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Prevalence and Significance of Autoantibodies in Children with Acute Liver Failure

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Abstract

Objectives—The purpose of this study is to estimate autoantibody (autoAB) frequency, clinical characteristics and 21 day outcome of participants in the Pediatric Acute Liver Failure Study Group (PALFSG) by ANA, SMA and LKM antibody status.

Methods—AutoAB were determined at local and/or central laboratories. Subjects were assigned to autoimmune hepatitis (AIH), indeterminate and other diagnoses groups.

Results—Between 1999 and 2010, 986 subjects were enrolled in the PALFSG. At least one autoAB result was available for 722 (73.2%). At least one autoAB was positive for 202 (28.0%). Diagnoses for autoAB+ subjects were: AIH (63), indeterminate (75) and other (64). AutoAB were more common in Wilson disease (12/32, 37.5%) vs other known diagnoses (52/253, 20.6%, $p=0.03$). LKM+ subjects were younger (median 2.4 vs 9.1 yrs, $p<0.001$) and more likely to undergo liver transplantation (53.3% vs 31.4% $p=0.02$) than other autoAB+/LKM- subjects. Steroid treatment of subjects who were autoAB+ was not significantly associated with survival and the sub group with known diagnoses other than AIH had a higher risk of death.

Conclusions—AutoABs are common in children with ALF, occurring in 28%. AutoAB+ subjects have similar outcomes to autoAB negative subjects. LKM+ children are younger and more likely to undergo liver transplantation compared to other autoAB+ subjects. Although AutoAB may indicate a treatable condition, positivity does not eliminate the need for a complete diagnostic evaluation as autoABs are present in other conditions. The significance of autoAB in PALF remains uncertain, but LKM+ appears to identify a unique population of children who merit further study.

Keywords

pediatric liver transplant; autoantibodies; pediatric liver failure

Introduction

Acute liver failure (ALF) in children is a rare disease with multiple causes (1). Autoimmune hepatitis (AIH) is a cause of ALF, yet consensus in establishing diagnostic criteria for this condition in the presence of severe coagulopathy and encephalopathy has not been achieved(2-4). A classic combination of elevated IgG, characteristic autoantibodies (autoAB) [anti-smooth muscle antibody (SMA) or anti liver kidney microsomal antibody (LKM)], and a liver biopsy showing a plasmacytic infiltrate with either centrilobular or interface hepatitis leading to a consensus diagnosis of autoimmune hepatitis (AIH) and

initiation of steroid therapy is infrequent in ALF(2,5). In adults with ALF, the presence of autoAB appears to be transient in many who have no other features characteristic of AIH (3,6,7). The significance of autoAB in the setting of ALF is unknown, and autoAB are often thought to be a non-specific response to antigens and not involved in the pathogenesis of liver injury (2).

AutoAB are present in settings other than AIH. Antinuclear antibody (ANA) is present in 3-12% of healthy children although SMA and LKM are rarely found (8,9). AutoAB are present in well characterized chronic liver diseases in children such as chronic viral hepatitis (ANA 8-23%, SMA 1-18%, LKM 1-4%) (10,11) and primary sclerosing cholangitis (ANA 43%, SMA 28%) (12) and in adults with non-alcoholic fatty liver disease (ANA 21%, SMA 5%) (13). AutoAB can also be present in acute or chronic presentations of Wilson disease (14). These autoAB are generally considered to represent epiphenomena and treatment is directed at the primary diagnosis rather than immunosuppression for AIH. It has been assumed that autoAB in children with ALF may arise similarly from non-specific liver injury. However, some children with ALF and positive autoABs have an acute presentation of AIH and respond to steroid therapy. To date, the frequency and significance of autoAB presence in PALF have not been investigated.

The Pediatric Acute Liver Failure Study Group (PALFSG) is a NIH funded international consortium of pediatric centers that has been collecting data on children with ALF since 1999. Previous data from our group has shown that testing for autoAB is variable (15). The goals of this study are to describe the prevalence, associated clinical features and outcomes of the finding of positive autoAB in children in PALF using locally available tests, supplemented by testing using a standardized central laboratory.

