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Natural History of Liver Disease in a Large International Cohort of Children with

Alagille syndrome: Results from The GALA Study

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ABBREVIATIONS

ALGS, Alagille syndrome

GGT, γ -glutamyl transferase

JAGGED1, *JAG1*

ASBT, apical sodium-dependent bile acid transporter

GALA, Global Alagille Alliance

NLS, native liver survival

LT, liver transplantation

REDCap, Research Electronic Data Capture

DCC, data coordinating center

STROBE, Strengthening the Reporting of Observational Studies in Epidemiology

ACMG, American College of Medical Genetics and Genomics

ULN, upper limit of normal

TB, total bilirubin

CB, conjugated bilirubin

ALT, alanine aminotransferase

AST, aspartate aminotransferase

TC, total cholesterol

TG, triglyceride

PLT, platelet count

APRI, AST to PLT Ratio Index

Echo, echocardiogram

MRI, Magnetic resonance imaging

CT, computerized tomography

PHT, portal hypertension

CEPH, Clinically Evident Portal Hypertension

IQR, inter-quartile ranges

KPE, Kasai portoenterostomy

AIC, Akaike information criterion

HR, Hazard ratios

CI, confidence interval

SPSS, Statistical Package for the Social Sciences

BA, biliary atresia

Conflict of interest statement

Shannon M. Vandriel [nothing to disclose], Li-Ting Li [nothing to disclose], Huiyu She [nothing to disclose], Jian-She Wang [consultant for Mirum Pharmaceuticals, Albireo Pharma, Inc., Intercept, and Ethypharm], Melissa A. Gilbert [consultant for Travers Therapeutics], Irena Jankowska [nothing to disclose], Piotr Czubkowski [nothing to disclose], Dorota Gliwicz-Miedzińska [nothing to disclose], Emmanuel M. Gonzales [nothing to disclose], Emmanuel Jacquemin [nothing to disclose], Jérôme Bouligand [nothing to disclose], Nancy B. Spinner [consultant for Mirum pharmaceuticals and Travers therapeutics], Kathleen M. Loomes [consultant for Mirum Pharmaceuticals, Albireo and Travers Therapeutics], David A. Piccoli [nothing to disclose], Lorenzo D'Antiga [nothing to disclose], Emanuele Nicastro [nothing to disclose], Étienne Sokal [nothing to disclose], Tanguy Demaret [nothing to disclose], Noelle H. Ebel [consultant for Mirum Pharmaceuticals], Jeffrey A. Feinstein [nothing to disclose], Rima Fawaz [nothing to disclose], Silvia Nastasio [nothing to disclose], Florence Lacaille [nothing to disclose], Dominique Debray [nothing to disclose], Henrik Arnell [consultant for Albireo, Baxter, Mirum, Shire], Björn Fischler [nothing to disclose], Susan Siew [nothing to disclose], Michael Stormon [nothing to disclose], Saul J. Karpen [consultant for Albireo, Intercept, Mirum and Vertex], Rene Romero [nothing to disclose], Kyung Mo Kim [nothing to disclose], Woo Yim Baek [nothing to disclose], Winita Hardikar [nothing to disclose], Sahana Shankar [nothing to disclose], Amin J. Roberts [nothing to disclose], Helen M. Evans [nothing to disclose], M.

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Shannon M. Vandriel, participated in the study concept and design, acquisition of data, analysis and interpretation of data, drafting of the manuscript, and critical revision of the manuscript for important intellectual content, final approval of the version to be published; Li-Ting Li, data acquisition, critical revision of the manuscript for important intellectual content, and final approval of the version to be published; Huiyu She, data acquisition, critical revision of the manuscript for important intellectual content, and final approval of the version to be published;

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ABSTRACT

BACKGROUND: Alagille syndrome (ALGS) is a multisystem disorder, characterized by cholestasis. Existing outcome data are largely derived from tertiary centers and real-world data are lacking. This study aimed to elucidate the natural history of liver disease in a contemporary, international, cohort of children with ALGS.

METHODS: Multicenter retrospective study of children with a clinically and/or genetically confirmed ALGS diagnosis, born Jan-1997 - Aug-2019. Native liver survival (NLS) and event-free survival rates were assessed. Cox models were constructed to identify early biochemical predictors of clinically evident portal hypertension (CEPH) and NLS.

RESULTS: 1433 children (57% male) from 67 centers in 29 countries were included. 10 and 18-years NLS rates were 54.4% and 40.3%. By 10 and 18-years, 51.5% and 66.0% of ALGS children experienced ≥ 1 adverse liver-related event (CEPH, transplant or death). Children (>6 and ≤ 12 months) with median total bilirubin (TB) levels between ≥ 5.0 and <10.0 mg/dL had a 4.1-fold (95% CI 1.6 – 10.8) and those ≥ 10.0 mg/dL had an 8.0-fold (95% CI 3.4 – 18.4) increased risk of developing CEPH compared with those <5.0 mg/dL. Median TB levels between ≥ 5.0 and <10.0 mg/dL and >10.0 mg/dL were associated with a 4.8 (95% CI 2.4 – 9.7) and 15.6 (95% CI 8.7 – 28.2) increased risk of transplantation relative to <5.0 mg/dL. Median TB <5.0 mg/dL were associated with higher NLS rates relative to ≥ 5.0 mg/dL, with 79% reaching adulthood with native liver ($p < 0.001$).

