Autoantibody Profile of Adult Patients With Childhood Onset Type 2 Autoimmune Hepatitis

Marek Woynarowski,¹ Małgorzata Woźniak,¹ Bożena Cukrowska,¹ Aldona Wierzbicka,¹ and Simon D. Lytton^{2*}

¹Children's Health Memorial Institute, Warsaw, Poland

²SeraDiaLogistics, Munich, Germany

Background: Anti-liver kidney microsome (anti-LKM) autoantibodies are a distinguishing feature of type II autoimmune hepatitis (AIH-2). However, the levels of anti-LKM-1 in adult AIH-2 patients and their role in liver immunopathology remain equivocal. The aim of the study was to survey the autoantibody profile and the activity of liver disease in adult patients diagnosed with AIH-2 at childhood. **Methods:** The autoantibody profile of adults was compared with the autoantibodies of the pediatric period. Liver function test, Immunoglobulin G (IgG), and gamma globulins were evaluated at the AIH presentation, at the age of 18 years, and at the current adult visit. **Results:** All ten patients tested positive for LKM-1 at least once during the pediatric period. At the adult visit, four patients lost autoantibody positivity. LKM-1 was positive in four, liver cytosol antigen 1 (LC-1) in two, soluble liver antigen in one, and antinuclear antigen in one patient. Additionally three patients with

LKM-1 and one patient without LKM-1 were positive for AMA-M2 (where AMA is antimitochondrial antibodies) Immunoglobulin M (IgM). Liver function markedly improved at 18 years and adult visit compared with initial diagnosis of AIH with only a mild decrease of IgG. The six adult patients positive for at least one autoantibody had statistically lower aspartate aminotransferase (AST) and gamma-glutamyltranspeptidase (GGTP) than the four patients autoantibody negative (AST: 52 vs. 88 IU/l, $P < 0.05$; GGTP 19 vs. 163 IU/l, $P < 0.05$). **Conclusion:** LKM-1 positivity is not a stable condition in all patients with AIH-2. Patients who remained autoantibody positive had better liver function tests than those who lost their positivity. The presence of AMA-M2 autoantibodies suggest that development of AIH/Primary Biliary Cirrhosis (PBC) overlap syndrome should be considered. J. Clin. Lab. Anal. 30:590–596, 2016. © 2015 Wiley Periodicals, Inc.

Key words: LKM-1; autoantibodies; autoimmune hepatitis

Abbreviations

AIH-2	=	type II autoimmune hepatitis
ALT	=	alanine aminotransferase
ANA	=	antinuclear antigen
AST	=	aspartate aminotransferase
AZA	=	azathioprine
GGTP	=	gamma-glutamyltranspeptidase
HAV	=	hepatitis A virus
HBV	=	hepatitis B virus
HCV	=	hepatitis C virus
LC1	=	anti-liver cytosol antigen 1
LKM-1	=	anti-liver kidney microsome 1
SMA	=	antismooth muscle antigen

INTRODUCTION

Autoimmune hepatitis (AIH), a necrotizing inflammation in the liver involving lymphocyte and plasmocyte

infiltrates (1, 2), is driven by a T-cell-mediated immune attack that prevails under a deficit in the number and function of regulatory T cells (3–7). The immune genetics and the environmental trigger of molecular mimicry of liver autoantigens after viral infection induce liver autoantibodies.

The diagnosis of AIH has been confounded by several decades of controversy over consensus on serological criteria that define a specific pattern of liver autoreactivity associated with hypergammaglobulinemia and elevated serum transaminases (7–9). The scoring system codified

*Correspondence to: Simon D. Lytton, SeraDiaLogistics, Benediktenwandstr 7, 81545 München, Germany. E-mail: simon.lytton@t-online.de