Methods

PALF Study Group

Enrollment in the PALF study cohort began in December 1999. The PALF study protocol has been previously described (1). IRB approval was secured at each of the clinical sites. Briefly, following informed consent, demographic, daily clinical, and laboratory information was recorded on case report forms for up to 7 days following enrollment and outcome was assessed up to 21 days or until death, liver transplantation, or hospital discharge without liver transplantation (survival with native liver) if occurring 21 days or less after enrollment (1). Diagnostic evaluation and medical management strategies were consistent with the standard of care at each site and were not determined by the PALF protocol. Completed data forms were forwarded to the Data Coordinating Center. Patients from birth through 17 years of age were eligible for enrollment if they met the following criteria: (1) no known evidence of chronic liver disease, (2) biochemical evidence of acute liver injury, and (3) hepatic-based coagulopathy defined as PT $\geq$ 15 seconds or INR $\geq$ 1.5 not corrected by vitamin K in the presence of hepatic encephalopathy or PT $\geq$ 20 seconds or INR $\geq$ 2.0 regardless of the presence or absence of clinical hepatic encephalopathy. Following enrollment, a single serum sample was to be collected daily for up to seven days, or until death, liver transplantation, or discharge from hospital. The serum sample was divided into aliquots, frozen at -80°C at the enrollment site and batch-shipped to the National Institute of Diabetes

and Digestive and Kidney (NIDDK) Disease bio-repository for long-term storage. The frequency and volume of serum that could be collected for research purposes was dependent upon participant weight, hemoglobin, and the daily volume of blood required for diagnosis and patient management. Given these safety restrictions, research samples were not available at all potential time points for all participants. The underlying etiology of liver dysfunction, based on standard laboratory tests obtained clinically at each center and investigator judgment, included indeterminate, acetaminophen (APAP) toxicity, AIH, infectious, non-APAP drug induced liver disease, metabolic liver disease, shock, and other (e.g. Budd-Chiari syndrome, hemophagocytic syndrome, leukemia, gestational alloimmune liver disease, and veno-occlusive disease) based on agreed upon criteria in the PALF study.

Autoantibody determination

AutoAB was measured at the discretion of individual centers at their laboratory of choice. For these analyses, laboratory-specific cutoffs were used to consider a value positive. In addition, supplemental funding was utilized to obtain more complete data for PALF participants enrolled between January 1, 2000 and November 23, 2007. This testing was not available for other participants. Samples were batch shipped to a central laboratory to perform the assays. All three markers were to be measured on all participants with available samples. However, if the sample had insufficient volume to measure all markers, ANA was the highest priority, followed by SMA then LKM. Central autoimmune marker testing occurred for PALF participants with a sufficient sample, regardless of autoimmune marker results from local laboratories. The central lab used an enzyme-linked immunosorbent assay to measure LKM (INOVA Diagnostics, San Diego, Revision 1, March 2000) and ANA (Bio-Rad Laboratories, Hercules CA) while SMA was measured using indirect immunofluorescence. Titers were not available for many of the subjects, thus AutoAB were recorded as either positive or negative based on local or central lab cutoffs. For this report, if any of the tested autoantibodies was positive, the participant was considered to be autoAB+. If all of the tested autoantibodies (which ranged from 1 to 3 for each participant) were negative, the participant was considered to be autoAB-. Each autoAB+ and autoAB- subject was classified into one of 3 diagnostic groups: autoAB+ AIH (based on the investigator final diagnosis, which did not utilize the international AIH score as it was not validated for PALF and there were limited data on IgG levels), autoAB+ or autoAB- with no identified diagnosis (Indeterminate) or autoAB+ or autoAB- with a specific diagnosis other than AIH (Other). If there were discrepant results between the local laboratory and the central laboratory, the autoAB was considered to be positive if any result was positive. We defined steroid treatment as prednisolone or prednisone therapy and excluded hydrocortisone for stress dosing. Prior to 2006, steroid treatment was not collected in this study.

Statistics

Pearson chi-square tests or exact chi-square tests were used to test for differences in the proportions of participants in each category of demographics, encephalopathy, steroid use and outcome among three final diagnosis groups and among the groups with different types of autoAB+. The Wilcoxon rank-sum test or Kruskal-Wallis test was used to test for differences in the distributions of continuous age and laboratory values by the final diagnosis groups and among the groups with different types of autoAB+. These same statistical tests

were used when subsets were tested. A multinomial logistic regression model was used to estimate the association of type of autoAB+ and of final diagnosis with outcome, adjusting for age. The p-values for post-hoc analysis of differences between final diagnosis groups for each variable were adjusted using the Holm-Bonferroni method (16). $P < 0.05$ determined statistical significance. All authors had access to the study data and reviewed and approved the final manuscript.