CONCLUSIONS: In this large international cohort of ALGS, only 40.3% of children reach adulthood with their native liver. A TB <5.0 mg/dL between 6-and-12-months of age is associated with better hepatic outcomes. These thresholds provide clinicians with an objective tool to assist with clinical decision-making and in the evaluation of novel therapies.

INTRODUCTION

Alagille syndrome (ALGS) is an autosomal dominant disorder, primarily characterized by hepatic involvement manifesting as high- γ -glutamyl transferase (GGT) cholestasis with variable extrahepatic involvement (1-3). Two causative genes encoding components of the Notch signalling pathway have been identified in ALGS: *JAGGED1* (*JAG1*) and *NOTCH2*.

Genetic testing yields a pathogenic *JAG1* variant or deletion in 94.3% of patients with ALGS meeting phenotypic criteria, while pathogenic variants in *NOTCH2* account for 2.5% of clinical cases (4). The prevalence of ALGS has historically been estimated to be approximately 1 in 70,000 live births, however, based on genetic analyses the true disease burden is likely closer to 1 in 30,000 (5).

There is considerable variation in the clinical course of ALGS, and there are no known genotypic predictors of liver disease progression. To date, the phenotype and natural history of ALGS have been reported from single centers, often without molecular characterization of patients and these data are somewhat outdated. A single recent multicenter study of ALGS comes from tertiary referral liver centers in North America and represents the most severely affected cholestatic patients (6). As a result, the full spectrum of ALGS-related liver involvement remains unknown and detailed analyses of real-world natural history data are lacking. This is a crucial unmet need in an era when novel therapeutic strategies are being developed to target cholestasis-induced pruritus, such as the apical sodium-dependent bile acid transporter (ASBT) inhibitors (7).

The Global Alagille Alliance (GALA) Study Group was formed to elucidate the natural history of liver disease in a contemporary, international cohort of children with ALGS. Specifically, we

sought to investigate rates of native liver survival (NLS) in children with ALGS and a history of neonatal cholestasis and to identify early laboratory predictors of long-term hepatic outcomes. Furthermore, we aimed to evaluate global patient and graft survival following liver transplantation (LT) in children with ALGS.

PATIENTS AND METHODS

The GALA Study was established in 2018 and presently consists of 67 pediatric centers from 29 countries. Children with a clinically and/or genetically confirmed ALGS diagnosis born between Jan-1997 and Aug-2019 were eligible for inclusion. A diagnosis of ALGS was made in accordance with standard clinical criteria (Supplementary Table 1). Children with ALGS born before Jan-1997 and who had a pathogenic or likely pathogenic variant in *JAG1* or *NOTCH2* were also included. The study was approved by the ethics committee at each participating center.

Data collection

From May 2018 to July 2021, the medical records of patients with ALGS were retrospectively reviewed at each participating center and each site abstracted data directly into REDCap (Research Electronic Data Capture), a remote web-based database (8). To ensure the collection of rigorous, high-quality data, the GALA data coordinating center (DCC) at The Hospital for Sick Children, Toronto, Ontario, Canada implemented several quality assurances measures and procedures as described in the supplementary material. This study followed the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) reporting guideline for cohort studies (9).

Study definitions and variables

The pathogenicity of reported variants was classified according to the American College of Medical Genetics and Genomics (ACMG) guidelines (10). Neonatal cholestasis was defined as having at least one of the following features during the first three months of life: (1) direct/conjugated bilirubin greater than >2.0 mg/dL (34.0 μ mol/L); (2) serum bile acids or GGT greater than 3 times the upper limit of normal (ULN); (3) fat-soluble vitamin deficiency, otherwise unexplainable (6). Liver involvement at any age was defined as a history of neonatal cholestasis, elevated liver transaminases, histological abnormalities, history of pruritus and/or xanthomas or underwent hepatobiliary surgery. Native liver biopsy reports were reviewed to characterize histopathological findings. Hepatic fibrosis was reported as a dichotomous variable (absent or present) based on review of histopathology reports. Pruritus and xanthomas were reported at each follow-up visit as a categorical variable (present, absent, or unknown). Biochemical parameters including serum bile acids, total bilirubin (TB), conjugated bilirubin (CB), alanine aminotransferase (ALT), aspartate aminotransferase (AST), GGT, total cholesterol (TC) and triglyceride (TG), platelet count (PLT) were collected during the first year of life in those who presented with neonatal cholestasis. The AST to PLT Ratio Index (APRI) was calculated utilizing laboratory data from the first year of life (11). Cardiac involvement was determined by review of echocardiogram (ECHO) reports. Magnetic resonance imaging (MRI) and/or computerized tomography (CT) scan reports were reviewed to determine the presence of cerebral and/or systemic vascular anomalies. Clinically Evident Portal Hypertension (CEPH) was defined as the combination of splenomegaly, as noted on ultrasound and thrombocytopenia (PLT count below 150×10^9 /L) and/or ascites requiring treatment with diuretics or esophageal or gastric varices requiring intervention at endoscopy (12). NLS was calculated from the date of

