

Autoantibody Profiling in a Cohort of Pediatric and Adult Patients With Autoimmune Hepatitis

Danilo Villalta,^{1*} Elia Girolami,² Maria Grazia Alessio,³ Maria Concetta Sorrentino,⁴ Marilina Tampoia,⁵ Ignazio Brusca,⁶ Massimo Daves,⁷ Brunetta Porcelli,⁸ Giuseppina Barberio,⁹ Mariaelisabetta Conte,¹ Lisa Pantarotto,¹ and Nicola Bizzaro on behalf of the Study Group on Autoimmune Diseases of the Italian Society of Laboratory Medicine, Italy¹⁰

¹Allergologia e Immunologia Clinica, A.O. "S. Maria degli Angeli", Pordenone, Italy

²Dipartimento di Oncoematologia Pediatrica e Medicina Trasfusionale, Ospedale Bambino Gesù, Roma, Italy

³Laboratorio Analisi Chimico-Cliniche, Ospedali Riuniti di Bergamo, Italy

⁴Laboratorio Analisi Chimico-Cliniche e Microbiologiche, ISMETT, Palermo, Italy

⁵Laboratorio di Patologia Clinica, Policlinico Consorziale di Bari, Italy

⁶Patologia Clinica, Ospedale Buccheri La Ferla, Palermo, Italy

⁷Laboratorio Centrale, Ospedale Civile, Merano (BZ), Italy

⁸Dipartimento Biotecnologie Mediche, Università di Siena, Italy

⁹Patologia Clinica, Ospedale Cà Foncello, Treviso, Italy

¹⁰Patologia Clinica, Ospedale Civile, Tolmezzo (UD), Italy

Background: Autoimmune hepatitis (AIH) is a rare condition characterized by the presence of autoantibodies distinctive of type 1 AIH (AIH-1) and type 2 AIH (AIH-2). The aim of this study was to evaluate the autoantibody profile in a cohort of pediatric and adult AIH patients, using both indirect immunofluorescence (IIF) and a new multiplexed line-blot assay. **Methods:** Sera from 63 pediatric and 53 adult AIH patients were tested for antinuclear (ANA), antismooth muscle (SMA), anti-liver kidney microsome 1 (anti-LKM1), anti-liver cytosol 1 (anti-LC1) autoantibodies using IIF methods; for anti-LKM1, anti-LC1, and soluble liver antigen/liver-pancreas (anti-SLA/LP) autoantibodies using the line-blot; for anti-F-actin autoantibodies using IIF both on VSM47 cell-line and on rat intestinal epithelial cells. **Results:** AIH-1 was the most common type of AIH in the adult cohort (73.6%), while AIH-2 was the most com-

mon AIH in the pediatric cohort (61.9%). Both in adult and pediatric AIH-2 anti-LKM1 were the prevalent autoantibodies. In pediatric AIH-2 anti-LC1 autoantibodies were more frequent than in adult AIH-2 (59 vs. 28.6%), and in 35.9% of cases they were present alone. In 17 patients anti-LC1 autoantibodies were detected only with the line-blot assay. The levels of anti-LKM1 and of anti-LC1 were not different between adult and pediatric AIH, and the overall agreement between the results obtained with the two IIF methods for F-actin detection was 98.8% (CI 95%: 94.4–99.7%). **Conclusions:** The line-blot assay showed a higher sensitivity than IIF for anti-LC1 detection. Anti-LKM1 and anti-LC1 autoantibody levels are not different in adults and children. An almost perfect agreement between the two IIF methods for anti-F-actin detection has been observed. *J. Clin. Lab. Anal.* 30:41–46, 2016. © 2014 Wiley Periodicals, Inc.

Key words: autoimmune hepatitis; autoantibodies; multiplexed line-blot; indirect immunofluorescence; anti-F-actin; reference intervals

*Correspondence to: Danilo Villalta, Allergologia e Immunologia Clinica (DML), A.O. "S. Maria degli Angeli" Via Montereale 24, 33170. E-mail: danilo.villalta@aopn.sanita.fvg.it

Received 28 May 2014; Accepted 25 August 2014

DOI 10.1002/jcla.21813

Published online in Wiley Online Library (wileyonlinelibrary.com).