Results

Of the 986 subjects enrolled in PALF as of December 2010, 722 (73.2%) had results from at least one autoAB test (ANA, SMA, LKM) available: 68 with AIH, 369 with indeterminate ALF and 285 with other diagnoses. Across diagnoses, 28.0% (202/722) of participants were autoAB+. The percentage of participants who were autoAB+ by diagnostic group (Table 1) was: 92.6% in AIH (5 autoAB- excluded from further analysis), 20.3% in indeterminate and 22.5% for participants with a known diagnosis other than AIH (19 with metabolic [12 Wilson disease, 7 other], 12 viral infections, 9 acetaminophen and 24 other diagnoses). For the 285 participants with other known diagnoses, the percentage with autoAB present was higher in Wilson disease (12/32, 37.5%) than those with non-Wilson disease (52/253, 20.6%, $p = 0.03$).

AutoAB+ children were older, slightly more likely to be female ($p = 0.047$) and Hispanic ($p = 0.01$) compared to those who were tested and autoAB negative (Table 1). Children who were autoAB+ with indeterminate ALF were younger than children with AutoAB+ AIH ($p = 0.03$) or other diagnoses ($p = 0.03$, Table 1). Children with autoAB+ AIH underwent liver biopsy more frequently than children with either Indeterminate ($p = 0.02$) or other diagnoses ($p < 0.0001$) with positive autoAB (Table 1). The autoAB patterns by group are shown in Table 2. The patterns varied among the three diagnostic groups. Subjects with AIH were more likely to have at least two positive autoAB with 31.8% with 2 autoAB for AIH vs 14.7% for indeterminate ($p = 0.03$) and 12.5% for other diagnoses ($p = 0.03$).

We investigated autoAB concordance between the central laboratory and the local laboratories. Of the 722 subjects, 410 had autoAB results available only from local testing, 100 subjects had results available only from testing at the central laboratory and 212 subjects had results available from both local and central laboratory testing. From subjects with testing at both local and central laboratories, 53 were autoAB+ by local testing, with only 20 (37.7%) of these 53 subjects autoAB+ by central testing. Conversely, 159 were autoAB- by local testing and 139 (87.4%) of these were autoAB- by central testing, leaving 20 subjects who were autoAB- by local testing who were autoAB+ by central testing.

Clinical and laboratory features are shown in Table 3. Compared to autoAB+ participants with indeterminate or other specified diagnosis, a larger percentage of subjects with AIH tended to have no or mild encephalopathy ($p = 0.003$ compared to indeterminate and 0.007 to other diagnoses) and higher likelihood of treatment with steroids ($p < 0.0001$ compared to indeterminate or other diagnoses). A higher percentage of autoAB+ participants with indeterminate diagnosis had ICU stays in the first 7 days following enrollment than autoAB+ participants with AIH ($p < 0.001$) or other specified diagnosis ($p = 0.04$).

Among autoAB+ positive participants diagnosed with AIH, a greater percentage of participants survived with native liver at least 21 days (73.0%) and smaller percentage died (1.6%) or underwent liver transplantation (25.4%) compared to those who were autoAB+ with an indeterminate (42.7%, 9.3%, 48.0%, $p=0.002$) diagnosis (all outcomes across all diagnostic groups $p=0.002$, Table 3). There was not a significant difference in outcome by autoAB status in those with indeterminate or other diagnosis (Table 3).

LKM positivity is associated with type 2 AIH and we sought to determine if there were differences in those participants who were LKM+ (with or without ANA and or SMA positivity). LKM+ did not differ significantly with respect to sex, race, ALT, or PT at presentation compared to AutoAB+/non LKM+ participants (Table 4). LKM+ subjects had a similar rate of undergoing a liver biopsy prior to liver transplant (8/16, 50%) compared to SMA+ and/or ANA+ without LKM+ (32/54 59%) subjects. The subjects with LKM+ tended to be younger ($p<0.0001$). Liver transplantation was less likely in the subjects with SMA+ and/or ANA+ without LKM+ than the subjects with LKM+ (age adjusted odds ratio=0.39, p -value=0.03).