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by the International Autoimmune Hepatitis Group assign features of liver histology, abnormal levels of serum transaminases, autoantibodies, viral markers, and alcohol or drug exposures to either “definitive” or “probable” diagnostic criteria (8,9). The American Association for the Study of Liver Disease (AASLD) guidelines promulgated in 2010 offer a semiquantitative scoring system with greater emphasis on autoantibodies that distinguish AIH type 1 with antinuclear antigen (ANA) and antismooth muscle antigen (SMA) antibodies and AIH type 2 with liver kidney microsome-1 (LKM-1) and liver cytosol antigen 1 (LC1) antibodies (10). An aggregate score >15 as definitive and 10–15 as probable is based on female gender, ratio of alkaline phosphatase: aspartate or alanine amino transferase, elevated gamma globulins, levels of ANA/SMA and/or LKM-1, antimitochondrial antibodies (AMA-M2), viral markers, drug and alcohol exposure, Human Lymphocyte Antigen (HLA) DR3 or DR4, thyroid immune disorders, other autoantibodies such as against soluble liver antigen (SLA)/actin/liver cytosol antigen 1 (LC1)/perinuclear neutrophil antigen (pANCA), classification of the liver histology and treatment response (10,11).

The critical need for initial diagnosis of AIH is exclusion of chronic liver disease attributed to α 1-antitrypsin deficiency, hemochromatosis, Wilson’s disease, viral infections (hepatitis A virus [HAV], hepatitis B virus [HBV], or hepatitis C virus [HCV]), malignancy, and drug-induced hepatotoxicity. Patients receiving steroids and/or azathioprine (AZA) at early onset of AIH usually respond well to treatments. The rates of biochemical and clinical remission are improved by extension of therapy for 2 years with cyclosporin or tacrolimus. Immune suppressive drugs may influence the anti-CYP450 autoantibody profile among pediatric patients as previously reported (12). The risk of relapse after treatment withdrawal and the increased viral infection and malignancy associated with prolonged immune suppressive drugs remain major concerns in management of AIH (13–16). Due to the heterogeneity of AIH diagnosis and reports that aminotransferase levels normalize despite continuing liver disease activity at the histological level, a more simplified criteria has been recently recommended to assess the responses to treatment (17).

AIH is chronic and long-lasting disease. In case of AIH with pediatric onset the observation period is usually limited to the moment when patient’s reach the adulthood and are transferred for further therapy from pediatric to adult care. In this study we managed to extend the observation of patients with pediatric onset of AIH beyond the age of the switch from pediatric to adult care. The aim of this study was to check the autoantibody profile of the adults with AIH type 2 diagnosed at childhood. The adult autoantibody profile was compared with the pediatric one. We wanted to check the relationship between the

presence of autoantibodies or the activity of anti-LKM-1 and liver function tests IgG or gamma globulins.

METHODS

Single-center, noninterventional, retrospective analysis of the course of LKM-1-positive AIH diagnosed at childhood in patients who reached 18 years of age and then were contacted as adults for observation at hepatology clinic. The presence of autoantibodies during childhood and adolescents was compared with the current adult autoantibody profile. The values of liver function tests at the three time points were compared: presentation of the disease, the age of 18 years, and the adult care visit.

Patient Population

The Children’s Health Memorial Institute (Warsaw, Poland) laboratory records from the period of 2003–2012 were checked for patients positive for anti-LKM-1 autoantibodies. Within this period, 28 anti-LKM-1 autoantibody-positive patients who reached the adulthood and were transferred to adult care were identified. Ten of them (three males and seven females) responded to telephone or e-mail contact and accepted the invitation for adult follow-up visit with laboratory and autoantibody evaluation. Nine patients had the onset of liver disease between the age 2 and 15 years and one patient had de novo autoimmune liver disease in transplanted organ diagnosed at the age of 16 years (7 years after liver transplantation due to end stage liver disease related to Allagill’s syndrome with HCV coinfection).

The diagnosis of AIH was based on typical laboratory and liver histology abnormalities. All biopsies were assessed according to Batts and Ludwig classification (18) by single pathologists in order to avoid interobserver error (19).