INTRODUCTION

Autoimmune hepatitis (AIH) is a chronic inflammatory disease, characterized serologically by hypertransaminasemia, elevated levels of immunoglobulin G (IgG), and presence of non-organ and liver-specific autoantibodies, and histologically by interface hepatitis. The diagnosis of AIH can be established using the criteria developed by the International Autoimmune Hepatitis Group (IAIHG, (1–3); a critical component of these criteria is the detection of autoantibodies. From a practical standpoint, AIH has been broadly categorized into two distinct disease subtypes on the basis of antibody profiles: antinuclear (ANA) and/or antismooth muscle (SMA) autoantibodies define type 1 AIH (AIH-1), while anti-liver kidney microsome 1 (anti-LKM1) and/or anti-liver cytosol 1 (anti-LC1) autoantibodies characterize AIH type 2 (AIH-2). The latter is mainly a pediatric condition, with a higher risk of acute liver failure. The above-described autoantibody profiles are, as a rule, mutually exclusive. In addition, autoantibodies against soluble liver antigen/liver-pancreas (anti-SLA/LP) have been included in the diagnostic criteria (3) being highly specific for AIH (4, 5) and associated with a more severe course of disease (6). Anti-SLA/LP autoantibodies are detectable in AIH-1 adult patients and in both AIH-1 and AIH-2 pediatric population, with variable prevalence depending on the method used for their detection (7, 8).

ANA and SMA are detected in up to 85% of AIH patients: such a high sensitivity is associated with a low specificity (9, 10). SMA/F-actin autoantibodies are commonly regarded as more specific markers of AIH-1 (11), but a reference method for their detection is not yet available. Enzyme-immunoassay (ELISA) and indirect immunofluorescence (IIF) methods using vascular smooth muscle (VSM47) and rat intestinal epithelial cell lines have been recently proposed for anti-F-actin detection (12, 13), providing higher specificity than the classical IIF method on rat tissue.

In children and adolescents, AIH often presents acutely and has a more aggressive course than in adults (14). Some authors reported that the scoring system developed by IAIHG is not easily applicable to pediatric AIH, in which relevant autoantibody titers are frequently lower with respect to cut-off value established in adults (15).

The aim of the present study was to evaluate the autoantibody profiles in a large cohort of pediatric and adult patients with AIH using different detection methods: the classical IIF on HEp-2 and rat tissue sections, a newly developed multiplexed line-blot assay, and two different IIF methods for anti-F-actin autoantibodies. A further objective of this study was to evaluate if the levels of anti-LKM1 and anti-LC1 autoantibodies are different in pediatric and adult AIH.

TABLE 1. Demographic and Laboratory Features of Adult Patients With AIH-1 and AIH-2

	Adult AIH	
	AIH-1	AIH-2
Number	39 (73.6%)	14 (26.4%)
Mean age (range)	48.5 (21–75)	40.1 (19–70)
Male	8 (20.5%)	4 (28.6%)
Female	31 (79.5%)	10 (71.4%)
ANA (HEp-2-IIF)	24 (61.6%)	1 (7.1%)
<i>homogeneous</i>	12 (50.0%)	1 (100%)
<i>speckled</i>	6 (25.0%)	–
<i>nucleolar</i>	2 (8.3%)	–
<i>few dots</i>	2 (8.3%)	–
<i>dense fine speckled</i>	1 (4.2%)	–
<i>CENP-B</i>	1 (4.2%)	–
SMA (rat tissue-IIF)	24 (61.5%)	–
ANA + SMA	14 (35.9%)	–
F-actin (VSM47-IIF)	19 (48.7%)	–
F-actin (rat epithelial cells-IIF)	16 (41.0%)	–
SLA/LP (line-blot)	2 (5.1%)	–
LKM1 IIF	–	11 (78.5%)
LKM1 (line-blot)	–	10 (71.4%)
LC1 IIF	–	1 (7.1%)
LC1 (line-blot)	–	4 (28.6%)
LKM1 (line-blot) alone	–	8 (57.1%)
LC1 (line-blot) alone	–	2 (14.2%)
LKM1 + LC1 (line-blot)	–	2 (14.2%)
AMA (line-blot)	1 (2.5%)	–
sp-100 (line-blot)	2 (5.1%)	–
gp210 (line-blot)	–	–
PML (line-blot)	1 (2.5%)	–