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birth until LT, death or date of last clinical follow-up, whichever event occurred first. To evaluate geographic differences, the cohort was stratified into one of seven geographic regions - (1) Africa; (2) Asia; (3) Europe; (4) the Middle East; (5) North America; (6) Oceania (Australia and New Zealand); and (7) South America and compared. The time to the first adverse liver-related event was calculated from the date of birth until one of the following events: CEPH, LT, death, or date of last known clinical follow-up. Patient and graft survival following LT were calculated from the date of LT until retransplantation, death or date of last known clinical follow-up. Overall survival (all-cause mortality, including post-transplant) was calculated from the date of birth until death or date of last known clinical follow-up, whichever event occurred first. In an analysis of the association between biochemistry data from the first year of life and long-term events (manifestations of PHT, CEPH or NLS), time was calculated from 12 months of age until the event of interest or date of last known clinical follow-up, whichever occurred first. Causes of death were classified into one of the following categories: Liver or LT-related complications, cardiac-related complications, non-cardiac vascular complications, multi-organ failure, sepsis, bleeding, other or unknown. All follow-up data were censored on August 31st, 2019. A summary of the data elements is provided in Supplementary Table 2.

Statistical Analysis

Descriptive statistics were summarized with medians and inter-quartile ranges (IQR) and categorical variables are reported as counts and percentages. Clinical characteristics between groups were compared using Chi-square test or Fisher's exact test, as appropriate. Laboratory values were log-transformed to normalize distributions and adjusted for regional differences by site-specific upper and lower limits of normal. For children who underwent Kasai

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portoenterostomy (KPE) during the first year of life, laboratory data were only reported up until the surgical procedure. All analyses were conducted in children with ALGS and a history of neonatal cholestasis and a secondary analysis were performed in the entire cohort to determine the rate of all-cause mortality. To assess the risk of NLS and LT in the presence of the competing risk of death, the Fine and Gray approach was applied (13). Time-dependent Cox proportional-hazards models were constructed to determine NLS in those who underwent a KPE or surgical BD. Event-free survival, patient and graft survival following LT and overall patient survival rates were determined using the Kaplan-Meier method and between group comparisons were made with log-rank test. To determine the utility of liver biochemistry as a prognostic marker of developing manifestations of PHT (ascites or varices), CEPH, and NLS, median serial laboratory measurements from the first year of life were calculated and potential associations were assessed using log-linear modeling. If a relationship was identified, thresholds were visually derived from the laboratory data distributions and the Akaike information criterion (AIC) and C-statistic values were compared. Cox proportional-hazards models were then used to assess the effect of the threshold on the event(s). Hazard ratios (HRs) and 95% confidence intervals (CIs) were estimated using univariate and multivariate Cox regression analysis and adjusted for sex, year of birth and geographical region by including this variable as a stratum. This allows each region to have its own background hazard rate, thereby allowing and repairing for heterogeneity. Those who had an event of interest (manifestations of PHT, CEPH or LT/death) in the first year of life or follow-up that ended before 1 year of age were excluded. A p -value <0.05 was considered statistically significant and the analysis was performed using Statistical Analysis System (SAS) version 9.4 and Statistical Package for the Social Sciences (SPSS, Chicago, IL) version 25.

RESULTS

Study Cohort

At the time of data extraction, a total of 1543 children with ALGS were reported in the GALA database. Of these subjects, 110 (7%) did not meet the study eligibility criteria and were excluded from further analysis. Ascertainment of the cohort is summarized in Fig. 1. The study cohort consisted of 1433 (57% male) clinically and/or genetically confirmed children with ALGS, with a median follow-up of 6.0 years (IQR 2.6 – 12.0). The largest number of patients were ascertained from Europe, 34% (n=487), followed by North America, 29% (n=420), and Asia, 23% (n=333). Evaluable results of molecular genetic testing were available in 62% (n=892/1433) of patients with ALGS and a pathogenic variant in *JAG1* or *NOTCH2* was identified in 98% (n=878/892). Among genotyped patients, 2% (n=14) were negative for a pathogenic variant in *JAG1* or *NOTCH2*, despite meeting clinical criteria for ALGS (≥ 3 clinical characteristics). The majority of ALGS cases were *de novo* (56%).

Table 1 summarizes the clinical characteristics of the entire study cohort and those with a history of neonatal cholestasis. Liver involvement at any age was reported in 95% (n=1321/1387) of children with ALGS and 85% (n=1184/1387) presented with neonatal cholestasis. During the study period, pruritus was reported in 74% (n=761/1028) of children with ALGS and first manifested at a median age of 12-months (IQR 6.2 – 26.0). Nearly one in four children with ALGS reported xanthomas (24%, n=236/980), first manifesting at a median age of 25 months (IQR 16.0 – 43.2). At the first presentation of the xanthomas, patients with ALGS had a median serum cholesterol of 646.0 mg/dL (IQR 398.0 – 1021.0; n=198).