At the initial workup viral liver disease (HAV and HBV) was excluded in all patients while HCV infection was present in one patient who developed AIH in transplanted liver only. Alpha-1 antitrypsin and ceruloplasmin were normal in all subjects, 170 ± 33 mg/dl and 36 ± 15 mg/dl, respectively. Except patients on immunosuppression for liver transplantation other patients had no history of hepatotoxic medication. Due to the age none of the patient was at risk of alcohol liver injury. All patients except one with AIH in transplanted liver reached at least 19 points (range 19–25 points) in AIH scoring system before therapy and at least 20 points (range 20–28 points) after treatment.

Biochemical and Autoantibody Tests

The autoantibodies at pediatric period were detected in sera by commercial indirect immunofluorescent tests

TABLE 1. Patient Characteristics at Initial Diagnosis and Follow-Up Visits

Features	Initial diagnosis Median (range) <i>n</i> = 10	Last pediatric visit Median (range) <i>n</i> = 10	Adult care Median (range) <i>n</i> = 10	<i>P</i> -value ^a
Age, years	8.5 (1–14)	18 (16–19)	21 (19–26)	NR
INR	1.44 (0.97–1.57)	1.06 (0.91–1.16)	1.04 (0.91–1.31)	0.04
ALT, IU/l	524 (85–1,510)	28 (11–91)	60 (14–177)	0.002
AST, IU/l	529 (88–680)	32 (19–83)	45 (18–131)	0.002
GGTP, IU/l	74 (50–396)	49 (16–123)	80 (11–300)	0.92
				0.005 ^b
γ-Globulins, g/l	20 (13.2–58)	14.6 (8–38)	13 (9.8–28)	0.01
IgG, g/l	19.4 (10–54)	16.1 (8–33)	14.8 (9.4–28)	0.01
Total bilirubin, mg/dl	1.6 (0.16–18.9)	0.74 (0.23–1.4)	0.77 (0.2–2.29)	0.004
Direct bilirubin, mg/dl	n.a.	0.24 (0.49)	0.28 (0.09–0.9)	0.06
Liver biopsy				
Piecemeal necrosis	2/10	n.a.	n.a.	NR
Fibrosis + necrosis	3/10	n.a.	n.a.	NR
G2S2	1/10	n.a.	n.a.	NR
G3S0	1/10	n.a.	n.a.	NR
G1S3	1/10	n.a.	n.a.	NR
G3S3	2/10	n.a.	n.a.	NR
Duration of therapy, years	n.a.	9 (2–16)	3.5 (1–8)	NR
Liver Tx, tacrolimus	NR	1/10	1/10	NR
Relapse	NR	6/10	n.a.	NR

^aWilcoxon rank sum test of the matched data sets of initial diagnosis versus adult care visit.

^bPediatric visit versus adult care. NR, not relevant for comparison; n.a., data not available.

using HEp-2 cells for ANA, and rat panel substrates (stomach, liver, and kidney) for ASMA, AMA-M2, and LKM-1 (Mosaic Basic Profile, Euroimmun, Germany). The initial screening dilution was 1:40, and positive samples were further diluted in order to determine the maximum titer.

Autoantibodies of the adult patients were assessed in semiquantitative ELISA (Aesku Diagnostics AESKULISA[®] AMA-M2 G, AMA-M2-M, LC1, LKM-1, and SLA), with range 0–300 IU/l and cut-off value 15 IU/l, IgG immunoblot (Aesku Diagnostics Wendelsheim, Germany ASKUBLOT[®] Liver Pro AMA-M2, Sp100, gp210, LKM1, LC1, and SLA), and indirect immune fluorescence detection of nuclear and cytoplasmic antibodies (Aesku Diagnostics ASKUSLIDE[®] ANA Hep-2 cells). The activity of anti-LKM-1 autoantibodies were quantified using Quanta Lite[®] ELISA, which demonstrated 100% (22/22) sensitivity for specimens that were AIH type 2 positive, LKM-1 Immunofluorescence assay (IFA) positive, and HCV negative and 100% specific (34/34) for 34 specimens that were found to be LKM-1 IFA positive but that were clinically not AIH-2 positive and were not HCV positive (Inova Inc., San Diego).

