Anti-smooth muscle and anti-actin antibodies are indirect markers of histological and biochemical activity of autoimmune hepatitis

CA Couto[1,2], PL Bittencourt[3], G Porta[4], CP Abrantes-Lemos[5], FJ Carrilho[6], BD Guardia[6], ELR Cançado[5,6].

1- Universidade Federal de Minas Gerais, Department of Internal Medicine, School of Medicine, Belo Horizonte, Minas Gerais, Brazil[1];

2- Universidade Federal de Minas Gerais, University Hospital, Alfa Institute of Gastroenterology, Belo Horizonte, Minas Gerais, Brazil[2];

3- Unit of Gastroenterology and Hepatology, Portuguese Hospital, Salvador, Bahia, Brazil[3];

4- Children Institute of University of Sao Paulo School of Medicine, Sao Paulo, Brazil[4];

5- Laboratory of Medical Investigation (LIM 06) of Institute of Tropical Medicine of University of Sao Paulo, Sao Paulo, Brazil[6];

6- Department of Gastroenterology of University of Sao Paulo School of Medicine, São Paulo, Brazil[5].

Authors' email:

Claudia Alves Couto – cacouto@uol.com.br

Paulo Lisboa Bittencourt – plbbr@uol.com.br

Gilda Porta – gildaporta@gmail.com

Clarisse P Abrantes-Lemos- cpal@uol.com.br

Flair Jose Carrilho -fjcarril@usp.br

Bianca Della Guardia – biancadg@einstein.br

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Corresponding author: Eduardo Luiz Rachid Cançado, Rua Alves Guimaraes 1133/102, CEP 0510-002, Bairro Jardim America, São Paulo, Brasil; tel 55 11 2661-6447; mobile 55 11 996069545; eduardocancado@usp.br, edulrc@uol.com.br

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List of Abbreviations:

AAA – anti-actin antibodies

AIH - autoimmune hepatitis

ALP - alkaline phosphatase

ALT - alanine aminotransferase

AMA - anti-mitochondrial antibodies

ANA - antinuclear antibodies

Anti-LC1 - anti-liver cytosol antibodies type 1

Anti-LKM1- anti-liver kidney microsome antibodies type 1

Anti-SLA/LP – anti-soluble liver antigen/liver-pancreas antibodies

ASMA – anti-smooth muscle autoantibodies

AST - aspartate aminotransferase

GGT - gamma glutamyl transpeptidase

IB - immunoblotting

IIF- indirect immunofluorescence

UNV - upper normal value

Abstract

Reactivity and titers of autoantibodies vary during the course of autoimmune hepatitis (AIH), and some autoantibodies have been associated with disease activity and adverse outcomes after treatment. The aim of this study was to assess the autoantibody behavior in AIH and its significance as predictors of biochemical and histological remission. A total of 117 patients with AIH (mean age 18.6 [4-69] years) were evaluated and tested for autoantibodies at disease onset and successively (mean 3.2 [2-6] times) after a mean follow-up evaluation of 70 [20-185] months. Anti-smooth muscle (ASMA), anti-liver kidney microsome type 1 (anti-LKM1), anti-liver cytosol type 1 (anti-LC1), anti-mitochondrial, antinuclear (ANA) and anti-actin antibodies (AAA) were determined at disease onset and 379 other times during the follow-up evaluation through indirect immunofluorescence in rodent tissues, HEp-2 cells and human fibroblasts. Anti-SLA/LP were assessed 45 times in the follow-up evaluation of 19 patients using ELISA. Upon admission, AIH types 1 and 2 were observed in 95 and 17 patients, respectively. Five subjects had AIH with anti-SLA/LP as the sole markers. Patients initially negative for AAA did not develop these antibodies thereafter. ANA was detected *de novo* in six and three subjects with AIH types 1 and 2, respectively. After treatment, only ASMA (>1:80) and AAA (>1:40) were significantly associated with biochemical (76.9% and 79.8%) and histological features (100% and 100%) of disease activity ($p<0.001$). **Conclusions**: With the exception of ANA, the autoantibody profile does not markedly vary in the course of AIH. The persistence of high titers of ASMA and/or AAA in patients with AIH is associated with disease activity.

Introduction

Autoimmune hepatitis (AIH) is a chronic inflammatory liver disease that, when untreated, leads to cirrhosis and liver failure. AIH primarily affects women and typically responds to immunosuppressive therapy with clinical and biochemical remission (1, 2). The disease is classified into at least two types based on the autoantibody profile (3). Thus, AIH-1 is characterized by the presence of anti-smooth muscle antibodies (ASMA) with specificity to F-actin (AAA) and/or antinuclear (ANA) antibodies, whereas reactivity to anti-liver kidney microsome antibodies type-1 (anti-LKM1) and/or anti-liver cytosol antibodies type-1 (anti-LC1) defines AIH-2 (1-4). Antibodies against soluble liver-pancreas antigens (anti-SLA/LP) were observed in some patients with AIH in association with ASMA or ANA, and in a small subset of individuals with AIH without the aforementioned autoantibodies (5-7).

