suggested by some studies, UDCA could improve biochemical response in OS through a decrease of toxic bile acid excretion, associated with immunoregulatory properties. In OS between AIH and PBC, immunosuppressors such as corticosteroids and azathioprine could have synergistic effects with UDCA in decreasing inflammatory infiltrate leading to liver injury. This combination therapy could be the more appropriate treatment of OS if our results are confirmed by further studies in a similar group of patients.

In summary, according to their status for AMA, two distinct homogeneous subgroups of patients overlapping between PBC and AIH who respond favourably to UDCA associated to immunosuppressors can be identified.

### Table IV

<table>
<thead>
<tr>
<th>Group 1 PBC (N=33)</th>
<th>Group 2 AIH (N=28)</th>
<th>Group 3 Overlap syndrome (N=14)</th>
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<tbody>
<tr>
<td><strong>AMA negative</strong></td>
<td><strong>AMA positive</strong></td>
<td><strong>AMA negative</strong></td>
</tr>
<tr>
<td>Complete response</td>
<td>11 (33.3%)</td>
<td>17 (60.7%)</td>
</tr>
<tr>
<td>Response ≥ 50%</td>
<td>20 (60.6%)</td>
<td>25 (89.3%)</td>
</tr>
</tbody>
</table>

*P<0.05 vs group 2

**Complete response:** Normal serum ALT, gamma-glutamyl transpeptidase and alkaline phosphatase level after one year of treatment.

**Response ≥50%**: decrease ≥50% of serum ALT, gamma-glutamyl transpeptidase and alkaline phosphatase level after one year of treatment.

### REFERENCES