Biochemical markers (alanine aminotransferase [ALT], aspartate aminotransferase [AST], and gamma-glutamyltranspeptidase [GGTP]) were assessed by enzymatic-colorimetric method using Roche COBAS p 512 analyzer. Total IgG was measured using nephelometric method. All samples were tested with the Siemens

BNII analyzer utilizing Siemens (total IgG) reagents and capillary zone electrophoresis of serum proteins was performed on a MiniCap instrument (Sebia) to measure gamma globulin concentration.

Statistical Analysis

Statistical analysis was done with MedCalc version 14 for Windows (MedCalc Software, Mariakerke, Belgium). Comparisons of the values between patient groups were assessed by nonparametric Wilcoxon sum rank test. A *P*-value of 0.05 was considered to be statistically significant with differences in values between matched groups.

Ethical Consideration

This study did not require any other than standard of care physical examination and blood sampling for laboratory evaluations procedures. Informed consent was obtained from each patient included into the study and the study protocol conforms to the ethical guidelines of the 1975 Declaration of Helsinki as reflected in a priori approval by Ethics Committee of the Children's Health Memorial Institute no. 192/KBE/2015.

RESULTS

Clinical Evaluation

The first liver biopsy showed inflammatory activity in all subjects and seven of ten patients had liver fibrosis.

Fig. 1. Serum enzyme levels in AIH-2 patients. Box-whisker plots of liver aminotransaminases; alanine aminotransferase, ALT (A); aspartate aminotransferase, AST (B); and gamma glutamyltranspeptidase, GGTP (C) in serum of ten patients. The enzyme activity (open circles) median and mean are indicated by the horizontal line and filled square respectively. * $P < 0.05$ are the significant differences in Wilcoxon rank sum test between the values at initial pediatric diagnosis versus matched values at 18 years or versus matched values at adult care or between values at pediatric care and matched values at adult care. NS = no significance. The reference ranges for ALT and AST 10–31 U/l and GGTP 5–61 U/l.

Follow-up biopsies were performed every 2–3 years. The standard therapy during the pediatric period included prednisone and AZA with dosage adjustment according to laboratory liver function tests and steroid-related side effects. Three patients received 1 year treatment with budesonide and AZA within clinical trial (16). Three other patients showing poor responses to steroids and/or AZA were switched to cyclosporine A.

At the end of pediatric period all subjects received continuous immunosuppressive treatments. At the current

adult visit, the clinical condition of adult patients was good and symptoms of liver disease did not impair their ability to study or carry out professional work. Two patients had osteoporosis with a history of vertebral fractures. One patient had type I diabetes mellitus and one patient had history of glucose intolerance that resolved after tapering of steroids. Two patients with recurrent chronic diarrhea were endoscopically diagnosed as ulcerative colitis and showed overlap with primary sclerosing cholangitis. One patient had mild anemia (hematocrit 33%, hemoglobin 10.2 g/dl), and four patients had thrombocytopenia (platelets count 84–144 K/ μ l).

Liver Function Tests

Liver function tests, gamma globulins, and IgG concentration were compared at three time points (Table 1). At the time of initial diagnosis, the serum enzymes were demonstrably elevated in all patients (median ALT 524 IU/l [95% CI 300–925 IU/l] and median AST 529 IU/l [95% CI 250–769 IU/l]). Median ALT and AST activity underwent over tenfold decline at 18 years of age. At this time point four patients had increased ALT and five patients had increased AST activity. ALT and AST activity remained stable at the adult care visit, however the elevation of ALT was present in six and AST in six subjects at this time point (Fig. 1).