The nature of autoantibodies varies during the course of AIH-1, and the titers of these autoantibodies were shown to fluctuate before and after immunosuppressive therapy (8, 9). Previous studies have shown an association between the persistance of biochemical (8, 9) and histological (9) measures of disease activity and the persistence of autoantibody reactivity after the institution of treatment, but the relevance of these findings remains unsettled.

In addition, some autoantibodies were associated with a more aggressive clinical course or adverse outcomes (10, 11). Specifically, AAA have been associated with a higher frequency of death and an increased need for liver transplantation in subjects with AIH (10). Moreover, anti-SLA/LP antibodies were also associated with a more severe clinical course and adverse outcomes after treatment (11, 12). However, until recently, few studies have properly addressed the course of autoantibodies in the long-term follow-up of patients with AIH-1 (8, 9) and AIH-2 (13, 14) and their association with the clinical and laboratory parameters of disease activity currently employed to define the treatment response.

In this study, we conducted a longitudinal evaluation (at least 20 months) of the reactivity and titers of autoantibodies in patients with AIH-1 and AIH-2, and in a subset of subjects with anti-SLA/LP and AAA-positive AIH, before and after the initiation of treatment with prednisone either alone or with azathioprine. In addition, we also correlated the occurrence and titers of these antibodies with clinical, biochemical and histological indexes of disease activity.

Patients and methods

Study population: The medical and laboratory records from all subjects diagnosed with AIH at the Department of Gastroenterology and Pediatric Hepatology from the University of Sao

Paulo School of Medicine were reviewed in order to select patients with the following inclusion criteria: probable or definite diagnosis of AIH according to international parameters (15); reactivity for one or more autoantibodies, including ASMA, AAA, ANA, anti-SLA/LP, anti-LC1, anti-LKM1, at accession; availability of frozen serum samples at –20°C, collected at diagnosis and thereafter at different time points during immunosuppressive therapy and after liver transplantation; adherence to immunosuppressive treatment with corticosteroids with or without azathioprine; and clinical follow-up of at least 20 months after the institution of therapy. All subjects with the diagnosis of overlapping AIH and primary sclerosing cholangitis (PSC) as well as AIH and primary biliary cirrhosis syndromes were excluded from the study. It is important to highlight that either endoscopic or magnetic resonance cholangiography was performed only in those subjects with AIH with clinical, laboratory and histological features of concurrent AIH and PSC.

All patients tested negative for the hepatitis B surface antigen (ELISA, Abbott Laboratories, North Chicago, IL) and anti-HCV antibodies (ELISA II or III, Ortho Diagnostic System, Raritan, New Jersey). The exclusion of HCV infection was performed through either a third generation immunoblot assay (INNO-LIA III, Innogenetics, Ghent, Belgium) or HCV-RNA (Amplicor, Roche, Basel, Switzerland) in all AIH-2 patients.

ASMA, ANA, anti-LKM1, anti-LC1 and anti-mitochondrial (AMA) antibodies were tested by indirect immunofluorescence (IIF) on unfixed sections of liver, kidney and stomach of female rats, and on HEp-2 cells (4). Titers of autoantibodies ≥1:40 were considered positive. AAA were detected by IIF in acetone-fixed human fibroblasts using the heat serum inactivation technique, and titers>1:10 were considered positive (4, 16, 17). Anti-LC1 and anti-LKM1 antibodies were also determined by immunoblotting (IB) using human liver cytosol and Wistar rat liver microsomal fractions as the source of antigens, respectively (18). All previously collected frozen serum samples were tested for ASMA, ANA, AAA, anti-LKM1, anti-LC1 and AMA. Anti-SLA reactivity was sequentially evaluated in a subset of 19 patients with reactivity for these antibodies at admission, as determined by ELISA according to the manufacturer's instructions (Euroimmun, Lubeck, Germany).

According to the protocol, all subjects were submitted to physical examination at presentation and at 1 to 2-month intervals during the first year of therapy, and subsequently every 3-6 months. All patients received immunosuppressive therapy consisting of corticosteroid alone (12%) or combined with azathioprine (88%) according to international guidelines (19). Pediatric patients received prednisone (1–1.5 mg/kg body weight/day) alone or prednisone (0.5–1.5 mg/kg/day) in combination with azathioprine (0.5–2.0 mg/kg body weight/day). All

patients were systematically questioned for adherence at each outpatient visit by one of the authors.