Table 5 shows 21-day outcome of the first event to occur, among death, liver transplantation and survival with native liver, between autoAB+ participants who were treated with steroids and those who were not. Steroid treatment data were only available for 94 (47%) of the autoAB+ subjects. Data regarding dose was even more limited, so it was not examined. Steroid treatment was not significantly associated with outcome in autoAB+ subjects with steroid treatment data recorded ($p = 0.19$) with 57% survival with native liver in steroid-treated vs. 53% in untreated. For the 33 (of 63, 52%) with AIH and the 29 (of 75, 39%) with indeterminate and reliable steroid data, outcomes were not significantly different between those with or without steroid treatment, but with only 4/33 not treated, the ability to detect even moderate differences is low. In the 32 of 64 (50%) with other established diagnoses and steroid data, 21 day outcomes were distributed differently between the participants who received steroids and those who did not, with more deaths and less spontaneous survival in those receiving steroids ($p=0.046$). 21-day outcomes did not differ significantly ($p=0.13$) by autoAB type (Table 6), but only 2/10 (20%) of LKM+ participants who received steroids vs. 4/5 (80%) who did not receive steroids underwent transplantation.

Discussion

This is the first pediatric study to report the frequency of autoAB in a large cohort of children with ALF. Our aims were to describe features of autoAB+ and autoAB- cohorts and reveal challenges of interpreting results of autoAB testing when performed by a local or central laboratory. While it is commonly assumed that autoABs can occur in the setting of acute liver failure, we found only 28% of PALF participants had at least one autoAB present among those who had testing performed either by local or central laboratory. This is lower than the 62% frequency of autoAB among adults with indeterminate ALF (3), but similar to the 32% frequency of autoAB reported in another study of adults with ALF (6). As expected, autoAB were more common in the children with a final diagnosis of AIH (93%) than children with other causes of ALF. More of the children with AIH (64%) underwent a liver biopsy as part of their evaluation than did those with an indeterminate diagnosis (43%).

We do not have data to explain this discrepancy. Liver biopsy histology may lead to the consideration of AIH, however, the role of liver biopsy in children with ALF who are also autoAB+ is not clear from our data. Diagnostic criteria for adults with AIH presenting with ALF or acute severe hepatitis are evolving (2-4), but similar criteria have not been validated in children.

Among participants with autoAB testing at local and central laboratories, we found the concordance of autoAB results between the two testing facilities to be weak. Many factors likely contribute to this, including samples taken at different times, disease progression and assay differences. While the interlaboratory variation in autoAB methods and reporting is a weakness in this study, our goal in this study was not to compare autoAB assays, so we included any evidence of autoAB+ in the definition. Therefore, in the absence of a “gold standard” methodology being available at all sites, interpretation of autoAB results remains with the clinician at the bedside.

An important question is whether the presence of one or more autoAB is associated with outcomes. In that regard, we found that having autoABs was not associated with a different 21-day outcome compared to subjects without autoAB. However, PALF autoAB+ subjects with AIH had a greater likelihood of surviving with their native liver than non-AIH autoAB+ patients (73% vs. 51% $p=0.004$). Also, those autoAB+ with AIH had a greater spontaneous survival rate than autoAB- subjects with indeterminate or other diagnoses (73% vs. 54% $p=0.005$). This may be related to differences in medical management or to a less aggressive course for AIH-associated PALF. Steroid treatment is generally the initial treatment for AIH. Due to study design, steroid treatment data was limited to the first seven days of the study and was only available on 94 autoAB+ subjects. For the 33 subjects diagnosed with AIH for whom steroid treatment information was available, 88% received steroids in the first week whereas only 34% of indeterminate autoAB positive and 25% of other diagnoses who were autoAB positive received steroids in the first week following enrollment. Although there was not a significant difference in outcome between those autoAB+ subjects who received steroid treatment during the first seven days and those who did not, steroid treatment information was only available for 47% of those who were autoAB positive which may preclude an accurate assessment of the effect of steroids in autoAB+ PALF.

