META-ANALYSIS

Incidence of *de novo* autoimmune hepatitis in children and adolescents with increased autoantibodies after liver transplantation: a meta-analysis

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SUMMARY

The objective of this meta-analysis was to assess the incidence of *de novo* autoimmune hepatitis (AIH) in children and adolescents with increased autoantibodies after liver transplantation. We systematically retrieved studies from PubMed, Embase, Central, CNKI, VIP and Wanfang published before February 1, 2020. All analyses were conducted using R-4.0.1 statistical package (Meta). Seven studies with high quality were pooled in our final analysis (N = 251 participants). The incidence of *de novo* AIH was 9% [95% confidence interval (CI) 1–23%, I^2 = 86%]. Subgroup analysis suggested that publications not using the International Autoimmune Hepatitis Group (IAIHG) criteria have marginally significantly higher incidence of *de novo* AIH than those using IAIHG criteria (P for interaction = 0.08). The incidence of chronic rejection was 8% (95% CI 2–17%, I^2 = 72%). Meta-regression indicated significant correlation (P = 0.04; estimate: 1.51) between the incidence of *de novo* AIH and the rate of increase of antibodies to liver/kidney microsome (anti-LKM). It is still challenging to distinguish *de novo* AIH and chronic rejection in children and adolescents with increased autoantibodies after liver transplantation. The diagnostic criteria for *de novo* AIH in children and adolescents and the role of anti-LKM in the development of *de novo* AIH deserve future investigation.

Transplant International 2021; 34: 412–422

Key words
adolescent, autoantibodies, autoimmune, child, hepatitis, liver transplantation, meta-analysis

Received: 10 September 2020; Revision requested: 21 October 2020; Accepted: 9 December 2020

Introduction

Autoimmune hepatitis (AIH) is a chronic progressive inflammatory liver disease that is characterized by the presence of hypergammaglobulinemia and autoantibodies (for example, anti-smooth muscle antibodies (SMA) or anti-nuclear antibodies (ANA)) in serum, and typical histological features. *de novo* AIH is a disease that has similar characteristics to AIH that occurs after liver transplantation for causes other than autoimmune liver disease [1]. *de novo* AIH is not the same in children and adolescents as in adults in some aspects. The incidence of *de novo* AIH is 5–10% in children and adolescents compared with 1–2% in adults. While common causes for liver transplantation in children and adolescents involve biliary atresia, metabolic liver disease and acute liver failure, alcoholic liver disease and non-alcoholic steatohepatitis-cirrhosis are the main indications

doi:10.1111/tri.13801

for liver transplantation in adults. The pathogenesis and therapy are also different between children and adolescents and adults with *de novo* AIH [2]. *de novo* AIH may lead to poor outcomes such as graft failure, retransplantation and even death, which largely affect patients' prognosis [3]. The International Autoimmune Hepatitis Group criteria, which were established and updated in 1993 and 1999 [4], are now applied to diagnose *de novo* AIH. However, the IAIHG report states explicitly that the criteria are only applicable to the diagnosis of AIH but not to other types of AIH like recurrent AIH after liver transplantation. It remains to be verified if the IAIHG criteria are applicable to the diagnosis of *de novo* AIH.

The increase in autoantibodies (e.g., ANA and anti-smooth muscle antibody; SMA), which is common in *de novo* AIH, is an important diagnostic indicator. However, the increase in autoantibody titer alone is not sufficient to diagnose *de novo* AIH. In other words, definitive diagnosis of *de novo* AIH is made considering other diagnostic indicators such as histological characteristics. Additionally, the increase in autoantibody titer can be caused by many factors, such as chronic graft rejection. It was reported that the rate of increase in autoantibodies reached 70% in patients after liver transplantation [5]. However, the clinical significance of the increase in autoantibodies is not clear. Hence, the systematic analysis of the incidence of *de novo* AIH and chronic graft rejection in children and adolescents with increased autoantibodies after liver transplantation can help us to determine the significance of the increase in autoantibodies after liver transplantation.

There have been several reviews [6–8] describing the correlation between increased autoantibodies and *de novo* AIH. It was thought that autoantibodies themselves were not sufficient to diagnose *de novo* AIH despite high rates of increase in patients with *de novo* AIH. There were no reports of the incidence of *de novo* AIH in patients with increased autoantibodies after liver transplantation. Moreover, search strategies and eligibility criteria were not available in these reviews and systematic analyses were not conducted.

Considering the higher incidence of *de novo* AIH in children and adolescents compared with adults, we systematically retrieved and analyzed studies that investigated the increase in autoantibodies after liver transplantation for reasons other than autoimmune liver disease in children and adolescents. We measured the incidence of *de novo* AIH in children and adolescents with increased autoantibodies after liver transplantation.