Laboratory liver tests, including alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase, gamma glutamyl transpeptidase, albumin, γ-globulins, INR and total bilirubin, were performed at each evaluation. The autoantibodies were determined at accession in all patients, and the sera were collected at different time points during the follow-up each year and at the time of liver biopsy to assess histological disease remission. The sera were collect and frozen at -20^{o}C.

The treatment end-points were defined based on international parameters (15, 20). Briefly, a complete response was defined by the presence of normal aminotransferase levels sustained for at least 18 months under maintenance therapy (biochemical remission) and absent or minimal disease activity (histological remission) in liver biopsy specimens collected in patients with biochemical remission at the end of this period. A partial response was characterized by improvement of symptoms, more than 50% reduction in AST and ALT levels after the initiation of treatment without achievement of normal levels or a liver biopsy showing disease activity, whereas no response was defined as less pronounced reductions in the AST and ALT levels or no change in histological parameters of disease activity after at least six months of immunosuppressive treatment. Treatment failure was considered as the presence of clinical and/or biochemical deterioration despite therapy. In patients who achieved complete response, the elevation of aminotransferase values more than twice the normal levels was considered as relapse of AIH at any time during maintenance therapy or after cessation of immunosuppressive therapy. Treatment withdrawal was considered only in patients who had complete biochemical and histological remissions.

Histopathological analysis was performed blindly by liver pathologists using a standardized grading system (21,22). Follow-up liver specimens were obtained according to protocol after at least 18 months of biochemical remission and also when justified by clinical judgment.

To correlate the antibody reactivity with complete response to treatment, the profiles of the autoantibodies and their titers at the time of histological assessment in the follow-up were considered. For patients with no complete remission, the results of the autoantibody testing at the time of the last follow-up visit were considered as this group of patients was not subjected to histological assessment.

The local ethics committees approved this study.

Statistical analyses

Comparisons of the categorical variables were performed using chi-square or Fischer's exact tests. Kruskal-Wallis and McNemar tests were used as appropriate for statistical comparison. *P* values <0.05 were considered as significant. The sensitivity of the autoantibodies for laboratory and histological indexes of disease activity was determined by calculating the frequency of seropositivity when the laboratory and histological features of the disease were present. The specificity of the autoantibodies for the laboratory and histological indexes of disease activity was determined by calculating the frequency of seronegativity when the laboratory and histological features of the disease were absent. Positive predictability indicated the frequency of seropositivity reflected in the presence of laboratory and histological features of the disease, and negative predictability indicated the frequency of seronegativity reflected in the absence of laboratory and histological features of the disease.

Results

One hundred and seventeen patients with AIH, 104 (88%) females, mean age at disease onset of 18.6 ± 4.9 years met the inclusion criteria. Table 1 shows the clinical, laboratorial and histological characterization of the patients. As expected, patients with AIH-2 when compared to their counterparts with AIH-1, had significantly lower age at disease onset. There were only two adult patients with AIH-2.

Data concerning clinical and laboratory features of patients with AIH-1 according to age at disease onset are depicted in table 2. Of note, patients with early disease onset, arbitrarily defined as age less than 16 years, had a significantly higher prevalence and titer of SMA and AAA and more frequently had an acute presentation (Table 2), whereas subjects with later disease onset had a higher prevalence of ANA and of concurrent autoimmune diseases. To more clearly describe this cohort, a table showing the frequency of concurrent autoimmune diseases is included as a supplement.

ANA, ASMA, anti-LKM1, anti-LC1, AMA and AAA were assessed 117 times before treatment and 379 times during treatment. In regard to disease types, those autoantibodies were determined 299, 66 and 14 times, respectively, in patients with AIH-1, AIH-2 and AIH with isolated reactivity to anti-SLA/LP. Autoantibodies were assessed 14 times after liver transplantation. Anti-SLA/LP was assessed 45 times in the follow-up of 19 patients, showing reactivity before treatment. At diagnosis, the liver samples were obtained through percutaneous needle biopsy in all but 3 patients.