Outcomes of Liver Disease

Among the 1184 patients with a history of neonatal cholestasis, 345 underwent an isolated LT and 4 underwent combined liver-kidney transplantation during the study period. Of note, an additional 14 patients with ALGS underwent LT who did not present with neonatal cholestasis, or their initial liver presentation was unknown.

To determine the rate of NLS, LT or risk of death without LT in those with a history of neonatal cholestasis (n=1184) a competing risk analysis was performed to evaluate the risk of each of these three independent outcomes (Fig. 2). At 5, 10, and 18-years, the rate of NLS was 66.8%, 54.4%, and 40.3%. The cumulative incidence of LT at 5, 10, and 18-years was 27.1% (95% CI, 24.3 – 30.1), 37.8% (95% CI, 34.2 – 41.3) and 50.4% (95% CI, 45.4 – 55.2). The risk of death without transplantation was 6.1% (95% CI, 4.7 – 7.7), 7.8% (95% CI, 6.1 – 9.8) and 9.3% (95% CI, 7.1 – 11.8). There were no significant differences between males and females in terms of NLS rates (log-rank, $p=0.35$). Rates of NLS were significantly different across the seven geographic regions (log-rank, $p<0.001$; Supplementary Figure 2A and Supplementary Table 3).

Among children with ALGS, 9% (n=102/1184) underwent a KPE at a median of 61 days (IQR 40.0 – 75.0; n=99). All KPE patients met the classic clinical criteria for ALGS, and the mutation detection rate was 88% (n=66/75). Infants who underwent KPE (n=99) were found to have a 3.2-fold increased risk of LT/death (95% CI 2.5 – 4.3, $p<0.001$). To account for the increased risk of LT/death among children who underwent a KPE, a sensitivity competing risk analysis was conducted to determine whether these patients were influencing the overall rate of NLS. In this analysis, children who underwent KPE were truncated at the time of their surgical procedure,

and the cumulative risk for LT/death was found to be comparable to the primary analysis - at 5, 10, and 18-years the rate of NLS was 70.7% (95% CI 26.2 – 32.5), 58.3% (95% CI 37.8 – 45.5) and 43.0% (95% CI, 51.5 – 62.2) (Supplementary Fig. 1).

A surgical BD was reported in 5% (n=56/1184) of children with ALGS, at a median age of 2.4 years (IQR 1.9 – 4.4; n=53). NLS rates in patients with ALGS who underwent a surgical BD, revealed a 1.9-fold greater risk of LT/death (95% CI 1.3 – 3.0; $p<0.001$).

The median age of LT was 2.8 years (IQR 1.6 – 5.4) and 72% (n=247) of transplants were performed during the first 5 years of life. The primary indications for LT were complications of persistent cholestasis (intractable pruritus, growth failure, xanthomas, metabolic bone disease, and/or fat-soluble vitamin deficiency) in 72% (n=235/328) and manifestations of PHT (ascites or varices) in 30% (n=97/328; Supplementary Table 4). Not surprisingly, 53% (n=174/328) of ALGS transplant recipients had more than one indication for LT. Of the 97 patients who underwent LT due to manifestations of PHT, 39% (n=38/97) also reported pruritus as an indication for LT. PHT as the indication for transplantation was more prevalent among older ALGS transplant recipients (median 4.1 years vs. 2.5 years, $p=0.001$).

The majority of children with ALGS underwent a deceased donor transplant (68%) and the median follow-up duration after LT was 4.0 years (IQR 1.3 – 8.6). Fig. 3 illustrates patient and graft survival rates following isolated LT. Patient survival rates following LT at 5, 10, and 20-years were 92.0%, 91.0%, and 88.0%, respectively (Fig. 3A). Graft survival rates in children with ALGS at 5, 10, and 20-years after LT were 88.0%, 86.3%, and 83.4% (Fig. 3B).

Analysis of Adverse Liver-related Events

Six hundred and fifty (650) adverse liver-related events were reported in 471 children with ALGS and a history of neonatal cholestasis. By 5, 10, and 18-years, 36.3%, 51.5% and 66.0% of children with ALGS experienced at least one adverse liver-related event (Fig 4.). At 10 and 18-years, there were no significant differences between males and females in terms of rates of event free survival (53.6% vs. 47.2% and 69.3% vs. 63.4%, respectively; log-rank, $p=0.22$).

To further explore the progression of liver disease in this ALGS cohort, the development of CEPH was investigated (Fig 5). The cumulative incidence of developing a combination of ultrasound-confirmed splenomegaly and $PLT < 150 \times 10^9/L$ was 39.2% at 10-years and 65.8% at 18-years. By 18-years of age, 22.3% of children with ALGS developed ascites requiring treatment with diuretics and 12.7% developed varices requiring intervention at endoscopy. By 18-years of age, 68.9% of patients developed CEPH.

Predictors of native liver survival

Among the 1184 patients with ALGS, serial laboratory measurements were available in 605 with 3777 follow-up visits during the first year of life. The median laboratory values were evaluated at the following 3 time points: (1) ≤ 6 months; (2) between >6 and ≤ 12 months; and (3) 12 months.