Materials and methods

Search strategy

Two of our authors conducted a search of PubMed, Embase, Central, CNKI, VIP and Wan Fang for articles published from inception to February 1, 2020. An examination of the electronic databases and a hand search of the literature were both carried out, including the retrieval of references from selected articles. We also searched Clinical Trials.gov to recognize ongoing or unpublished eligible trials. We combined subject words and free words for the search strategy with language restriction in Chinese and English, and adjusted according to the specific database. The search terms included "autoantibodies", "autoantibody", "liver transplantation", "liver transplant", "hepatic transplantation", "liver transplantations", "child", "children", "pediatrics", "pediatrics", "pediatrics", "kid", "youngster" (see File S1: Table S1).

Inclusion and exclusion criteria

The inclusion criteria were as follows. The study type was classified as case–control or cohort. The study subjects should meet following conditions: children and adolescents (age < 20 years) who underwent liver transplantation; all or some of the subjects were tested for serum autoantibodies at least once after surgery: ANA, SMA, antineutrophil cytoplasmic antibody, asialoglycoprotein receptor antibody, liver membrane antigen antibody, liver-specific protein antibody, soluble liver antigen antibody, liver–pancreas antigen antibody, antibody to liver/kidney microsome (anti-LKM), antibody to liver cytosol antigen type 1, and antimitochondrial antibody. Information about *de novo* AIH onset, chronic rejection onset, abnormal results in liver biochemical tests and/or histopathological tests was available.

The exclusion criteria were as follows: (i) it was not possible to exclude that patients were transplanted for autoimmune liver diseases; (ii) language was not Chinese or English; (iii) if multiple studies reported the same data, we selected the one with the largest sample size or most detailed information; and (iv) outcome measures mentioned above were not available, or the data were incomplete.

Study selection and data collection process

After removing duplicates, two independent reviewers screened all titles and abstracts according to the

inclusion/exclusion criteria. Full texts of potentially eligible articles were obtained to be further assessed for final inclusion. Disagreements were resolved by discussion or by consultation with a third author.

We used a standard data extraction form to collect information from the studies included. The following information was extracted: (i) basic information including first author, publication year, study area, sample size, sex, age, follow-up time, donor type, study design, and diagnostic criteria for *de novo* AIH; (ii) causes of liver transplantation, type and titer of autoantibodies, use of immunosuppressive agents, biochemical markers relevant to liver functions, adverse events related to liver diseases (death or retransplantation), onset of *de novo* AIH and chronic rejection. When a study reported an outcome of interest without sufficient details, we contacted the authors for the data.

Assessment of study quality

We established a quality assessment scale according to the Institute of Health Economics-18 checklist [9] and Agency for Healthcare Research and Quality [10] checklist (Table 2). The scale consisted of 15 items, appraising the research objectives, subjects, outcome measurements and statistical analysis. For each item, a score of 1 was given for "yes" and 0 for "no" or "uncertain", with a full score of 15. Two researchers scored independently and cross-checked the results.

Statistical analysis

The following measures of effect were counted: (i) incidence of *de novo* AIH (for articles using IAIHG criteria, we calculated the incidence of "definite" *de novo* AIH, while for the ones without using IAIHG criteria, we calculated the incidence of reported *de novo* AIH); (ii) incidence of chronic rejection; (iii) abnormal rate of liver biochemical tests; (iv) rate of elevated IgG; and (v) rate of increase of different kinds of autoantibodies. We performed statistical analyses using the meta package in R (version 4.0.1; R Project for Statistical Computing). A double arcsine transformation was used to make original rate (r) comply with the normal distribution, then we performed a meta-analysis on the transform rate (tr) to acquire the final rate (R) and 95% CI. The I^2 test was used to estimate heterogeneity. If significant heterogeneity was not present ($I^2 < 50\%$), we used fixed effects models; when significant heterogeneity was present, we used random effects models ($I^2 \geq 50\%$). To detect the sources of heterogeneity, we performed subgroup analyses and meta-regression of random effects according to sample size (<50 and ≥50), experimental area (Europe and Asia), study design (case–control study and cohort study), and diagnostic criteria (IAIHG criteria and non-IAIHG criteria). The length of follow-up and sex ratio were not tested for limited information. Sensitivity analyses were also conducted. In addition, we further investigated the relationship between the rate of increase of various antibodies and incidence of *de novo* AIH by meta-regression of random effects. Due to the small number of included articles, publication bias could not be assessed. $P < 0.05$ was considered statistically significant.

Results

Eligible studies and study characteristics

We initially identified 879 records, and included seven eligible publications in the final meta-analysis, including 254 patients. Figure 1 shows a summary of the included articles. One of the studies was from Asia, the rest from Europe. Five were case–control studies and two were cohort studies. IAIHG criteria were adopted in five articles, one used self-revised diagnostic criteria, and one did not report the diagnostic criteria in detail [1,11–16]. Table 1 gives details of those publications.