PATIENTS AND METHODS

Sera from 63 pediatric (mean age, 8.5 years; range, 2–17 years) and 53 adult AIH patients (mean age, 46.8 years; range, 19–75 years) classified according to the IAIHG criteria (2, 3) have been consecutively collected in ten different centers in Italy. AIH type and male/female ratio are shown in Tables 1 and 2. As control group sera from 53 patients with primary biliary cirrhosis (PBC), 11 patients with primary sclerosing cholangitis (PSC) and 67 patients affected by nonautoimmune chronic liver diseases (39 liver steatosis, 11 chronic alcoholic liver diseases, 9 cryptogenetic cirrhosis, 8 toxic nonalcoholic liver diseases; mean age, 42.5 years; range, 5–88 years) were selected. ANA, SMA, anti-LKM1, and anti-LC1 were assayed using IIF methods on HEp-2 and rat stomach/liver/kidney sections (Euroimmun, Lübeck, Germany) at a starting serum dilution of 1:40. Anti-LKM1, anti-LC1, and anti-SLA/LP were assayed using the new multiplexed line-blot Autoimmune Liver Disease Profile 2 (ALD2, Euroimmun). Anti-F-actin autoantibodies were tested using both IIF on VSM47 cell line, obtained from rat thoracic aorta (Euroimmun, Fig. 1) and IIF on rat intestinal

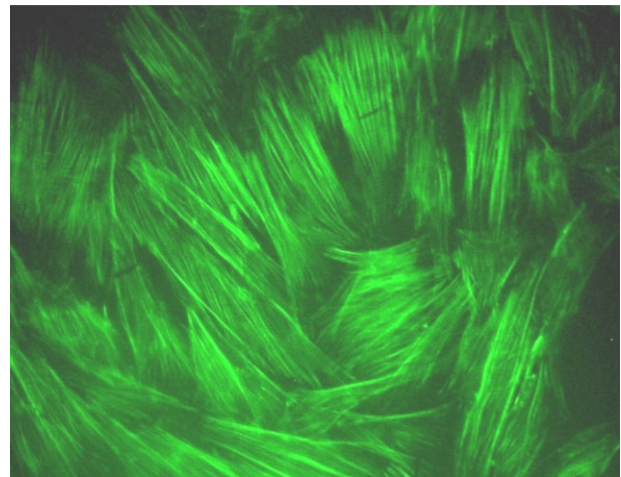
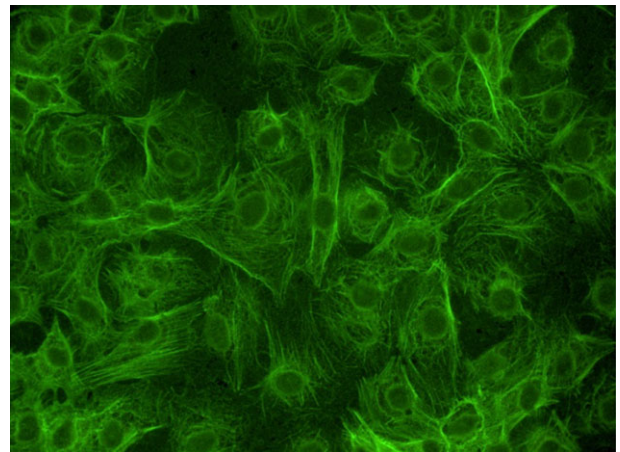
TABLE 2. Demographic and Laboratory Features of Pediatric Patients With AIH-1 and AIH-2