The LKM+ subjects with PALF were younger and more likely to be diagnosed with AIH (57%) compared with SMA+ or ANA+ without LKM+ (27%) subjects. While the data are limited by small numbers, it is interesting that none of the 15 LKM positive participants whose steroid treatment status was known died, regardless of whether they had steroid treatment ($n=10$) or not ($n=5$). However, 4 out of the 5 who did not have steroid treatment and only 2 of 10 who received steroids underwent transplantation. We suggest that the issue of steroid treatment in LKM+ subjects with PALF deserves further study in a larger cohort.

Overall, steroid treatment was not significantly associated with improved 21 day survival with native liver in the autoAB+ group as a whole compared to those who did not receive steroids. Of concern, there was a trend towards more deaths in autoAB+ subjects with other diagnoses who received steroids compared to those who did not, suggesting that in some causes of PALF, steroids may be detrimental or that steroids are more commonly used in

children with more severe illness outside of AIH. In the adult ALF study the association of steroid treatment on spontaneous survival in AIH, indeterminate and drug induced ALF was investigated, independent of autoAB status. Steroid treatment also did not improve spontaneous or overall survival with a trend towards increased mortality in the patients with high MELD who received steroids(17). Although steroid treatment did not impact 21 day outcomes, it is important to note that outcomes after 21 days were not assessed in this study. At this point, steroid use cannot be routinely recommended outside of treatment for AIH associated ALF in children, except in the context of a clinical trial that evaluates both short and longer term outcomes.

This study is the largest series to investigate autoAB in children with ALF. Most of the autoAB determinations were performed at local labs. Thus there are likely a variety of methods and cutoffs for positive values that were used. In addition, there was variability in the timing of the autoAB determination.

Despite these issues, we have shown that autoAB are common in children with ALF. Only 1/30 (3.3%) children who were LKM+ regardless of ANA or SMA status died, whereas 14/172 (8.1%) of those who were either ANA+ or SMA+, but LKM- or LKM unknown died. We propose that the role of autoAB in the pathophysiology of ALF in children and their association with specific outcomes should be the subject of further investigation.

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References

1. Squires RH Jr, Shneider BL, Bucuvalas J, et al. Acute liver failure in children: the first 348 patients in the pediatric acute liver failure study group. *J Pediatr*. 2006; 148:652–658. [PubMed: 16737880]
2. Czaja AJ. Acute and acute severe (fulminant) autoimmune hepatitis. *Dig Dis Sci*. 2013; 58:897–914. [PubMed: 23090425]
3. Stravitz RT, Lefkowitz JH, Fontana RJ, et al. Autoimmune acute liver failure: proposed clinical and histological criteria. *Hepatology*. 2011; 53:517–526. [PubMed: 21274872]
4. Yeoman AD, Westbrook RH, Al-Chalabi T, et al. Diagnostic value and utility of the simplified International Autoimmune Hepatitis Group (IAIHG) criteria in acute and chronic liver disease. *Hepatology*. 2009; 50:538–545. [PubMed: 19575457]
5. Di Giorgio A, Bravi M, Bonanomi E, et al. Fulminant hepatic failure of autoimmune aetiology in children. *J Pediatr Gastroenterol Nutr*. 2015; 60:159–64. [PubMed: 25304891]
6. Bernal W, Ma Y, Smith HM, et al. The significance of autoantibodies and immunoglobulins in acute liver failure: a cohort study. *J Hepatol*. 2007; 47:664–670. [PubMed: 17602781]
7. Leung PS, Rossaro L, Davis PA, et al. Antimitochondrial antibodies in acute liver failure: implications for primary biliary cirrhosis. *Hepatology*. 2007; 46:1436–1442. [PubMed: 17657817]
8. Hilario MO, Len CA, Roja SC, et al. Frequency of antinuclear antibodies in healthy children and adolescents. *Clin Pediatr (Phila)*. 2004; 43:637–642. [PubMed: 15378151]
9. Martini A, Lorini R, Zanaboni D, et al. Frequency of autoantibodies in normal children. *Am J Dis Child*. 1989; 143:493–496. [PubMed: 2784621]
10. Molleston JP, Mellman W, Narkewicz MR, et al. Autoantibodies and autoimmune disease during treatment of children with chronic hepatitis C. *J Pediatr Gastroenterol Nutr*. 2013; 56:304–310. [PubMed: 23439301]
11. Pop TL, Miu N, Burac L, et al. Influence of autoantibodies in chronic viral hepatitis in children. *J Pediatr Gastroenterol Nutr*. 2006; 42:e65.
12. Feldstein AE, Perrault J, El-Youssif M, et al. Primary sclerosing cholangitis in children: a long-term follow-up study. *Hepatology*. 2003; 38:210–217. [PubMed: 12830004]
13. Loria P, Lonardo A, Leonardi F, et al. Non-organ-specific autoantibodies in nonalcoholic fatty liver disease: prevalence and correlates. *Dig Dis Sci*. 2003; 48:2173–2181. [PubMed: 14705824]
14. Weiss, KH. Wilson Disease. In: Pagon, RA, Adam, MP, Ardinger, HH., editors. *GeneReviews(R)*. Seattle (WA): 1993.
15. Narkewicz MR, Dell'Olio D, Karpen SJ, et al. Pattern of diagnostic evaluation for the causes of pediatric acute liver failure: an opportunity for quality improvement. *J Pediatr*. 2009; 155:801–806 e1. [PubMed: 19643443]
16. Holm S. A Simple Sequentially Rejective Multiple Test Procedure. *Scand J Statist*. 1979; 6:65–70.
17. Karkhanis J, Verna EC, Chang MS, et al. Steroid use in acute liver failure. *Hepatology*. 2014; 59:612–621. [PubMed: 23929808]