TB, CB, ALT, AST and APRI showed a significant log-linear relationship with LT or death (Supplementary Table 5). Based on the distribution of TB values, three thresholds were applied: (1) < 5.0 mg/dL; (2) ≥ 5.0 and < 10.0 mg/dL; (3) ≥ 10.0 mg/dL. As shown in Fig. 6A, a univariate

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cox-regression analysis showed children with ALGS (>6 and ≤ 12 months) with median TB levels <5.0 mg/dL had significantly higher rates of NLS compared to those ≥ 5.0 mg/dL, with 79.0% reaching adulthood with their native liver compared to 31.6% and 18.2% (log-rank, $p < 0.001$). Median TB levels between ≥ 5.0 and <10.0 mg/dL were associated with a 4.8-fold (95% CI 2.4 – 9.7) increased risk of LT. When utilizing a median serum TB cut-off of ≥ 10.0 mg/dL, the risk of LT increased to HR 15.6 (95% CI 8.7 – 28.2) relative to those <5.0 mg/dL.

To further explore the predictive value of TB, the applied thresholds were used to investigate the risk for developing CEPH. This analysis revealed children with ALGS (>6 and ≤ 12 months) with median TB levels between ≥ 5.0 and <10.0 mg/dL had a 4.1-fold (95% CI 1.6 – 10.8) increased risk of developing CEPH. A median serum TB threshold of ≥ 10.0 mg/dL, the risk was HR 8.0-fold (95% CI 3.4 – 18.4) versus <5.0 mg/dL; Supplementary Fig. 3).

The prognostic significance of the serum TB thresholds were also assessed for predicting manifestations of PHT (ascites or varices). Children (>6 and ≤ 12 months) with median TB levels between ≥ 5.0 and <10.0 mg/dL and those with ≥ 10.0 mg/dL had a significant increase in the likelihood of developing manifestations of PHT, 4.2-fold (HR 95% CI 0.9 – 19.0) and 4.5-fold (95% CI 1.1 – 18.0), respectively.

Based on the distribution of AST, a median AST value ≥ 152 U/L between >6 and ≤ 12 months of life was identified and associated with an increased risk of LT (HR 1.9, 95% CI 1.4 – 2.6; Fig 6.C). No other specific cut-offs for serum liver biochemistries, including serum bile acids, from the first year of life, were significantly associated with LT or death.

To explore potential bias, a sensitivity analysis was performed to assess rates of NLS between children with (n=600) and without TB data (n=406) in the first year of life using the Kaplan-Meier method and a log-rank. In this analysis, there were no significant differences in rates of NLS between those with and without available laboratory data (log-rank, $p=0.78$)

Hepatocellular carcinoma (HCC)

Histologically proven HCC was reported in <1.0% (n=9/1433) of the study cohort and the median age of diagnosis was 4.1 years (IQR 1.5 – 8.3). Hepatic fibrosis was reported in 55% (n=5/9) of native liver biopsies and 71% (n=5/7) had CEPH. Among these patients, 6 underwent LT, 2 died before LT, and one patient underwent surgical resection and remains alive at last clinical follow-up. The majority of patients were diagnosed with HCC prior to LT (n=4/6).

Histopathology

Seven hundred and fifty-four native liver biopsy reports were retrospectively reviewed in 604 patients with ALGS and a history of neonatal cholestasis. Bile duct paucity was reported in only 65% (n=202/311) of liver biopsies performed during the first 3 months of life. Features of biliary obstruction, including bile duct proliferation and/or ductular bile plugs, were reported in 22% (n=69/311) of patients aged ≤ 3 months. Among these 69 patients with ALGS and histologic features of obstruction, 44 underwent genetic testing and a pathogenic variant in *JAG1* or *NOTCH2* was identified in 31 patients (70%). In a subgroup analysis, patients were stratified according to the presence or absence of baseline bile duct paucity (Supplementary Table 6). Children with ALGS without baseline bile duct paucity were significantly more likely to have giant cell transformation ($p<0.001$) and features of biliary obstruction ($p<0.001$).

Histological features were compared based on age at first native liver biopsy: (1) ≤ 6 months; (2) between >6 and ≤ 24 months; and (3) >24 months. As shown in Supplementary Table 7, the presence of bile duct paucity and fibrosis increased significantly with advancing age ($p < 0.001$; $p < 0.001$). As expected, the frequency of giant cell transformation significantly decreased with age ($p < 0.001$).

Of the 604 children with ALGS, 14% ($n=85/604$) underwent two or more native liver biopsies at least 12 months apart during the study period (Supplementary Table 8). The patient's first and second biopsies were compared and the median interval between biopsies was 31.0 months (IQR 20.5 – 61.0). Second biopsies were significantly more likely than baseline biopsies to show liver fibrosis (28% vs. 62%, $p=0.01$), but not bile duct paucity (66% vs. 86%, $p=0.53$). Bile duct paucity was not reported on either baseline or follow-up biopsy in 6% ($n=5/86$) of children with ALGS who underwent a second biopsy. Among these 5 patients without bile duct paucity on either biopsy, a pathogenic variant in *JAG1* or *NOTCH2* was identified in 80% ($n=4/5$).