Characterization and frequencies of autoantibodies before the treatment of AIH-1

Upon admission and before treatment, 95 patients had AIH-1 with either ANA (n=15) or ASMA/AAA (n=33) or ASMA/AAA/ANA (n=42) or ASMA/ANA (n=1) or isolated ASMA (n=4). Most of the patients had these autoantibodies in high-titers (≥1:160) (Table 3). Antinuclear antibodies were observed in 58 (61%) subjects with the following IIF patterns on HEp-2 cells: speckled (50%), homogeneous (32.8%), centromeric (1.7%), nucleolar (13.8%) and nuclear dots (1.7%). On the other hand, ASMA were positive in 80 (84.2%) of AIH-1 patients. Seventy-five patients (93.7%) were also reactive for AAA, detected particularly in those 60 (80%) subjects with ASMA reacting with vessels, glomeruli and also fibrils of tubular cells (VGT-pattern). Patients reactive for ASMA, but not for AAA (n=5), presented with low titers of ASMA (1:40-1:80) reacting with either vessels alone (V-pattern) or vessels and glomeruli (VG-pattern). Concurrent autoantibodies observed in this group of patients included ANA in one and anti-SLA/LP in another four ASMA positive patients without AAA. On the contrary, none of the ASMA negative patients had AAA reactivity.

No patients with AIH-1 presented anti-LKM1 reactivity, and anti-LC1 reactivity was observed in only one patient at accession. This subject also had ASMA ≥1:320 with a VGT-pattern as well as AAA and was classified as AIH-1. Anti-SLA/LP was positive in 14 (28.6%) cases of AIH-1. AMA reactivity was detected in three patients before treatment with a clear-cut diagnosis of AIH type 1.

Characterization and frequencies of autoantibodies before the treatment of AIH-2 and AIH with anti-SLA/LP as the only marker

Seventeen patients with AIH-2 had anti-LKM1. Of those, six patients also had anti-LC1 reactivity and two patients had high titers of ANA. Five subjects were characterized as having anti-SLA/LP without any other serological markers.

Sequential analyses of autoantibodies

Reactivity of anti-LKM1 antibodies was not detected at any time during follow-up of patients with AIH-1. Anti-LC1 reactivity was observed in only one patient with AIH-1 during the follow-up. Furthermore, those AIH-2 patients, initially negative for anti-LKM1 did not develop anti-LKM1 in the follow-up period. Likewise, those AIH-2 patients initially negative for anti-LC1 showed no anti-LC1 reactivity in the course of the disease. Similarly, the reactivity for ASMA was not observed thereafter in the follow-up of patients initially negative for ASMA (15/95) and AAA (20/95). However, ANA reactivity was observed in six and three patients in the course of AIH-1 and AIH-2, respectively. ANA were positive in one or more

consecutive tests, and the titers varied from 1:40 to ≥1:320. Two AIH-2 patients with ANA reactivity at diagnosis maintained this reactivity in follow-up evaluations. Changes in the IIF patterns of ANA were observed in 7/58 (12.1%) patients. All AMA positive subjects with AIH-1 at diagnosis had persistent reactivity for AMA during the follow-up period.

Autoantibodies and treatment response in AIH

A complete biochemical response was observed in 68% (65/95) of patients with AIH-1 after a mean follow-up of 35 [12-75] months. Five patients were excluded from this analysis, as they had normal biochemical tests for only 12 months and consequently they had no histological assessment at the end of the study. Thus, sixty patients underwent repeat percutaneous liver biopsies. Of those, 75% (45/60) of patients were classified as complete responders according to histological findings and 25% (15/60) showed persistent inflammatory activity. All patients with complete response (liver biopsy specimen showing absence or minimal disease activity) were evaluated for treatment withdrawal. Treatment was discontinued in 71% (32/45) of the complete responders. Immunosuppressive treatment was not withdrawn in 13 patients due to concurrent autoimmune diseases (n=5) and patients' preference to maintain treatment (n=8). A subsequent relapse was observed in 15 (46.8%) patients, while 17 (53%) patients had sustained remission after a mean time of 24 [2-60] months.

Considering biochemical and histological findings on liver biopsies, 45/95 patients (46.7%) had no complete response due to persistent elevation of aminotransferase levels (n=24), liver failure despite normal aminotransferase levels (n=6) and evidence of inflammatory activity at biopsies despite biochemical remission (n=15). At the end of the follow-up evaluation, 11 out of 95 patients required liver transplantation and two patients died.

Table 4 shows the association of ASMA and the AAA titers and the failure of treatment to produce complete response and Table 5 shows the titers of AAA, ANA and ASMA at the time of evaluation of the histological response to treatment. The persistence of ASMA (titers >1:80) and AAA (titers >1:40) was significantly associated with the biochemical (60.3% and 63.1%) and histological features (100% for both) of AIH activity ($p<0.001$). Table 6 shows the high specificity of ASMA (>1:80) and AAA (>1:40) in predicting failure of treatment to produce complete response, which was observed in patients with early (<16 years) and late disease onset. However, the ANA reactivity and titers were not significantly associated with biochemical or histological parameters of inflammatory activity. The analysis of the reactivity of ANA either alone or in combination with ASMA showed no association with the inflammatory activity (Table 4). The ANA behavior was different from that of ASMA. No

correlation was observed between disease activity and ANA patterns. The accuracy of the ANA homogeneous pattern in predicting complete response was low, with 15.3% specificity, 66.6% sensitivity, 26.6% positive predictability and 50% negative predictability (data not shown).