Study quality

We assessed the quality of all seven publications using the self-revised quality assessment scale (Fig. 2). Two articles were rated as 8 points, three as 9 and two as 10 points.

Primary outcomes

Incidence of de novo AIH

All seven articles reported sufficient information to calculate the incidence of *de novo* AIH. Statistically significant heterogeneity was found among the studies ($I^2 = 86\%$, P: 0.01), thus a random effects model was used for meta-analysis. The incidence of *de novo* AIH was 9% (95% CI 1–23%; Fig. 3).

Sensitivity analysis

Sensitivity analysis was performed in which statistics were recombined after excluding each study successively. There was neither directional change in result nor significant change in I^2, indicating that the results were relatively stable.

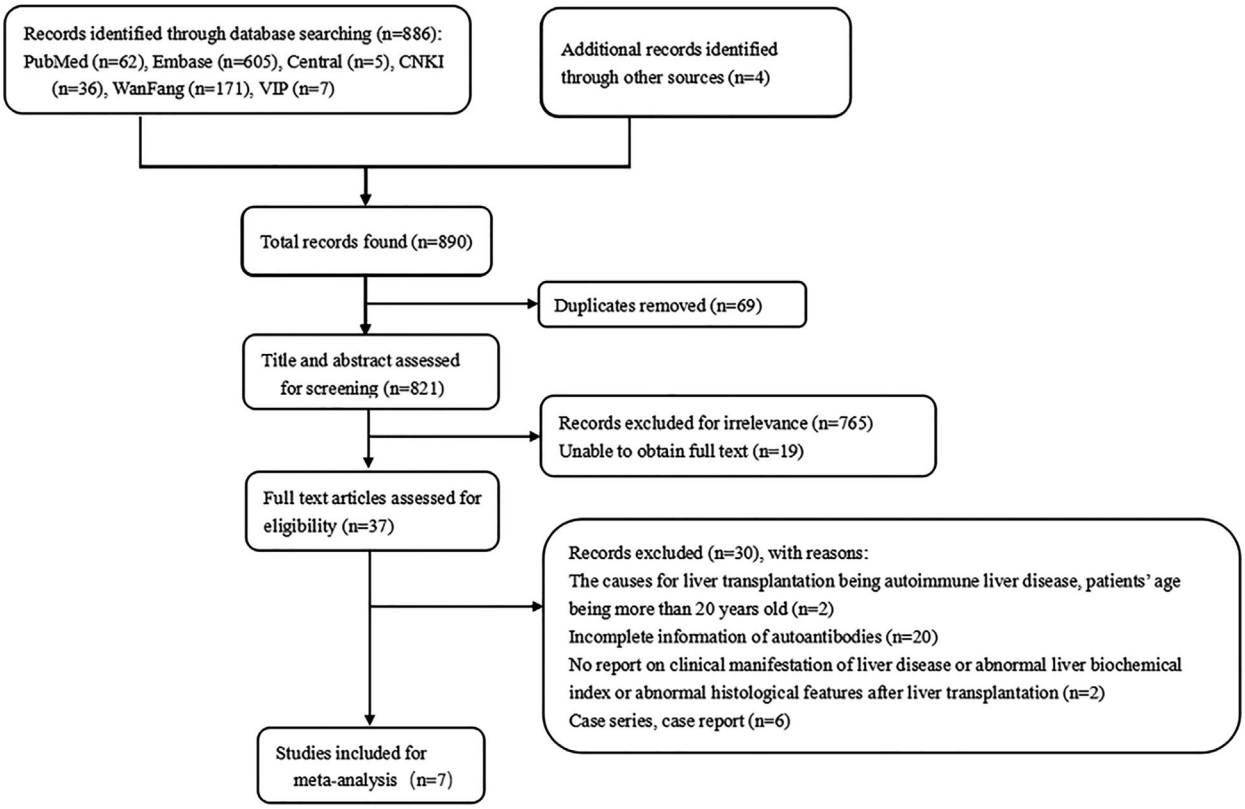

Figure 1 Flow chart of the study selection process. The flow chart demonstrates the study selection process in the meta-analysis.

Subgroup analysis and meta-regression analysis

Subgroup and meta-regression analyses found marginally significant differences between subgroups when sample size (P for interaction in subgroup analysis = 0.07, P for interaction in regression analysis = 0.08) and diagnostic criteria (P for interaction in subgroup analysis = 0.08, P for interaction in regression analysis = 0.10) were taken as grouping factors (Table 2). The incidence of *de novo* AIH was higher among publications with <50 patients (18%, 95% CI 5–37%) than in publications with ≥50 patients (4%, 95% CI 0–16%). Publications not using IAIHG criteria (18%, 95% CI 5–37%) have higher incidence of *de novo* AIH than those using IAIHG criteria (4%, 95% CI 0–16%). However, when experimental area, study type and study quality score were used as grouping factors, no significant difference was found between groups.