Mortality

During the study period, 108 deaths were reported in all 1433 children with ALGS. At 5, 10 and 18-years the rate of overall patient survival was 92.8%, 91.2%, and 88.1%, respectively (Supplementary Fig. 4). Significant differences in overall survival rates across geographic regions were observed (log-rank, $p=0.002$; Supplementary Figure 2B). The median age of death was 2.6 years (1.2 – 4.7). Liver-related complications, including LT complications, were the leading cause of death (22%; median age 2.8 years (IQR 1.6 – 6.7), followed by cardiac-related complications in 18% (median age 1.1 years, IQR 0.5 – 4.5), and multi-organ failure (median age

3.9 years, IQR 3.0 – 7.0) and non-cardiac vascular complications (median age 2.2 years, IQR 1.5 – 3.2) accounted for 15% each.

A survival analysis was performed to compare overall survival in ALGS with (n=1184) and without a history of neonatal cholestasis (n=203). A Kaplan–Meier analysis showed the cumulative rate of survival at 10- and 18-years was significantly lower in ALGS children who presented with neonatal cholestasis in comparison with those who did not (log-rank $p<0.001$; Supplementary Fig. 5). The 10- and 18 patient survival rates in patients with ALGS presenting with cholestasis were 89% and 86%, compared to 100% and 97%, respectively.

DISCUSSION

The GALA database is the largest cohort of children with ALGS ever ascertained and encompasses a broad spectrum of sites in terms of center size and geography, with 29 countries represented. This first analysis of the GALA dataset provides unique insights into the liver disease natural history and outcomes of this complex disorder. In this real-world, global view, 40% of children with ALGS-cholestasis reached adulthood with their native liver. A competing risk analysis revealed that this rate of NLS is largely driven by LT and perhaps surprisingly not death, despite the multi-system nature of this disorder with substantial cardiac involvement. As expected, heterogeneity in rates of NLS were observed across geographic regions and likely represents differences in allocation policies and clinical resources. The burden of liver disease is greatest among young children with the median age of LT being only 2.8 years and almost three-quarters of all LTs occurring in the first 5 years of life. The majority of early LTs occur due to complications associated with cholestasis, including pruritus, with a smaller number occurring

later in childhood due to the onset of PHT. The NLS of 40% at 18 years is higher than the 24% rate recently reported by the Childhood Liver Disease Research Network (ChILDRen) which is comprised of North American tertiary referral centers and therefore may select a more severely affected group of patients (6). In addition to LT, a time to first adverse liver-related event analysis demonstrates the extent of clinically significant liver co-morbidities occurring during childhood with more than 60% of children experiencing an adverse liver event during the study period. These data highlight the consequences of profound cholestasis arising from developmental defects of the biliary tree and reveal the unmet need to develop targeted therapies for ALGS-related cholestasis to the youngest children. Since the majority of transplants in ALGS have pruritus as a leading indication, anti-pruritic agents which target cholestasis (rather than fibrosis) may also offer hope to change disease biology and extend NLS. The natural history data and outcomes presented here are essential to evaluate therapies already in clinical trials and to identify rational novel therapies.

Serum TB level in children with ALGS aged 6 and ≤ 12 months was identified as a prognostic marker for long-term hepatic outcomes. Median TB levels < 5.0 mg/dL during this early stage of life were associated with significantly higher rates of NLS, with 79% reaching adulthood with their native liver. Children with ALGS (> 6 and ≤ 12 months) and median TB levels between ≥ 5.0 and < 10.0 mg/dL had an almost 5-fold increased risk of LT and > 10 was 15.6-fold as compared to < 5.0 mg/dL. It is important to note that these TB thresholds are not only associated with LT, but also appear to be associated with progressive liver disease, as shown by their association with the development of CEPH. Children with ALGS (> 6 and ≤ 12 months) with median TB levels between ≥ 5.0 and < 10.0 mg/dL had a 4.1 increased risk of developing CEPH

and those with TB >10.0 mg/dl had a 8.0-fold increased risk. It is intriguing that a median AST >152 in the first year of life is also associated with NLS which may portend the onset of PHT (13).

These data provide novel and clinically valuable information to the clinician managing infants with ALGS liver disease. One of the challenges associated with the management of ALGS liver disease is the decision-making surrounding timing of LT. Unlike biliary atresia (BA), in which cholestasis is often progressive even after KPE, in ALGS, a patient's cholestasis can resolve or stabilize (14). Therefore, not all cholestatic children with ALGS inevitably require LT and there is a strong need for early predictors of NLS. In a previous study, Mouzaki et al identified a serum total bilirubin cut-off of 3.8 mg/dL between 12 and 24 months of life as a threshold for poor hepatic outcome (surgical biliary diversion or LT), however this threshold was applied at an older age and is therefore less clinically useful early on. Furthermore, the sample size was limited in this analysis and patients were stratified into outcome groups at the age of 10 years, thereby not accounting for events beyond this age (14).

It may be considered surprising that serum bile acids in the first year of life were not predictive of NLS in ALGS. Observations from real world practice at GALA sites clearly show that serum bile acid levels are not routinely sent on a clinical basis in ALGS. Only 247 patients had serum bile acid levels available in the first year of life for this analysis. This is in stark contrast to other laboratory variables evaluated as predictors for which levels were available for >600 patients. Therefore, the lack of association of serum bile acids and liver disease outcomes

in ALGS warrants further prospective investigation, especially in an era when ASBT inhibitors that target serum bile acid levels are now approved therapies (15).