	Pediatric AIH	
	AIH-1	AIH-2
Number	24 (38.1%)	39 (61.9%)
Mean age (range)	9.1 (2–17)	8.1 (2–15)
Male	6 (25.0%)	8 (20.5%)
Female	18 (75.0%)	31 (79.5%)
ANA (HEp-2-IIF)	16 (66.6%)	15 (38.4%)
<i>homogeneous</i>	8 (50.0%)	3 (20.0%)
<i>speckled</i>	6 (37.5%)	8 (53.3%)
<i>nucleolar</i>	2 (12.5%)	1 (6.6%)
<i>centriole</i>	–	1 (6.6%)
<i>dense fine speckled</i>	–	1 (6.6%)
<i>CENP-F</i>	–	1 (6.6%)
SMA (rat tissue-IIF)	11 (45.8%)	–
ANA + SMA	4 (16.7%)	–
F-actin (VSM47-IIF)	7 (29.1%)	–
F-actin (rat epithelial cells-IIF)	7 (29.1%)	–
SLA/LP (line-blot)	1 (4.2%)	–
LKM1 IIF	–	26 (66.7%)
LKM1 (line-blot)	–	25 (64.1%)
LC1 IIF	–	7 (17.9%)
LC1 (line-blot)	–	23 (59.0%)
LKM1 (line-blot) alone	–	16 (41.0%)
LC1 (line-blot) alone	–	14 (35.9%)
LKM1 + LC1 (line-blot)	–	9 (23.0%)
AMA (line-blot)	–	1 (2.5%)
sp-100 (line-blot)	–	–
gp-210 (line-blot)	1 (4.2)	–
PML (line-blot)	–	–

epithelial cells (INOVA Diagnostics, San Diego, CA; Fig. 2), at a starting serum dilution of 1:40. All the assays were performed following manufacturers' instructions, and the slides were read visually by two expert operators (MC and DV) who worked independently.

With the multiplexed ALD2 line blot it was also possible to detect PBC-specific antimitochondrial (AMA), anti-gp210, anti-sp100, and anti-PML autoantibodies. In particular, for AMA detection ALD2 line blot uses two different autoantigens: (1) M2 natively purified from bovine heart containing the 74-kDa E2 subunit of the pyruvate dehydrogenase (PDH) complex; and (2) M2-3E-recombinant fusion protein comprising the immunogenic domains of the E2 subunits of PDH, of the branched-chain 2-oxo-acid dehydrogenase (BCOADH) complex and of the 2-oxo-glutarate dehydrogenase (OGDH) complex.

The ALD2 blot strips were digitalized using a camera and band intensities were determined by a computer program (EUROLineScan, Euroimmun). Autoantibody testing was centralized in the laboratory of one of the authors (DV). The study protocol followed the ethical guidelines of the Helsinki Principles and all the subjects enrolled in

**Fig. 1.** Immunofluorescence pattern of anti-F-actin antibodies on VSM47 cell line.**Fig. 2.** Immunofluorescence pattern of anti-F-actin antibodies on rat intestinal epithelial cell line.

the study provided written informed consent after being informed about the nature of the study.

Statistical Analysis

Diagnostic sensitivity and specificity were calculated for each AIH-associated autoantibody. Differences of anti-LKM1 and anti-LC1 levels between adult and pediatric AIG groups were analyzed by the Mann–Whitney nonparametric *t*-test. Cohen's kappa with 95% confidence interval (95%CI) was used to evaluate the analytical agreement among the IIF methods for SMA and anti-F-actin detection. *P*-values <0.05 were considered significant. MedCalc software (Mariakerke, Belgium) was used for statistical analysis.

RESULTS

Autoantibody profiles of adult and pediatric AIH-1 and AIH-2 patients are shown in Table 1 and Table 2, respectively. AIH-1 was the most common type of AIH in adult cohort (73.6%), while AIH-2 was more common in the pediatric cohort (61.9%). ANA were detected in approximately two-thirds of AIH-1 patients (adults and children) but also in 38.4% of pediatric AIH-2 patients. Both in adult and pediatric AIH-2 patients, anti-LKM1 were the prevalent autoantibodies. However, in pediatric AIH-2 patients, using the line-blot assay, anti-LC1 autoantibodies were present in higher percentage than in adults (59 vs. 28.6%) and in 35.9% of the cases they were the sole antibody. In 14 pediatric and three adult AIH-2 patients, anti-LC1 autoantibodies were detected only by the ADL2 method. This was not unexpected, since using IIF such antibodies are usually masked by the concurrent presence of anti-LKM1 antibodies. The specificity of the AIH-associated autoantibodies resulted low for ANA (65%), but very high for the other markers: 95.5% for SMA; 97.3% for both anti-F-actin assays; 98.5% for anti-LKM1; 99.3% for anti-LC1; and 100% for anti-SLA/LP. The levels of anti-LKM1 and anti-LC1, expressed as arbitrary units (AU) measuring the signal intensity of the respective lines in the multiplexed line blot, were similar between adult (mean 55.9 ± 23.4 AU and 81.2 ± 67.9 AU for anti-LKM1 and anti-LC1, respectively) and pediatric AIH (mean 54.4 ± 26.7 AU and 93.9 ± 44.3 AU for anti-LKM1 and anti-LC1, respectively).