Secondary outcomes

Incidence of chronic rejection

We gathered information from six articles providing sufficient data and calculated the incidence of chronic rejection. Statistically significant heterogeneity was found among studies (I^2 = 72%, P < 0.01), thus a random effects model was adopted for meta-analysis. The incidence of chronic rejection was 8% (95% CI 2–17%; Fig. 4).

Sensitivity analysis of the incidence of chronic rejection

Sensitivity analysis was conducted by recombining statistics after excluding each study in turn. There was neither directional change in result nor significant change in I^2, indicating that the results were relatively stable.

Rate of abnormal liver biochemical tests

Three articles reported an increase in alanine transaminase (ALT), with 127 patients in total (Table 3). Statistically significant heterogeneity was found among the studies (I^2 = 90%), thus a random effects model was used for meta-analysis, and the combined rate of increase of ALT was 67% (95% CI 36–92%). Three articles reported an increase in aspartate transaminase (AST), with 131 patients in total. No significant heterogeneity was found among the studies (I^2 = 0), so a fixed

Table 1. Characteristics of the articles included

First author (publication year)	Country	Study type	Study size	Median age (years)	Sex (female/male)	Mean follow-up	Donor type	Diagnostic criteria for *de novo* AIH
Andries (2001)	Belgium	Case–control	34	NA	NA	NA	Case: living liver transplant for 1, deceased donor liver transplant for 10 Control: NA	Non-IAIHG
Avitzur (2007)	Canada	Cohort	18	9	NA	18 months	NA	IAIHG
Chen (2013)	Taiwan, China	Case–control	51	3.6*	30/21	NA	Living liver transplant for 46, deceased donor liver transplant for 5	IAIHG
Herzog (2008)	Canada	Case–control	8	3.8	4/4	12 years	NA	IAIHG
Kerkar (1998)	Britain	Case–control	12	Case: 8.3 Control: 2.7	NA	Case: 5 years Control: 19 months	Case: deceased donor liver transplant Control: NA	IAIHG
Richter (2007)	Germany	Cohort	68	7.9	NA	4.3 years	NA	IAIHG
Riva (2006)	Italy	Case–control	60	2.7	NA	*de novo* AIH: 39.3 months, chronic rejection: 35.8 months, the rest NA	NA	Non-IAIHG

NA, not available.

*In Chen's study (2013), the mean age was 3.6 years, and the median age was unknown

effects model was used for meta-analysis, and the combined rate of increase of AST was 51% (95% CI 43–60%). Five publications reported the rate of increase of IgG, with 182 patients in total. Statistically significant heterogeneity was found among the studies (I^2 = 65%), thus a random effects model was used for meta-analysis, and the combined rate of increase of IgG was 20% (95% CI 35–92%).

Rate of increase in various autoantibodies

Six articles reported rates of increase of a single antibody (ANA, SMA or anti-LKM), with 191 patients in total (Table 4). Statistically significant heterogeneity was found among studies in all three analyses of rates of increase of a single antibody (I^2 = 93%, 90% and 70%), thus a random effects model was used. The rate of increase of ANA was 47% (95% CI 18–77%), SMA was 54% (95% CI 29–78%) and anti-LKM was 3% (95% CI 0–12%). Five articles reported simultaneous increases in ANA and SMA, with 173 patients in total. Significant heterogeneity was found among the studies (I^2 = 65%), so a random effects model was used for meta-analysis, and the rate of simultaneous increase of ANA and SMA was 8% (95% CI 1–20%).

Meta-regression

Meta-regression analysis showed that the incidence of *de novo* AIH was significantly correlated with the rate of increase of anti-LKM (P = 0.04; coefficient = 1.51) (see File S1: Fig. S1). However, neither the rate of increase of SMA (P = 0.55; coefficient = −0.21) nor the rate of increase of ANA (P = 0.99; coefficient = −0.01) was significantly correlated with the incidence of *de novo* AIH.

Discussion

Our meta-analysis revealed that the overall incidence of *de novo* AIH among children and adolescents with elevated autoantibodies after liver transplantation was 9%. Previous research has reported an incidence of *de novo* AIH of 1.2–8.3% among children with elevated autoantibodies after liver transplantation, which is similar to our result. This indicates that, although elevated autoantibodies are one of the criteria for the diagnosis of *de novo* AIH, *de novo* AIH is not the only reason for elevated autoantibodies in patients who have undergone liver transplantation for nonautoimmune liver disorders. Therefore, the accuracy and specificity of autoantibodies