This series represents the largest review of liver biopsy reports in ALGS. Examination of liver tissue histopathology in this extensive cohort also provides insights that will aid diagnosis. It is noteworthy that bile duct paucity was reported in only 65% of liver biopsies performed during the first 3 months of life, the period during which there are diagnostic challenges with distinguishing ALGS from syndromic BA. It is crucial for clinicians to appreciate that more than one-third of ALGS infants will not have bile duct paucity and that further, almost one-quarter have histologic features of biliary obstruction. These observations suggest that ductular proliferation and/or bile duct plugs are not pathognomonic of biliary atresia and are present in a sizeable subset of infants with ALGS, potentially leading to misdiagnosis. Clearly future histopathologic review of early ALGS liver biopsies is warranted, though even the current data alone can inform clinical practice. These data highlight the limitations of relying on histopathology for diagnostic purposes in cholestatic infants and support the incorporation of rapid genetic testing into diagnostic algorithms (16). Consistent with earlier reports, though in a substantially larger cohort, this study also found that the frequency at which bile duct paucity is identified on liver biopsy increases with advancing age (3). However, in individual patients who have undergone repeat liver biopsies that the progression of bile duct paucity over time did not reach statistical significance.

The GALA Study demonstrates that global patient and graft survival rates in children with ALGS are very good exceeding 83% at 18-years of age. These observations are comparable

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to two prior North American analyses of ALGS (17, 18). This reflects the global clinical practice of carefully selecting recipients with ALGS. Some children with ALGS may be deemed ineligible for LT due to a severe cardiac phenotype, though with expert input from cardiologists this number of patients should diminish over time (19). It is also notable that there is substantial early mortality 52% (<30 days post-LT) in ALGS. This concerning observation was also reported by the prior SPLIT study in which early post-LT mortality rates were significantly higher in ALGS in comparison to patients transplanted for BA (18).

In this contemporary cohort of children with ALGS, the all-cause mortality rate was 8.5%. This rate is considerably lower than earlier reports and likely reflects an increased availability of LT, earlier interventions for cardiac defects and subspecialty clinical teams working synergistically to manage the multisystem disease burden (1-3, 20, 21). Furthermore, although previous studies have estimated long-term outcomes in ALGS, most of them were conducted without considering competing risks. Among reported deaths, liver or LT related-complications were the most common, followed closely, by cardiac-related-complications, together accounting for almost half of all deaths. Notably, most deaths occurred during the first five years of life. Patient survival data in ALGS underscore the need for adult clinicians to be aware of ALGS and the medical needs of these individuals as they transition from pediatric to adult care. There is currently a paucity of research in this age-bracket and specific recommendations for the clinical management of ALGS adults are lacking.

While our study provides novel insight into the natural history and outcomes of ALGS, there are certain limitations that require comment. This is a retrospective study and therefore it

was not feasible to assess the severity and evolution of pruritus and xanthomas from medical records. This reflects the variability with which clinicians assess these two clinically important features of ALGS and speaks to the need for standardized methods to assess and record these on a clinical basis. In addition, the histopathology data reported were extracted from local liver biopsy reports rather than central review. Histologic staging systems clearly vary by site and to overcome this to some extent, fibrosis was reported as a binary variable for this analysis though this does limit its value. Finally, the prevalence and spectrum of hepatic involvement may still be underestimated as individuals who present with partial or subclinical disease expression may go undetected and are not represented in this analysis. Overall, despite the retrospective nature of the GALA, the data were rigorously collected and queried by the DCC to account for incompleteness.

CONCLUSIONS

The GALA study provides a comprehensive real-world analysis of ALGS liver disease in more than 1400 children with ALGS from 29 countries. A TB below 5.0 mg/dL between 6 and 12 months of age appears to be associated with better native liver survival and may help guide complex decisions regarding timing of LT which is challenging in ALGS. These natural history data are important to evaluate novel therapies which are currently in clinical trials for ALGS. With 40% of children with ALGS surviving to adulthood with native liver, adult hepatologists also need to be aware of ALGS and its complex manifestations.

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REFERENCES

1. Alagille D, Odievre M, Gautier M, Dommergues JP. Hepatic ductular hypoplasia associated with characteristic facies, vertebral malformations, retarded physical, mental, and sexual development, and cardiac murmur. *J Pediatr* 1975;86:63-71.
2. Alagille D, Estrada A, Hadchouel M, Gautier M, Odievre M, Dommergues JP. Syndromic paucity of interlobular bile ducts (Alagille syndrome or arteriohepatic dysplasia): review of 80 cases. *J Pediatr* 1987;110:195-200.
3. Emerick KM, Rand EB, Goldmuntz E, Krantz ID, Spinner NB, Piccoli DA. Features of Alagille syndrome in 92 patients: frequency and relation to prognosis. *Hepatology* 1999;29:822-829.
4. Gilbert MA, Bauer RC, Rajagopalan R, Grochowski CM, Chao G, McEldrew D, Nassur JA, et al. Alagille syndrome mutation update: Comprehensive overview of JAG1 and NOTCH2 mutation frequencies and insight into missense variant classification. *Hum Mutat* 2019;40:2197-2220.
5. Leonard LD, Chao G, Baker A, Loomes K, Spinner NB. Clinical utility gene card for: Alagille Syndrome (ALGS). *Eur J Hum Genet* 2014;22.