What is Known

- Autoantibodies occur in pediatric acute liver failure
- Autoantibodies may indicate autoimmune hepatitis

What is New

- Autoantibodies detected in 28% of children with acute liver failure and 94% of those with autoimmune hepatitis
- Autoantibodies are not significantly associated with 21 day outcomes
- While steroid treatment is not associated with survival in autoAB positive patients overall, the sub-group of patients with known diagnoses other than AIH had a higher risk of death.

Table 1
Demographics and liver biopsy status by autoAB status and diagnostic group (n=717*)

	AIH (n=63)		Indeterminate (n=369)				Other (n=285)			p value §
	AutoAB +	AutoAB -	AutoAB +	AutoAB -	p value &		AutoAB +	AutoAB -	p value #	
n	63	294	75	294			64	221		
Age (years)					0.41 ^c				0.29 ^c	0.01 ^a
Median	9.9	5.6		4.5			9.6	7.8		
25%, 75%	3.3, 13.9	2.0, 10.3		1.5, 11.1			4.6, 14.6	1.2, 14.8		
Min, Max	0.6, 17.5	0.1, 17.4		0.0, 17.9			0.0, 17.9	0.0, 17.9		
Sex					0.046 ^b				0.49 ^b	0.83 ^b
Male	30 (47.6)	34 (45.3)		171 (58.4)			27 (42.2)	104 (47.1)		
Female	33 (52.4)	41 (54.7)		123 (41.8)			37 (57.8)	117 (52.9)		
Race					0.97 ^b				0.12 ^b	0.65 ^b
Unknown	1	2		3			2	4		
White	45 (72.6)	53 (72.6)		212 (72.9)			41 (66.1)	165 (76.0)		
Other	17 (27.4)	20 (27.4)		79 (17.1)			21 (33.9)	52 (24.0)		
Hispanic					0.09 ^b				0.53 ^b	0.27 ^b
No	45 (71.4)	55 (73.3)		241 (82.0)			53 (82.8)	190 (86.0)		
Yes	18 (28.6)	20 (26.7)		53 (18.0)			11 (17.2)	31 (14.0)		
Liver Biopsy					0.58 ^d				0.59 ^d	<0.0001 ^d
Unknown	2	3		18			3	9		
Not Done	19 (31.1)	36 (50.0)		147 (53.3)			44 (72.1)	158 (74.5)		
Prior to LTx	39 (63.9)	31 (43.1)		109 (39.5)			14 (23.0)	42 (19.8)		
Explant	3 (4.9)	5 (6.9)		20 (7.3)			3 (5.0)	12 (5.7)		

* 5 AIH participants with autoAB- were excluded, LTx=liver transplant

& p-value was from the comparison between the groups of “autoAB+” and “autoAB-” within the “Indeterminate” group

p-value was from the comparison between the groups of “autoAB+” and “autoAB-” within the “Other” diagnosis group.

§ p-value was from the comparison among the participants with autoAB positive by AIH, indeterminate and other diagnosis groups.