	Andries(2001)	Avitzur(2007)	Chen(2013)	Herzog(2008)	Kerkar(1998)	Richter(2007)	Riva(2006)
1. State hypothesis, purpose and object of study clearly	1	1	1	1	1	1	1
2. Define the source of information (survey, record review)	1	1	1	1	1	1	1
3. Describe patients' characteristics	1	1	1	1	1	1	1
4. Collect multicenter cases	0	0	0	0	0	0	1
5. Make clear and reasonable inclusion and exclusion criteria	0	1	1	1	1	0	1
6. Include consecutive patients	0	0	1	0	0	0	1
7. Determine outcome of study in advance	1	1	1	1	1	1	1
8. Measure outcome indicators in objective and/or subjective way	1	1	1	1	1	1	1
9. Describe how confounding was assessed and/or controlled	0	0	1	0	0	0	0
10. Report follow-up time	1	1	0	1	1	1	0
11. Report people's number and reasons of loss to follow-up	1	1	1	1	1	1	0
12. Explain any patients exclusions from analysis	1	1	1	1	1	1	0
13. State conflict of interest and sources of support of study	0	0	0	0	0	0	0
14. Study prospectively	0	1	0	0	0	1	0
	8	10	10	9	9	9	8

Figure 2 Evaluation of study quality. The figure depicts detailed scoring of each literature included. The red blocks represent score 0, green represents score 1, and white represents summary score.

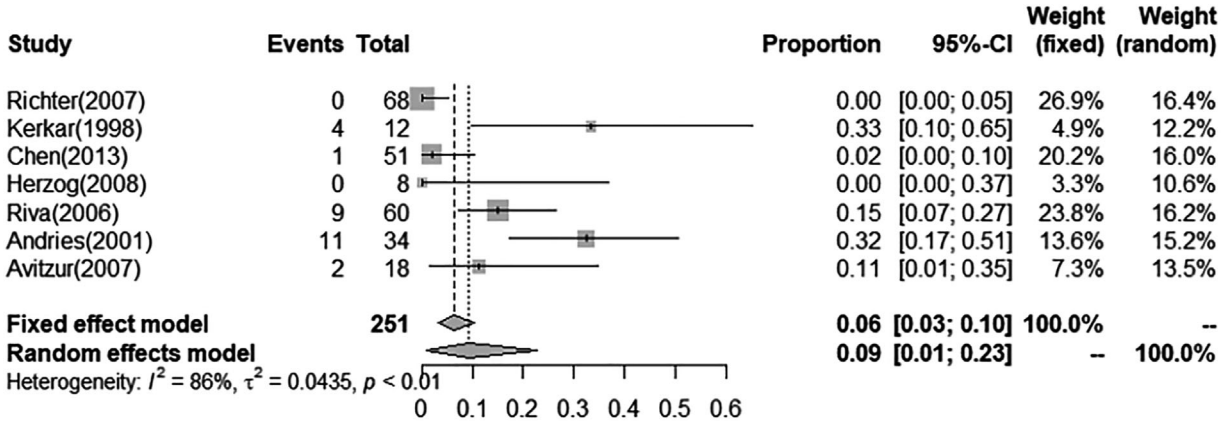

Figure 3 Forest plot of the incidence of *de novo* autoimmune hepatitis (AIH). The gray squares represent point estimates of incidence of *de novo* AIH, and the square size represents study weight. Horizontal bars indicate 95% confidence intervals. The gray diamond represents overall estimated incidence.

in diagnosis of *de novo* AIH after liver transplantation remain to be discussed [17]. Furthermore, exact differential diagnosis between *de novo* AIH and chronic rejection is needed because the incidence of chronic rejection in children and adolescents with increasing autoantibodies after liver transplantation can be up to 8% and the treatments for the two kinds of late graft dysfunction are different. Additionally, according to meta-regression analysis, the rate of increase of anti-LKM is positively related to the incidence of *de novo* AIH, which can be a reference for the correlation between anti-LKM and *de novo* AIH pathogenesis.

Autoantibodies are generated against certain epitopes not specifically associated with *de novo* AIH. Many factors can lead to elevated autoantibodies, including use of immunosuppressants such as calcineurin inhibitors, infection with viruses such as cytomegalovirus (CMV), and acute graft rejection [11]. The literature cited in our meta-analysis showed that patients with elevated autoantibodies were negative for CMV, but other virus infections were not excluded. Additionally, nearly all patients used immunosuppressive agents such as cyclosporine and tacrolimus (see File S2: Table S6). These factors may have contributed to increased autoantibodies in patients after liver transplantation without *de novo* AIH, which makes the incidence of *de novo* AIH in patients with elevated antibodies lower. This reveals that isolated elevation of autoantibodies

Table 2. Subgroup analysis and meta-regression analysis of the incidence of de novo AIH.