In the adult cohort, one AIH-1 patient was AMA-positive and two AIH-1 patients were sp100-positive. One of the latter was also PML-positive. In the pediatric cohort, one AIH-1 patient resulted gp210-positive and one AIH-2 patient was AMA-positive. The AMA-positive subject was positive for both AMA-M2 and M2-3E bands of the ALD2 line-blot, whereas in the pediatric patient the IIF was inconclusive for the simultaneous presence of anti-LKM1 autoantibodies. Only one of the five AIH patients that were positive for PBC markers (the adult AIH-1 AMA-positive) had both serological and histological signs of PBC and a diagnosis of AIH-PBC overlap was eventually formulated.

The overall agreement between the results obtained with the two IIF methods for F-actin detection was 98.8% (95% CI: 94.4–99.7%): positive agreement was 94.5% (95% CI: 88.4–100%) and negative agreement was 99.3% (95% CI: 98.5–100%). The Cohen's kappa (0.938; 95% CI: 0.879–1.00) showed almost perfect agreement. The overall concordance between SMA and anti-F-actin detected on VSM47 cells was 91.9% (95% CI: 87.8–94.9%), with a positive agreement of 70.6% (95% CI: 58.3–82.9%) and a negative agreement of 95.3% (95% CI: 93.2–97.4%). The Cohen's kappa (0.660; 95% CI: 0.524–0.791) showed sub-

stantial agreement. Finally, the overall agreement between SMA and anti-F-actin detected on rat intestinal epithelial cells was 92.3% (95% CI: 88.2–95.3%): positive agreement 70.8% (95% CI: 58.2–83.3%) and negative agreement 95.6% (95% CI: 93.6–97.6%). The Cohen's kappa (0.666; 95% CI: 0.529–0.803) showed substantial agreement.

DISCUSSION

Autoantibody detection is a very important tool for AIH diagnosis and the definition of autoantibody profiles enables the distinction between AIH-1 and AIH-2 (16). Most relevant autoantibodies are currently detected by the IIF method; however, recognition and interpretation of patterns can be challenging and errors are not infrequent due to lack of standardization and operator-dependency of the technique. For this reason, in 2004 the IAIHG Committee for Autoimmunity Serology drew up a consensus statement containing guidelines for appropriate and effective autoantibody testing in AIH (17): in this statement the use of tissue sections dried in air without further fixation both for ANA and anti-LKM1/anti-LC1 detection is recommended. However, some of these recommendations cannot be followed in most of autoimmunology laboratories, where commercially available tissue substrates and HEP2 cell lines treated with fixatives are used for anti-LKM1 and anti-LC1, and ANA detection, respectively. In recent years, ELISA and immunoblot assays to detect AIH-associated autoantibodies have been developed: such methods may be useful as confirmatory tests of IIF positivity.

In this study we evaluated the antibody profile in a relatively large cohort of pediatric and adult AIH using both the established IIF assays and a new multiplexed line-blot assay. We confirm that AIH-1 was the most common type of AIH in adults (73.6%), while AIH-2 was more common in the pediatric cohort (61.9%): the percentage of AIH-2 in childhood was higher than that reported in other studies (14, 18). The difference may be attributed to the higher percentage of children below 8 years (57.3%) in our cohort. Indeed, it has been previously reported that AIH-2 tends to present at younger age and AIH-1 around the puberty (19). Interestingly, in adult AIH-1, ANA and SMA were equally represented, while in pediatric AIH-1, ANA resulted the prevalent autoantibody. In both adult and pediatric cohorts approximately 80% of ANA-positive patients showed a homogeneous or speckled pattern, while the remainder 20% displayed different patterns (nucleolar, few dots, dense fine speckled, CENP-B, CENP-F, centriole), confirming that in AIH ANA target antigens are heterogeneous and not yet completely defined. The ANA/SMA association was significantly higher in adult than in pediatric AIH-1 (35.9 vs. 16.7%), at variance from the data obtained by Gregorio et al. (19),