Patients with AIH-2, on the other hand, had inconsistencies between the IIF and IB results four times. In this setting, the IB results were considered as more accurate. All but one patient showed biochemical response after one to four years of treatment. Anti-LKM1 reactivity was observed in 50% (3/6) of patients, despite histological remission. Among the patients who had undergone liver biopsy for assessing remission, three in six patients still showed anti-LKM1 reactivity despite the presence of histological remission. In spite of normal levels of aminotransferases, seven of 16 patients still harbored anti-LKM1 reactivity during follow-up. Overall, anti-LKM1 were reactive in 77.2% (51/66), while the aminotransferase levels were normal in 74.5%. In contrast, the reactivity of anti-LC1 disappeared in the majority of those re-assessed for these autoantibodies (68.5%; 13/19).

Anti-SLA/LP was persistently reactive in 66.7% of cases. Despite a complete response, five out of eight patients still showed anti-SLA/LP reactivity in the follow-up evaluation. Four patients in the anti-SLA/LP group had treatment failure. We did not observe a significant association between the biochemical response and anti-LKM1, anti-LC1 and anti-SLA/LP reactivity. AAA, SMA and ANA reactivity at the time of treatment withdrawal were not shown to predict relapse (data not shown).

Antibodies and prognosis of AIH

No significant differences were observed in the age, sex distribution, clinical onset, number of concurrent autoimmune disorders, and biochemical abnormalities at the time of diagnosis between the AAA positive and AAA negative patients. Similar results were observed for ASMA, ANA, anti-LKM1 and anti-LC1. In respect to AIH-1, there was no association between the biochemical and complete response frequencies and the reactivity of each antibody before the institution of treatment. However, patients with ANA had fewer therapy failures (6.9% vs. 24.3% p=0.016) when compared to their counterparts without ANA reactivity. There were no differences in response between AIH-1 patients with high (≥1:160) and low (<1:80) autoantibody titers before treatment, but only 6/95 (6.3%) of the patients had low titers.

Discussion

The current study sequentially examined autoantibodies in the course of AIH and separately analyzed the behavior and titers of each autoantibody in a large cohort of AIH patients. Our results suggested the potential clinical application of ASMA/AAA in the follow-up evaluation of patients with AIH-1. In this regard, persistence of ASMA/AAA with titers >1:80 were indicators of inflammatory activity in follow-up liver biopsies. Interestingly, differences between ASMA/AAA and ANA behavior during the course of AIH were observed. Thus, ASMA/AAA reactivity and titers were associated with inflammatory indexes of disease activity. In respect to ANA, the present study is in agreement with previous studies that have shown no specificity of ANA for AIH-1 either at the time of diagnosis or during the course of treatment (8, 23, 24). Moreover, our data shows that ANA behavior is not associated with signs of inflammatory activity in liver biopsies of patients with AIH. (8, 23, 24). Furthermore, ANA reactivity was detected in the course of AIH-1 and in 29% (5/17) of patients with AIH-2 either at presentation (n=2) or during the course of disease (n=3). In addition, significant changes in the ANA patterns in the same patient during the AIH course were observed in the present study but no correlation was observed between the disease activity and ANA patterns.

No reactivity for either ASMA or AAA in the present study was detected in the follow-up evaluation of patients who did not present these autoantibodies before treatment. Our findings were in contrast to those reported by Czaja et al. (9), who described de novo reactivity for ASMA in the follow-up evaluation of North-American patients with ANA-positive AIH-1 (AAA was not tested). It is clear that both cohorts of patients with AIH may not be comparable due to ethnic and clinical heterogeneity, but it is worthwhile to mention that different methods for autoantibody detection were performed in the aforementioned studies. In this regard, we have employed in the present study the currently standardized methodology recommended by the International AIH Study Group (4): this could have led to more accurate results concerning autoantibody screening. Notably, IIF reactions rely on the observer and could be influenced by such factors, probably leading to detection of autoantibodies in low titers in many diseases, such as hepatitis C and non-alcoholic steatohepatitis (25, 26). In this study, false positive reactions for autoantibodies were reduced by testing AAA and confirming anti-LKM1 reactivity using IB. Moreover, the detection of AAA using human fibroblasts has been performed using standardized techniques for many years in our laboratory, and AAA seropositivity has not been observed in the follow-up evaluation of any patient negative for this antibody at the onset the disease, considering patients with AIH-1, AIH-2 and patients with anti-SLA/LP as the sole markers. Thus, the present study showed

that high titers of ASMA/AAA do not develop during inactive AIH, as these antibodies were associated with disease activity before treatment onset.