Subgroup	No. of studies	Total no. of samples	Combined rate and 95% CI		Subgroup analysis			Meta-regression analysis	
			Fixed effects model	Random effects model	*P** for interaction in subgroup	I^2 (%)	*P**	Coefficient	*P**
Country									
Europe	6	200	0.08 (0.04–0.13)	0.11 (0.01–0.29)	0.10	87	<0.01	0.20	0.44
Asia	1	51	0.02 (0.00–0.08)	0.02 (0.00–0.08)					
Study type									
Case–control	5	165	0.12 (0.07–0.18)	0.13 (0.02–0.30)	0.27	81	<0.01	0.20	0.26
Cohort	2	86	0.00 (0.00–0.04)	0.02 (0.00–0.22)		82	0.02		
Sample size									
<50	4	72	0.21 (0.12–0.32)	0.18 (0.05–0.37)	0.07†	60	0.06	−0.26	0.08†
≥50	3	179	0.03 (0.01–0.07)	0.04 (0.00–0.16)		87	<0.01		
Diagnostic criteria									
IAIHG criteria	5	157	0.01 (0.00–0.04)	0.04 (0.00–0.16)	0.08†	76	<0.01	−0.25	0.10†
Non-IAIHG criteria	2	94	0.21 (0.13–0.30)	0.22 (0.08–0.41)		73	0.05		
Quality									
<10	5	182	0.08 (0.04–0.13)	0.11 (0.00–0.32)	0.42	89	<0.01	−0.12	0.24
≥10	2	69	0.03 (0.00–0.10)	0.04 (0.00–0.17)		55	0.14		

*$P < 0.05$ represents significant; $P < 0.10$ represents marginally significant.

†Marginally significant.

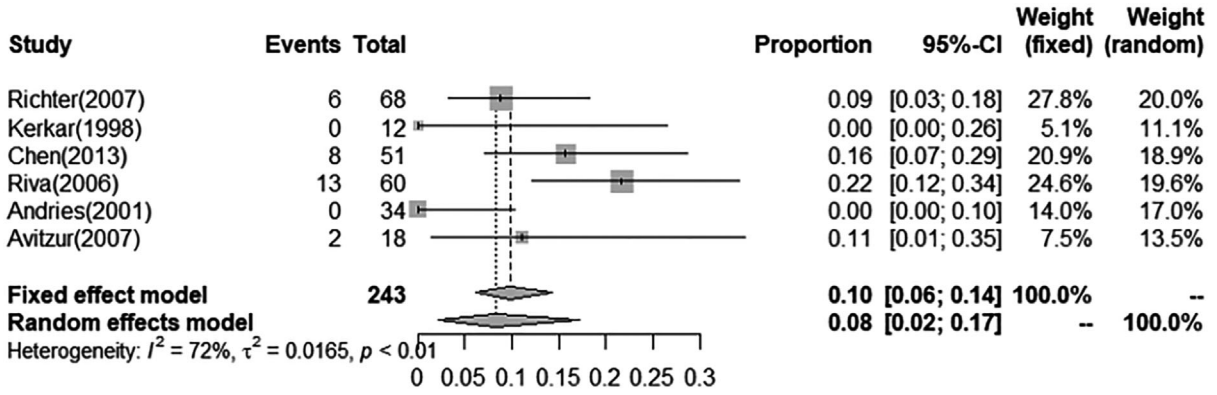

Figure 4 Forest plot of the incidence of chronic rejection. The gray squares represent point estimates of incidence of chronic rejection, and the square size represents study weight. Horizontal bars indicate 95% confidence intervals. The gray diamond represents overall estimated incidence.

does not have sufficient specificity for the diagnosis of *de novo* AIH.

The overall incidence of *de novo* AIH among children and adolescents with increased autoantibodies after liver transplantation in subgroups with <50 patients is significantly higher. As in these included studies with smaller sample size, not all patients after liver transplantation were tested for autoantibodies and long-term follow-up was not conducted after autoantibody measurement. This resulted in underestimation of the number of patients with elevated autoantibodies, making the incidence of *de novo* AIH higher.

Another factor with slight statistical significance is whether to use IAIHG diagnostic criteria. The prevalence of *de novo* AIH in the group using non-IAIHG criteria was higher than that in the group using IAIHG criteria. However, the diagnostic value of IAIHG criteria for *de novo* AIH in patients after liver transplantation is still unclear. Additionally, many of the criteria are not suitable for children, such as the standard for positive autoantibodies (different from adults, ANA and SMA $\geq$ 1:20, or anti-LKM-1 $\geq$ 1:10 are regarded positive in children), drug history and average alcohol intake. In two studies using non-IAIHG criteria for meta-analysis, Andries *et al.* considered using post-transplant AIH instead of AIH to interpret the disease, and excluded alkaline phosphatase, drugs and alcohol from the diagnostic criteria; and Riva *et al.* used 1:40 as the positive standard for autoantibodies, which is similar to the simplified AIH diagnostic scoring system. The revised criteria are more suited to the characteristics of patients after liver transplantation, and may be the main reason that the incidence of *de novo* AIH in the group with non-IAIHG criteria was higher than in the group with IAIHG criteria. At present, there is no uniform international standard for the diagnosis of *de novo* AIH, and more extensive research is needed to evaluate the IAIHG criteria for the diagnosis of *de novo* AIH.