who found ANA/SMA association in 43.7% of AIH-1 pediatric patients. This may be explained by the fact that Gregorio et al. used for both ANA and SMA IIF assays tissue sections at 1:10 starting dilution. Which should be the optimal starting serum dilution in children remains a matter of debate. In fact, some authors propose different autoantibody diagnostic cutoffs in adult and pediatric patients (15, 16). Taking into account that autoantibody positivity is extremely rare in healthy children, they indicate titers as low as 1:20 for ANA, and even lower (1:10) for SMA, anti-LKM1, and anti-LC1 as clinically relevant in pediatric patients. This is not supported by the results obtained in the present study, as the levels of anti-LKM1 and anti-LC1 antibodies, expressed as arbitrary units measuring the signal intensity of the respective lines in the multiplexed line-blot, did not show any difference between adult and pediatric AIH-2 patients. Therefore, we do not think it is advisable to report level of positivity below 1:40 in children. This is further confirmed by the fact that in our study all pediatric sera resulting anti-LKM1 positive by line-blot assay were positive also in IIF at a starting dilution of 1:40. On the other hand, both in adult and pediatric AIH-2 a certain number of anti-LC1 positive sera, without concomitant anti-LKM1 positivity, were detected only by the line-blot assay. This may be due either to higher sensitivity of line-blot or to the inability of the operator to identify the specific IIF pattern.

Thus, our results confirm the usefulness to associate an additive analytical method to IIF, both to reveal anti-LC1 when associated to anti-LKM1 autoantibodies, and to identify anti-LC1 autoantibodies when IIF results are inconclusive. Using the multiplexed line-blot assay, anti-SLA/LP autoantibodies were detected in two (5.1%) adult AIH-1 and in one (4.2%) pediatric AIH-1. In all three patients, anti-SLA/LP autoantibodies were detected in absence of conventional AIH autoantibodies and were determinant for the final diagnosis of AIH. The lower prevalence of anti-SLA/LP autoantibodies in our population compared to that (6–58%) reported in previous studies (7, 20) may be due to the characteristics of the line-blot method we used, which, however, proved to be very specific.

Finally, since SMA with F-actin specificity are commonly regarded as specific markers of AIH-1, we compared two different IIF methods for anti-F-actin detection, using VSM47 and rat intestinal epithelial cell lines, respectively, as substrate. The results showed an almost perfect agreement between the two methods. However, though the specificity of anti-F-actin autoantibodies was very high (97.3%), its sensitivity was lower than SMA, confirming the strong association between anti-F actin and AIH-1, but also that about 20–30% of SMA positive patients are anti-F-actin negative. For this reason, IIF on rat tissue section remains the first-level test in diagnosing

AIH. In the case of SMA positivity, or when interpretative doubts persist, a positive confirmation using one of the described IIF methods for anti-F actin increases the predictive value for AIH-1.

In conclusion, AIH-1 was the most common type of AIH in adults, while AIH-2 was the most common AIH subtype in the pediatric cohort. In pediatric AIH-2, anti-LC1 autoantibodies were more represented than in adult AIH-2 (59 vs. 28.6%) and in 35.9% of the cases they were present alone. The multiplexed line-blot showed a higher sensitivity than IIF on tissue rat sections for anti-LC1 detection. Using the line-blot assay we demonstrated that anti-LKM1 and anti-LC1 autoantibody levels do not differ in adults and children: this does not support the use of different starting dilutions for testing adult and pediatric populations. Finally, as an almost perfect agreement between the two IIF methods for anti-F-actin detection was found, the use of one or the other method is equally acceptable. Moreover, these data suggest that IIF methods using VSM47 or rat epithelial cell lines could represent an important step toward the standardization of anti-F-actin autoantibody detection.

ACKNOWLEDGMENTS

The authors thank Euroimmun AG and Inova Diagnostics for kindly providing the reagents for autoantibody detection free of cost, and Dr Daniela Lazzarini of Euroimmun Italy for technical assistance.