Previous longitudinal studies with smaller cohorts have shown an association between autoantibody seropositivity and biochemical activity of AIH. Gregorio et al. (8), evaluated 19 patients and showed that in AIH, ASMA and anti-LKM1 titers were associated with biochemical evidence of disease activity (8). However, they have described a positive correlation between the serum concentrations of AST, levels of anti-liver specific protein and anti-asialoglycoprotein, and titers of ASMA, but not ANA in children with AIH-1. Notably, their studied population was comprised mainly of children. No correlation was made with autoantibodies and histological indices of disease activity, which are clearly more reliable in order to assess either risk for disease progression or relapse after discontinuation of treatment. In the present study, we were able to describe similar findings in a larger cohort of patients with AIH-1. In this regard, the persistence of ASMA/ANA was associated not only with biochemical signs but also with histological indexes of disease activity. These findings were observed not only in children, but also in adults with AIH-1. We were not able to observe a relationship between the seropositivity for anti-LKM1, anti-LC1 or anti-SLA/LP and normal aminotransferase levels, but we cannot conclude whether the presence and/or titers of these antibodies could reflect disease activity due to the small number of patients studied.

Czaja et al. (9), on the other hand, showed that the disappearance of autoantibody reactivity, but not the autoantibody status, was associated with the improvement of laboratory and histological features of AIH. However, it must be mentioned that no distinction between ANA and ASMA reactivity were made in that study which makes their results hard to compare with ours. Indeed, some previous studies also showed no correlation between ANA status and the response to immunosuppressive therapy despite the disappearance of ANA in the majority of patients with AIH-1 (8, 9, 24). The results of the present study showed that ANA reactivity was not associated with disease activity, as these antibodies tested positive in as many as 50% of patients at the time of biochemical and histological remission. However, the ASMA and AAA reactivity in titers >1:80 and >1:40, respectively, were associated with evidence of histological disease activity. This finding should discourage the suspension of therapy in patients showing reactivity of these antibodies, although only a few patients would have these autoantibodies reactive in high titers in the context of normal biochemical liver tests. Thus, testing ASMA/AAA could be useful to avoid performing an unnecessary liver biopsy or interrupting therapy when ASMA reactivity is higher than 1:80. Indeed, incremental increase in the dosage of medications would represent a more logical approach. In spite of the

association between ASMA reactivity in high titers (>1:80) and disease activity, seronegativity for ASMA or AAA does not preclude liver biopsy, as signs of inflammatory activity were respectively detected in 12% (5/42) and 19% (8/41) of the instances when liver tests were abnormal in the absence of the aforementioned autoantibodies.

Altogether, there were no data on whether each autoantibody reactivity at diagnosis could influence prognosis (10, 27), but a significantly higher mortality was observed in patients negative for ANA. No significant differences in mortality were identified in the AAA reactive group when compared with their negative counterparts. However, this study was not designed to address this issue. A previous study has shown an association of AAA antibody reactivity with a greater frequency of treatment failure, death from liver disease, and requirement for liver transplantation when compared with ANA (10). However, in adult and pediatric with AIH-1 patients, Granito et al. (27) showed that AAA reactivity was associated with higher globulin and IgG levels, but the presence of these antibodies was not associated with any other clinical, biochemical, histological or immunogenetic parameters, suggesting that AAA do not identify a particular subset of disease among AIH-1 patients (27).

Autoimmune hepatitis type 1 in Brazil and Argentina is associated with HLA-DRB1*1301, and with a secondary association with DRB1*0301 (28). Those findings are different from those reported in Northern-Europe and the United States of America, leading some authors to speculate about the occurrence of different environmental triggers in South-America, particularly hepatitis A virus. Compared with North-American patients, Brazilian patients were younger and frequently showed AAA as a disease marker and had a more aggressive clinical course (29). It still remains speculative to link the described association of ASMA and/or AAA persistence with disease activity in only a subset of patients with AIH, for instance pediatric AIH or AIH in South-American patients, in as much as two other reports from the United Kingdom and the United States have disclosed this association, respectively in children and adults.

It is also important to highlight that AIH and sclerosing cholangitis overlap syndrome, also called autoimmune sclerosing cholangitis (ASC), was not entirely ruled out in all our patients with AIH-1. Indeed, ASC was shown to be common in children with AIH even in the absence of overt biochemical or histological signs of cholestasis (30). Most patients with ASC respond to immunossupressive therapy with biochemical and histological remissions, but it remains to be proven whether the persistence of ASMA and or AAA in this subset of patients with ASC could also be linked to disease activity.