^d by Pearson chi-square test, compare biopsy prior to LTX to Not Done groups

^c by Wilcoxon Rank-Sum test

^f by Pearson chi-square test,

^g by Kruskal-Wallis test,

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Table 2
Antibody positivity patterns by diagnostic group

AB(s) positive	AIH (n=63)	Indeterminate (n=75)	Other (n=64)	<i>p</i> value
	n (%)	n (%)	n (%)	0.004 ^a
ANA Alone	18(28.6)	28 (37.3)	23 (35.9)	
SMA Alone	13 (20.6)	28 (37.3)	31(48.4)	
LKM Alone	12 (19.1)	8 (10.7)	2 (3.1)	
ANA & SMA	15 (23.8)	8(10.7)	8 (12.5)	
ANA & LKM	3 (4.8)	2(2.7)	0 (0.0)	
SMA & LKM	2(3.2)	1(1.3)	0 (0.0)	

^aby Exact Pearson chi-square test

Table 3
Significant differences in clinical presentation and outcome by autoAB status and diagnostic group (n=717*)

	AIH	Indeterminate			Other			p-value \$
		AutoAB + n=63	AutoAB + n=75	AutoAB – n=294	p-value &	AutoAB + n=64	AutoAB – n=221	
		n (%)	n (%)	n (%)		n (%)	n (%)	
Encephalopathy					1.0 ^b			
Not done or not assessable	0		4	12		1	11	0.002 ^c
0	37 (58.7)	30 (42.3)	125 (44.3)			39 (61.9)	105 (50.0)	
I	22 (34.9)	21 (29.6)	79 (28.0)			8 (12.7)	54 (25.7)	
II	3 (4.8)	11 (15.5)	43 (15.2)			4 (6.4)	19 (9.1)	
III	1 (1.6)	6 (8.5)	23 (8.2)			9 (14.3)	19 (9.1)	
IV	0 (0.0)	3 (4.2)	12 (4.3)			3 (4.8)	13 (6.2)	
Total bilirubin (mg/dL)					0.35 ^d			0.004 ^a
n	57	73	276			60	205	
Median	12.4	14.5	14.1			7.6	4.6	
ALT (IU/L)					0.55 ^d			0.02 ^a
n	56	69	260			58	192	
Median	1158	1817	1895			1063.5	1614	
Steroids in the 1st 7 days					0.24 ^b			0.91 ^b
Missing	30	46	188			32	148	
Yes ^f	29 (87.9)	10 (34.5)	25 (23.6)			8 (25.0)	19 (26.0)	
ICU stay in the 1st 7 days					0.92 ^b			0.0006 ^b
Yes	25 (39.7)	54 (72.0)	210 (71.4)			34 (53.1)	162 (73.3)	
21-day outcome					0.80 ^b			0.002 ^c
Withdrawn	0	0	1			0	0	
Death	1 (1.6)	7 (9.3)	21 (7.2)			7 (10.9)	33 (14.9)	
Transplantation	16 (25.4)	36 (48.0)	140 (47.8)			18 (28.1)	41 (18.6)	
Survival with native liver	46 (73.0)	32 (42.7)	132 (45.1)			39 (60.9)	147 (66.5)	

*5 AIH participants with autoAB- were excluded

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percent is for number who received therapeutic steroids (prednisone or prednisolone) out of total with available data
p-value was from the comparison between the groups of "autoAB+", and "autoAB-", within the "Indeterminate" group
p-value was from the comparison between the groups of "autoAB+" and "autoAB-" within the "Other" diagnosis group.
p-value was from the comparison among the participants with autoAB positive by AIH, indeterminate and other diagnosis groups.
by Kruskal-Wallis test,
by Pearson chi square test,
by exact chi-square test,
by Wilcoxon Rank-Sum test

Table 4
Demographics, clinical characteristics and outcome between the participants with LKM+ and the ones with ANA+ and/or SMA+ excluding LKM+