REFERENCES

1. Johnson PJ, McFarlane IG. Meeting report: International Autoimmune Hepatitis Group. *Hepatology* 1993;18:998–1005.
2. Alvarez F, Berg PA, Bianchi FB, et al. International Autoimmune Hepatitis Group Report: Review of criteria for diagnosis of autoimmune hepatitis. *J Hepatol* 1999;31:929–938.
3. Hennes EM, Zeniya M, Czaja AJ, et al. Simplified criteria for the diagnosis of autoimmune hepatitis. *Hepatology* 2008;48:169–176.
4. Manns M, Gerken G, Kriatsoulis A, Staritz M, Meyer Zum Büschenfelde KH. Characterization of a new subgroup of autoimmune chronic active hepatitis by auto-antibodies against a soluble live antigen. *Lancet* 1987;1:292–294.
5. Stechemesser E, Klein R, Berg PA. Characterization and clinical relevance of liver-pancreas antibodies in autoimmune hepatitis. *Hepatology* 1993;18:1–9.
6. Czaja AJ, Donaldson PT, Lohse AW. Antibodies to soluble live antigen/liver pancreas and HLA risk factor for type I autoimmune hepatitis. *Am J Gastroenterol* 2002;97:413–419.
7. Vitozzi S, Djilali-Saiah I, Lapiere P, Alvarez F. Anti-soluble liver antigen/liver-pancreas (SLA/LP) antibodies in pediatric patients with autoimmune hepatitis. *Autoimmunity* 2002;35:485–492.
8. Meda F, Zuin M, Invernizzi P, Selmi C. Serum autoantibodies: A road map for the clinical hepatologist. *Autoimmunity* 2008;41:27–34.
9. Obermayer-Straub P, Strassburg CP, Manns MP. Autoimmune hepatitis. *J Hepatol* 2000;32:181–197.

10. Bogdanos DP, Invernizzi P, Mackay IR, Vergani D. Autoimmune liver serology: Current diagnostic and clinical challenge. *World J Gastroenterol* 2008;14:3374–3387.
11. Granito A, Muratori L, Muratori P, et al. Antibodies to filamentous actin (F-actin) in type 1 autoimmune hepatitis. *J Clin Pathol* 2006;59:280–284.
12. Villalta D, Bizzaro N, Da Re M, Tozzoli R, Komorowski L, Tonutti E. Diagnostic accuracy of four different immunological methods for the detection of anti-F-actin auto-antibodies in type 1 autoimmune hepatitis and other liver-related disorders. *Autoimmunity* 2008;41:105–110.
13. Toh BH, Taylor R, Pollok W, et al. Actin-reactive discriminated from non-actin-reactive smooth muscle auto-antibody by immunofluorescence reactivity with rat epithelial cell line. *Pathology* 2010;42:463–469.
14. Floreani A, Liberal R, Vergani D, Mieli-Vergani G. Autoimmune hepatitis: Contrast and comparison in children and adults—A comprehensive review. *J Autoimmun* 2013;46:7–16.
15. Mieli-Vergani G, Vergani D. Autoimmune hepatitis in children: What is different from adult AIH? *Semin Liv Dis* 2009;29:297–306.
16. Liberal R, Grant CR, Longhi MS, Mieli-Vergani G, Vergani D. Diagnostic criteria of autoimmune hepatitis. *Autoimmunity Rev* 2014;13:435–440.
17. Vergani D, Alvarez F, Bianchi FB, et al. Liver autoimmune serology. A consensus statement from the committee for autoimmune serology of the international Autoimmune Hepatitis Group. *J Hepatol* 2004;41:677–683.
18. Roberts EA. Autoimmune hepatitis from the pediatric perspective. *Liver Int* 2011;31:1424–1431.
19. Gregorio GV, Portmann B, Reid F, et al. Autoimmune hepatitis in childhood: A 20-year experience. *Hepatology* 1997;25:541–547.
20. Johanet C, Ballot E. Auto-antibodies in autoimmune hepatitis: Anti-soluble liver antigen (SLA). *Clin Res Hepatol Gastroenterol* 2012;36:244–246.