We found that the incidence of chronic rejection in children and adolescents with elevated autoantibodies after liver transplantation was 8%. Compared with the incidence of chronic rejection of $\leq$3% in general children and adolescents with or without elevation in autoantibodies after liver transplantation [18], this relatively high incidence suggests that increased autoantibodies may be one of the risk factors for chronic rejection after liver transplantation or vice versa: chronic rejection may also lead to presence of autoantibodies. According to the above outcomes, the incidence of chronic rejection is similar to that of *de novo* AIH in children and adolescents with elevated autoantibodies after liver transplantation (8% vs. 9%). As two common causes of chronic graft function injury, it is still challenging for the discrimination of chronic rejection and *de novo* AIH. Previous studies have proposed using onset time (median onset time of chronic rejection is 3–12 months, while that of *de novo* AIH is >5 years) and characteristic pathological manifestations for differentiation. However, it is still difficult for differential diagnosis in atypical cases. There is controversy regarding whether *de novo* AIH is an autoimmune response or a special type of chronic rejection. In the 7 articles included in our meta-analysis, the diagnosis of *de novo* AIH mainly depended on liver biopsy, and 3 articles reported the histological features in detail (see File S2: Tables S2–S5). The latest updated Banff standard suggests renaming *de novo* AIH as plasma-cell-rich rejection according to pathological features [19]. This suggests that the major pathological difference between chronic rejection and *de novo* AIH is proportion of

Table 3. Rates of abnormal liver biochemical tests.

Biochemical items	No. of studies	Total no. of samples	Combined rate and 95% CI		Heterogeneity analysis I^2 (%)
			Fixed effects model	Random effects model	
ALT	3	127	0.51 (0.43–0.60)	0.67 (0.36–0.92)	90.60
AST	3	131	0.51 (0.43–0.60)	0.51 (0.43–0.60)	0.00
IgG	5	182	0.17 (0.12–0.23)	0.20 (0.09–0.33)	65.70

ALT, alanine transaminase; AST, aspartate Aminotransferase; IgG, immunoglobulin G.

Table 4. Rates of increase in various autoantibodies.

Autoantibodies	No. of studies	Total no. of samples	Combined rate and 95% CI		Heterogeneity analysis I^2 (%)
			Fixed effects model	Random effects model	
ANA	6	191	0.32 (0.25–0.39)	0.47 (0.18–0.77)	93.70
SMA	6	191	0.70 (0.63–0.76)	0.54 (0.29–0.78)	90.30
Anti-LKM	6	191	0.02 (0.00–0.06)	0.04 (0.00–0.12)	70.60
SMA + ANA	5	173	0.08 (0.04–0.13)	0.08 (0.01–0.20)	71.80

ANA, antineutrophil antibody; Anti-LKM, antibodies to liver/kidney microsome; SMA, smooth muscle antibody.

plasma cells infiltrated and the extent of interface hepatitis and bile duct injury, but no cutoff level is provided [20]. Elena *et al.* analyzed the composition of various infiltrated cells in liver biopsies of patients with *de novo* AIH and chronic rejection with the aid of a new technique. They found that the proportion of infiltrated plasma cells in patients with *de novo* AIH had a characteristic increase (six times higher than that in patients with chronic rejection), and significantly decreased after treatment. Their hypothesis proposed that during surgery, intracellular antigens such as glutathione S-transferase θ 1 protein are released and activated B cells that continuously differentiate into plasma cells and generate related antibodies. At the time of diagnosis, the proportion of plasma cells in patients with *de novo* AIH is characteristic and supports the new concept of *de novo* AIH as antibody-mediated rejection [21]. It may be of interest to look for donor-specific antibodies (DSAs) in patients with chronic rejection or *de novo* AIH, and that possibly histologic criteria may need to get more emphasis in the diagnosis of both conditions.

We also found that in patients with elevated autoantibodies, those with ANA or SMA increase alone accounted for the majority, which is consistent with the level of serum antibodies in *de novo* AIH patients reported by Luo *et al.* [22]. Additionally, there was considerable elevation (>50%) of ALT and AST among this study population, suggesting that hepatocyte injury was common in patients with elevated antibodies after liver transplantation. Level of IgG can reflect the activity level of intrahepatic inflammation, and can be regarded as one of the characteristics of *de novo* AIH [23], as well as a measurement of its curative effect. Our meta-analysis found that the rate of increase of IgG in patients with elevated autoantibodies after liver transplantation was 20%. Hu *et al.* reported that 84.4% of 32 patients with newly increased ANA after liver transplantation was accompanied by elevation in IgG level. However, they only included patients with ANA titer > 1:80, and most of their subjects were adults [24]. Li *et al.* [25] showed that the positive rate of IgG in *de novo* AIH patients was 61.1%. In our study, the types and titers of autoantibodies were different, so the relationship between IgG and elevation of autoantibodies after liver transplantation still needs to be studied.