	LKM+ (n=30)	ANA+ and /or SMA+ excluding LKM+ (n=172)	p-value
	n (%)	n (%)	
Age (years)			0.0001 ^b
Median (25%, 75%) Min, Max	2.4 (1.2, 7.1) 0.5, 15.4	9.1 (3.6, 14.1) 0.0, 17.9	
Sex			0.55 ^a
Male	12 (40.0)	79 (45.9)	
Female	18 (60.0)	93 (54.1)	
Race			0.50 ^a
Unknown	1	4	
White	22 (75.9)	117 (69.6)	
Other	7 (24.1)	51 (30.4)	
Hispanic			0.43 ^a
No	21(70.0)	132 (76.7)	
Yes	9 (30.0)	40 (23.3)	
Coma Grade at Entry			0.79 ^d
Unknown	0	1	
Not assessable	0	4	
Grade 0	14 (46.7)	92 (55.1)	
Grade 1	10 (33.3)	41 (24.6)	
Grade 2	3 (10.0)	15 (9.0)	
Grade 3	2 (6.7)	14 (8.4)	
Grade 4	1 (3.3)	5 (3.0)	
Maximum Coma Grade			0.25 ^d
Unknown	0	4	
Not assessable	0	3	
Grade 0	8 (26.7)	71 (43.0)	
Grade 1	7 (23.3)	35 (21.2)	
Grade 2	6 (20.0)	20 (12.1)	
Grade 3	7 (23.3)	22 (13.3)	
Grade 4	2 (6.7)	17 (10.3)	
ALT at presentation (IU/L)			0.35 ^b
N	29	154	
Median (25%, 75%) Min, Max	1167 (597, 1770) 44, 4964	1493.5 (464, 2735) 12, 19918	
PT at presentation (sec)			0.56 ^b
N	26	146	
Median (25%, 75%) Min, Max	25.7 (22.8, 32.0) 16.2, 86.8	25.2 (21.6, 31.0) 12.8, 141.3	
21-day outcome			0.053 ^c

	LKM+ (n=30)	ANA+ and /or SMA+ excluding LKM+ (n=172)	p-value
	n (%)	n (%)	
Death	1 (3.3)	14 (8.1)	
Transplantation	16 (53.3)	54 (31.4)	
Survival	13 (43.3)	104 (60.5)	

^aby Chi-square test

^bby Wilcoxon rank-sum test

^cExact Chi-square test

^dExact Cochran-Armitage trend test

Table 5
Steroid treatment response as determined by 21 day outcomes by diagnosis

Diagnoses	Any Diagnosis AB+			AIH			Indeterminate AB+			Other Diagnosis AB+		
	Steroids (n= 47)	No steroids (n=47)	Unknown Steroids (n =108)	Steroids (n= 29)	No steroids (n=4)	Unknown Steroids (n = 30)	Steroids (n= 10)	No steroids (n=19)	Unknown Steroids (n = 46)	Steroids (n=8)	No steroids (n=24)	Unknown Steroids (n =32)
Outcomes												
Death	6(13%)	2(4%)	7 (6%)	0(0%)	0(0%)	1 (3%)	3(30%)	1 (5%)	3(7%)	3(38%)	1(4%)	3(9%)
Transplantation	14(30%)	20(43%)	36 (33%)	6(21%)	1 (25%)	9 (30%)	5(50%)	11(58%)	20(43%)	3(38%)	8(33%)	7(22%)
Survival	27(57%)	25(53%)	65(60%)	23(79%)	3(75%)	20 (67%)	2 (20%)	7(37%)	23(50%)	2 (25%)	15(63%)	22(69%)

Table 6
Steroid treatment response as determined by 21 day outcomes by type of autoantibody

Type of AB+	Steroids	No steroids	Unknown steroids
LKM	n = 10	n = 5	n=15
Death	0(0%)	0(0%)	1(7%)
Transplantation	2(20%)	4(80%)	10(67%)
Survival	8(80%)	1(20%)	4(27%)
SMA	n = 22	n= 24	n=60
Death	4(18%)	2(8%)	4(7%)
Transplantation	9(41%)	9(38%)	13(22%)
Survival	9(41%)	13 (54%)	43(72%)
ANA	n=28	n= 24	n=53
Death	2(7%)	1(4%)	4(8%)
Transplantation	9(32%)	8(33%)	16(30%)
Survival	17(61%)	15(63%)	33(62%)

No AB+	Steroids	No steroids	Unknown steroids
	n=45	n= 133	n=336
Death	2(4%)	11(8%)	41(12%)
Transplantation	20(44%)	45(34%)	116 (35%)
Survival	23(51%)	77(58%)	179(53%)