In conclusion, our data show that the autoantibody profile, with the exception of ANA, did not markedly vary during the course of AIH. In addition, these results indicate that in AIH-1, the ASMA/AAA titers were associated with biochemical and histological evidence of disease activity. This is potentially useful in the clinical follow-up of the affected patients to guide treatment strategies.

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Table 1: Clinical features of patients with type 1 and type 2 autoimmune hepatitis at onset and treatment response

Clinical features	Total (n=117) *	AIH-1 (n=95)	AIH-2 (n=17)
Female gender (%)	88.9	86.3	100.0
Age/years (mean)	18.6±4.9	19.6±14.7**	7.4±8.8**
Acute presentation (%)	83 (70.9)	65 (68.4)	15 (88.2)
Chronic presentation (%)	34 (29.1)	30 (31.6)	2 (11.8)
Concurrent autoimmune diseases (%)	31 (26.5)	25 (26.3)	4 (23.5)
Familiar autoimmune diseases (%)	24 (20.5)	16 (17.2)	6 (35.3)
Clinical presentation			
Jaundice (%)	75.9	74.5	88.2
Upper gastrointestinal bleeding (%)	6.0	5.3	0.0
Edema/ascites (%)	4.3	6.4	5.9
Biochemical abnormalities (%)	4.3	2.1	0.0
Number of liver biopsies	113	91	17
Cirrhosis	77 (68.1)	64 (66.3)	9 (52.9)
Response to treatment			
Complete biochemical response in 12 months (%)	41 (35)	29 (30.5)	10 (58.8)
Partial response 12 months (%)	56 (47.9)	49 (51.6)	51 (29.4)
No response 12 months (%)	20 (17.1)	17 (17.9)	20 (11.8)
Number of studied patients	102	90	7
Complete response any time (%)***	58 (56.9)	50 (55.5)	5 (71.4)
Death/therapeutic failure (%)	15 (12.8)	13 (13.7)	1 (5.9)

*5 patients had only anti-SLA/LP, ** p<0.001, ***biochemical and histological response

AIH-1: autoimmune hepatitis type 1; AIH-2: autoimmune hepatitis type 2

Table 2: Clinical and laboratory features of patients with AIH-1 according to age at disease onset

Clinical and serological features	AIH-1 (N=95)		P value
	< 16 years (N=54)	≥ 16 years (N=41)	
Age at disease onset (mean)	9.7 ± 3.1	32 ± 13.6	
Female gender	44 (81%)	41(93%)	*NS*
Clinical presentation			
Acute presentation (%)	44/54 (81%)	21/41 (51%)	0.0034
Concurrent autoimmune diseases (%)	7/54 (13%)	18/41 (44%)	0.0009
Familiar autoimmune diseases (%)	8/54 (15%)	8/41 (20%)	*NS*
Cirrhosis at presentation	41/52 (79%)	23/39 (59%)	*NS*
Autoantibody profiles at the onset of AIH			
Anti-smooth muscle antibodies- high titers ≥1:160 (%)	48/54 (91%)	22/41 (54%)	0.0002
Anti-actin antibodies - high titers ≥1:160 (%)	48/54 (89%)	22/41 (54%)	0.0001
Antinuclear antibodies – high titers ≥1:160 (%)	23/54 (43%)	28/41 (68%)	0.021
Antinuclear antibody Patterns			
Speckled	13/27 (46%)	15/28 (53%)	*NS*
Homogeneous	10/27 (53%)	9/19 (47%)	*NS*
Centromeric	0	1	
Nucleolar	4/27 (44%)	5/9 (55%)	*NS*
Nuclear dots	0	1	-
Anti-mitochondrial antibodies	1	2	-
Anti-liver cytosol antibodies type 1	1	0	-
Response to treatment			
Number of studied patients	50	40	
Complete response any time (%)*	27 (54%)	20 (50%)	*NS*
Death/therapeutic failure (%)	9/54 (17%)	4/41 (10%)	*NS*

* biochemical and histological response

AIH: autoimmune hepatitis; AIH-1: autoimmune hepatitis type 1; AIH-2: autoimmune hepatitis type 2

Table 3: Titers of autoantibodies before treatment

Autoantibody Titers	Anti-actin antibodies	Anti-nuclear antibodies	Anti-smooth muscle antibodies
1:40	2	3	1
1:80	2	5	9
1:160	32	5	10
1:320	12	6	60*
1:640	12	13	-
1:1280	7	13	-
1:2560	5	2	-
1:5120	1	5	-
1:10240	2	7	-
Total	75	58	80

*Titers≥1:320

Table 4: Measures of accuracy and performance of autoantibody titers in predicting failure of treatment to produce complete response (biochemical and histological)