SMA and ANA are commonly elevated antibodies after liver transplantation, but meta-regression analysis showed no association with the onset of *de novo* AIH. However, rate of increase of anti-LKM was positively correlated with the incidence of *de novo* AIH. This may have been due to the small sample size, but it also implies the correlation between elevated anti-LKM and the incidence of *de novo* AIH. In a study of 986 children with acute liver failure, Narkewicz *et al.* [26] found that compared with patients with SMA or ANA without anti-LKM (27%), patients with anti-LKM were younger and more likely to be diagnosed with AIH (57%), which

was consistent with the results of our study. Additionally, CYP2D6 is one of the target autoantigens of anti-LKM-1 and is expressed on the surface of rat liver cells, so the attack of anti-LKM-1 against CYP2D6 is one of the causes of *de novo* AIH and hepatocyte injury [23]. The relationship between anti-LKM and the development of *de novo* AIH requires further study.

Strengths and limitations

To our knowledge, this is the first meta-analysis to limit the study sample to children and adolescents with increasing autoantibodies after liver transplantation, compared with other studies that focused on general patients after liver transplantation at the same age. At the same time, we excluded patients with higher autoantibodies than normal before liver transplantation to prevent the influence of preoperative antibody elevation on postoperative antibody level. Additionally, multicenter studies with high quality were included in our study. Comprehensive subgroup and sensitivity analysis were conducted to explain the heterogeneity. The entire process of this study strictly followed the protocol from the Cochrane Collaboration and PRISMA statement.

Although we tried to enlarge the retrieval area through a combination of a variety of databases and manual searches, there were still some limitations to this study. This meta-analysis only consisted of seven articles and in some of these articles elevated autoantibodies and *de novo* AIH were considered for secondary outcomes. Hence, data collection for secondary outcomes may not be as complete as those for primary outcomes. Additionally, the measurement methods and cutoff value of positive autoantibodies differed among the studies. It was difficult to estimate the effect of immunosuppression after liver transplantation on elevated autoantibodies and occurrence of *de novo* AIH [27] because of the small number of studies and control groups. Therefore, more further studies will be needed to obtain comparative results.

Conclusions and implications

Increased autoantibodies are common in children and adolescents after liver transplantation. However, as a disease that can cause late graft dysfunction, the relationship between elevated autoantibodies and *de novo* AIH occurrence is still unclear. Our meta-analysis included seven studies and a total of 251 patients. The main results showed that the incidence of *de novo* AIH in children and adolescents with increasing autoantibodies after liver transplantation was 9%. Subgroup analysis inferred that the diagnosis criteria can lead to significant differences between groups. Secondary outcomes showed that the incidence of chronic rejection in children and adolescents with increasing autoantibodies after liver transplantation was 8%, and hepatic biochemical indicators (ALT, AST and IgG) also increased in these samples. The rates of increase of autoantibodies (ANA, SMA and anti-LKM) were 47%, 54% and 3%, respectively, while the rate of concurrence of increasing SMA and ANA was 8%. Meta-regression showed a positive correlation between the incidence of *de novo* AIH and the rate of elevation of anti-LKM.

Authorship

LM and ML: designed the study, collected the data. TZ and JQX: performed the analysis. LM, ML,TZ, JQX and XBC: wrote the final manuscript. SSW and LYS: help design the study and reviewed the final manuscript.

Funding

This study was funded by Beijing Municipal Science & Technology Commission (No. Z181100001718220).

Conflict of interest

The authors have declared no conflicts of interest.

Acknowledgements

The authors thank professor Li-Ying Sun for enlightening tutorial and professor Shan-Shan Wu for valuable methodological advice. We thank for the support of Beijing Municipal Science & Technology Commission (No. Z181100001718220).

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Table S1. Summary of search strategies in different databases.

Figure S1. Meta regression of the incidence of *de novo* AIH and the rate of increase of anti-LKM.

Table S2. Information related to liver biopsy in articles included.

Table S3. Histologic characteristics of patients underwent liver biopsy in article "Kerkar (1998)".

Table S4. Histologic characteristics of patients underwent liver biopsy in article "Herzog (2008)".

Table S5. Histologic characteristics of patients underwent liver biopsy in article "Andries (2001)".

Table S6. Immunosuppressive scheme of articles included.

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