No significant fluctuation of GGTP among initial diagnosis, 18 years of age, and adult care visit were observed. The number of patients with abnormal GGTP did not change (respectively, five, four, and five patients).

Initial IgG concentrations (Fig. 2B) were elevated above the reference range in all patients except one, who consistently gave IgG values within normal range and showed strong anti-LKM-1 reactivity. Total gamma globulins levels (Fig. 2A) were similar to IgG levels and followed the same distribution. There were no statistically significant differences in IgG or gamma globulins level between baseline and of pediatric period visit, while at adult visit both values were significantly lower than at AIH presentation.

Autoantibody Profile

During pediatric period the presence of autoantibodies was evaluated several times by indirect immunofluorescence. All subjects were at least once positive for anti-LKM-1 autoantibodies. ANA were positive in six, SMA in four, and SLA in four subjects. All patients were negative for AMA-M2 and LC-1 antibodies were not measured (Table 1).

At the adult visit six patients were found to be positive for autoantibodies (Table 2), and four of them were positive for LKM-1. These patients were also positive for LKM-1 at all tests done at pediatric period having titers

Fig. 2. Gamma globulins and IgG in AIH-2 patients. Box-whisker plots of gamma globulins (A) and IgG (B). The test of significance is described as in legend to Figure 1, * $P < 0.05$. The reference ranges for gamma globulins: 8–13.5 g/l and IgG 7–16 mg/l.

TABLE 2. Autoantibodies Characteristics at Pediatric and Adult Care Visits

	Pediatric care	Adult care
Autoantibody positivity	10 (100%)	6 (60%)
ANA	6 (60%)	1 (10%)
SMA	4 (40%)	n.a.
LKM-1	10 (100%)	4 (40%)
SLA	4 (40%)	1 (10%)
AMA-M2	0 (0%)	4 (40%)
LC-1	n.a.	3 (30%)

within the range of 1:40 to 1:2,560. In the remaining six patients the anti-LKM-1 reactivity was negative at one or more occasions during pediatric period. Three of four LKM-positive adult patients were positive for AMA-M2, one IgG and three IgM ELISA. The AMA-M2 IgG positivity was confirmed by immunoblot.

Two patients tested positive for anti-LC-1 in both ELISA and immunoblot and one patient had anti-SLA that displayed strong reactivity in ELISA and immunoblot (Fig. 3). None of the adult patients tested positive in immunoblots of sp100 and gp210 (Fig. 3B).

Patients positive for autoantibodies at the adult care visit had significantly lower AST and GGTP activity than patients negative for autoantibodies (respectively, AST: 52 vs. 88 IU/l, $P < 0.05$; GGTP: 19 vs. 163 IU/l, $P < 0.05$). ALT activity, IgG, and gamma globulin concentration in patients positive for autoantibodies were lower than in patients without autoantibodies positivity, but the results were not statistically significant (Fig. 4).

Relapse of liver disease six of ten (60%) occurred in five of six patients who maintained positive autoantibodies at adult care visit and in one of four who lost autoantibodies positivity, but this difference was of no statistical significance.

DISCUSSION

Adults With LKM-1-Positive AIH-2 in Childhood Whom Remain Autoantibody Positive Show Better Liver Function Than AIH-2 Patients Who Lose Their Autoantibody Reactivity

AIH type 2, typically of early childhood onset, is a chronic illness psychologically stressful both in terms of

Fig. 3. Distribution of type 2 autoantibody levels in adults. The levels of autoantibodies for each patient are absorption values in the AESKULISA™ (A) and units relative to the positive control (r.u.) in the Gastro AESKUBLOT™ (B). The dotted horizontal lines represent the clinical cutoff values for each autoantibody test.