Statistical measures of performance	Non-complete response		
	(≥1:40)	(≥1:80)	(≥1:160)
Accuracy of AAA			
Sensitivity	80.5	78.0	31.7
Specificity	87.5	100.0	100.0
Positive predictability	89.2	100.0	100.0
Negative predictability	77.8	78.0	46.7
False positive	10.8	0.0	0
False negative	22.2	22.0	53.3
Accuracy of ASMA			
Sensitivity	88.1	80.5	42.8
Specificity	70.6	91.2	100
Positive predictability	78.7	91.7	100
Negative predictability	82.8	77.5	58.6
False positive	21.3	8.3	0
False negative	17.2	22.5	57.2
Accuracy of ANA			
Sensitivity	60.7	60.7	53.6
Specificity	46.4	50.0	60.7
Positive predictability	53.1	54.8	57.7
Negative predictability	54.2	56.0	43.3
False positive	46.9	45.2	43.3
False negative	45.9	44.0	57.7
Accuracy of combined reactivity of ANA and ASMA			
Sensitivity	92.3	87.2	-
Specificity	42.6	57.5	-
Positive predictability	57.1	63.0	-
Negative predictability	87.0	84.4	-
False positive	42.9	37.0	-
False negative	13.0	15.6	-

ANA (anti-nuclear antibodies), AAA (anti-actin antibodies), ASMA (antismooth muscle antibodies)

Table 5: Titers of AAA, ANA e ASMA at the time of evaluation of histological therapeutic response

Titers	Complete responders			Non-responders		
	AAA (n = 32)	ANA (n = 28)	ASMA (n = 41)	AAA (n = 31)	ANA (n = 17)	ASMA (n = 32)
Negative	28	13	31	0	0	0
1:40	4	2	7	2	2	2
1:80	0	2	3	13	2	11
1:160	0	3	0	3	2	5
1:320	0	1	0	6	1	14*
1:640	0	3	0	3	3	0
1:1280	0	1	0	2	2	0
1:2560	0	0	0	0	1	0
1:5120	0	1	0	1	2	0
1:10240	0	2	0	1	2	0

*Titers≥1:320, ANA (anti-nuclear antibodies), AAA (anti-actin antibodies), ASMA (antismooth muscle antibodies)

Table 6: Measures of accuracy and performance of AAA and ASMA titers in predicting failure of treatment to produce complete response (biochemical and histological) according to the age at autoimmune hepatitis onset

Measures of accuracy	Titer ≥1:40		Titer ≥1:80		Titer ≥1:160	
	<16 years	≥16 years	<16 years	≥16 years	<16 years	≥16 years
For AAA						
Sensitivity	80.7	80.0	76.9	80.0	30.7	33.3
Specificity	90.1	80.0	100.0	100.0	100.0	100.0
Positive predictability	91.3	85.7	100.0	100.0	100.0	100.0
Negative predictability	80.0	78.6	78.5	76.9	45.0	50.0
False positive	8.7	14.3	0	0	0	0
False negative	20.0	11.4	21.5	23.1	55.0	50.0
For ASMA						
Sensitivity	88.4	87.5	92.3	75	46.1	37.5
Specificity	75.0	60.0	91.7	90.0	100.0	100.0
Positive predictability	79.3	77.7	92.3	84.6	100.0	100.0
Negative predictability	85.7	75.0	91.7	64.2	63.1	50.0
False positive	20.7	22.3	7.7	10.0	0	0
False negative	14.3	25.0	8.3	35.8	36.9	50.0

AAA: anti-actin antibodies; ASMA: anti-smooth muscle antibodies

Table supplemental: Concurrent autoimmune diseases in patients with AIH-1, AIH-2 and AIH associated with isolated anti-SLA.

Types of AIH	Concurrent autoimmune disease	Number of patients
AIH-1	autoimmune thyroiditis	4
	ulcerative colitis	3
	idiopathic thrombocytopenic purpura	1
	systemic lupus erythematosus	2
	vitiligo	2
	idiopathic thrombocytopenic purpura/celiac disease	1
	rheumatoid arthritis	1
	Sjogren Syndrome	1
	Crohn's disease	1
	systemic sclerosis	1
	hypoparathyroidism	1
	IgA deficiency	1
	parotiditis	1
	autoimmune anemia	1
	Henoch-Schönlein purpura	1
	nephritis	1
	type 1 diabetes mellitus	1
	interstitial pneumonitis	1
AIH-2	hypoparathyroidism, nephritis and IgA deficiency	1
	autoimmune thyroiditis	1
	autoimmune anemia	1
	vitiligo and IgA deficiency	1
AIH-anti-SLA isolated	celiac disease	2

AIH-1: autoimmune hepatitis type 1
AIH-2: autoimmune hepatitis type 2
SLA: soluble liver antigens