Fig. 4. Laboratory test results in patients positive and negative for autoantibodies. Box-whisker plots of ALT, AST, and GGTP (A); gamma globulins and IgG (B). The test of significance is described as in legend to Figure 1. The reference ranges for ALT and AST: 10–31 U/L; GGTP 5–61 U/L; gamma globulins: 0.8–1.35 g/dl and IgG 7–16 g/dl inscribed within gray box.

the need for continuous management of therapy and the high relapse rate after therapy withdrawal (15, 16). In this study, patients were selected in accordance with the criteria of positive anti-LKM-1 serology during the pediatric period and referral at 18 years of age to adult care hepatology clinics. The reliability of the AIH diagnosis is confirmed by the AIH score above threshold with positive liver histology in all cases. Additionally, the anti-LKM-1 autoantibodies detected by indirect immunofluorescence at initial diagnosis were positive at multiple visits during the pediatric period. The relapse in six of ten patients defined as a flare in aminotransferase levels and the reoccurrence of clinical symptoms either under treatment, following the minimum dose of maintenance therapy, or after withdrawal and the aggressive and rapidly progressive disease in childhood and teenage years compared with milder course among adults is consistent with previous reports (6).

Limitations and Advantages

The underlying limitation of this study is the small patient sample size and no reference values available for the ELISA and immunoblot reactivity in childhood years. However the study provides unique insight into the autoantibody profile of adult AIH-2; adults that test negative in anti-LKM-1 ELISA and in other type 2 autoantibody assays display worse liver function, elevated amino transaminases and γ -globulin, than the adults that were autoantibody positive. Moreover, the autoantibody-negative group shows strikingly higher levels of GGTP than the autoantibody-positive patients.

The records of liver biopsy, immune suppressive drug treatments, laboratory data, and serology were ostensibly complete. The ten AIH-2 patients, all long course of disease, similar treatment regimen, and predominantly female gender, are a relatively homogenous cohort for the

evaluation of autoantibody profile. This data set thus offers an advantage over the large patient national registry files that are often incomplete in terms of clinical laboratory data, autoantibody testing, and treatment history (9–11).

Key Findings

- (1) LKM positivity is not always a stable condition in patients with AIH, thus the classification of AIH into type 1 and 2 has a limited reliability.
- (2) In the presented group, patients who remained LKM positive had better liver function tests than those who lost LKM positivity.
- (3) The presence of AMA-M2 autoantibodies suggest that development of AIH/PBC overlap syndrome should be considered.

A shift from positive liver autoantibodies in the pediatric period to loss of autoantibody expression in adults with clinical suspicion of AIH does not necessarily indicate resolution of autoimmune liver inflammation. The ulcerative colitis comorbidity in two patients and type 1 diabetes in one patient other than the AMA-M2-positive patients indicate overlap of AIH-2 with gastrointestinal autoimmunity. In this study, only the known AIH autoantibodies and biomarkers of liver inflammation were evaluated and thus we cannot exclude nonconventional autoantibodies in the adults that test autoantibody negative. For example, a recent report of a new anti-asialoglycoprotein receptor (ASGPR) antibody in ELISA found ASGPR autoantibodies at prevalence of 17–19% in autoimmune liver disease. Interestingly higher anti-ASGPR reactivity was detected among AIH-2 patients with immune suppressive treatments than untreated AIH-2 patients (20). In order to confirm our current findings of increased serum enzymes among AIH-2 adults that test

seronegative for autoantibodies after immune suppressive therapy, a statistically powered prospective study of comprehensive autoantibody profile and liver function in cohorts of AIH patients during pediatric and adult disease is now in progress.

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CONFLICT OF INTEREST

The authors confirm that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome.

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AUTHOR'S CONTRIBUTION

Marek Woynarowski, the principle investigator of the study, was responsible for patient recruitment, patient information, and all clinical and laboratory records. Małgorzata Woźniak, Bożena Cukrowska, and Aldona Wierzbicka contributed to the retrieval of the patient records and review of the manuscript content. Simon D. Lytton and Marek Woynarowski made equal contributions to the conception and design of the study. Simon D. Lytton was responsible for the autoantibody testing of adult patients, the data analysis, and the intellectual content in drafting of the manuscript.

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