- Accepted Article
6. Kamath BM, Ye W, Goodrich NP, Loomes KM, Romero R, Heubi JE, Leung DH, et al. Outcomes of Childhood Cholestasis in Alagille Syndrome: Results of a Multicenter Observational Study. *Hepatol Commun* 2020;4:387-398.
 7. Shneider BL, Spino C, Kamath BM, Magee JC, Bass LM, Setchell KD, Miethke A, et al. Placebo-Controlled Randomized Trial of an Intestinal Bile Salt Transport Inhibitor for Pruritus in Alagille Syndrome. *Hepatol Commun* 2018;2:1184-1198.
 8. Harris PA, Taylor R, Thielke R, Payne J, Gonzalez N, Conde JG. Research electronic data capture (REDCap)--a metadata-driven methodology and workflow process for providing translational research informatics support. *J Biomed Inform* 2009;42:377-381.
 9. Aghazadeh-Attari J, Mobaraki K, Ahmadzadeh J, Mansorian B, Mohebbi I. Quality of observational studies in prestigious journals of occupational medicine and health based on Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) Statement: a cross-sectional study. *BMC Res Notes* 2018;11:266.
 10. Richards S, Aziz N, Bale S, Bick D, Das S, Gastier-Foster J, Grody WW, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genet Med* 2015;17:405-424.

- Accepted Article
11. Wai CT, Greenson JK, Fontana RJ, Kalbfleisch JD, Marrero JA, Conjeevaram HS, Lok AS. A simple noninvasive index can predict both significant fibrosis and cirrhosis in patients with chronic hepatitis C. *Hepatology* 2003;38:518-526.
 12. Bass LM, Shneider BL, Henn L, Goodrich NP, Magee JC, Childhood Liver Disease Research N. Clinically Evident Portal Hypertension: An Operational Research Definition for Future Investigations in the Pediatric Population. *J Pediatr Gastroenterol Nutr* 2019;68:763-767.
 13. Shneider BL, Abel B, Haber B, Karpen SJ, Magee JC, Romero R, Schwarz K, et al. Portal hypertension in children and young adults with biliary atresia. *J Pediatr Gastroenterol Nutr* 2012;55:567-573.
 14. Mouzaki M, Bass LM, Sokol RJ, Piccoli DA, Quammie C, Loomes KM, Heubi JE, et al. Early life predictive markers of liver disease outcome in an International, Multicentre Cohort of children with Alagille syndrome. *Liver Int* 2016;36:755-760.
 15. Gonzales E, Hardikar W, Stormon M, Baker A, Hierro L, Gliwicz D, Lacaille F, et al. Efficacy and safety of maralixibat treatment in patients with Alagille syndrome and cholestatic pruritus (ICONIC): a randomised phase 2 study. *Lancet* 2021;398:1581-1592.
 16. Karpen SJ, Kamath BM, Alexander JJ, Ichetovkin I, Rosenthal P, Sokol RJ, Dunn S, et al. Use of a Comprehensive 66-Gene Cholestasis Sequencing Panel in 2171 Cholestatic Infants, Children, and Young Adults. *J Pediatr Gastroenterol Nutr* 2021;72:654-660.

- Accepted Article
17. Arnon R, Annunziato R, Schiano T, Miloh T, Baisley M, Sogawa H, Contreras AG, et al. Orthotopic liver transplantation for adults with Alagille syndrome. Clin Transplant 2012;26:E94-E100.
 18. Kamath BM, Yin W, Miller H, Anand R, Rand EB, Alonso E, Bucuvalas J, et al. Outcomes of liver transplantation for patients with Alagille syndrome: the studies of pediatric liver transplantation experience. Liver Transpl 2012;18:940-948.
 19. Luong R, Feinstein JA, Ma M, Ebel NH, Wise-Faberowski L, Zhang Y, Peng LF, et al. Outcomes in Patients with Alagille Syndrome and Complex Pulmonary Artery Disease. J Pediatr 2021;229:86-94 e84.
 20. Hoffenberg EJ, Narkewicz MR, Sondheimer JM, Smith DJ, Silverman A, Sokol RJ. Outcome of syndromic paucity of interlobular bile ducts (Alagille syndrome) with onset of cholestasis in infancy. J Pediatr 1995;127:220-224.
 21. Quiros-Tejeira RE, Ament ME, Heyman MB, Martin MG, Rosenthal P, Hall TR, McDiarmid SV, et al. Variable morbidity in alagille syndrome: a review of 43 cases. J Pediatr Gastroenterol Nutr 1999;29:431-437.

Author names in bold designate shared co-first authorship

FIGURE LEGENDS

Fig. 1. Ascertainment of The GALA Study cohort stratified by Alagille syndrome (ALGS) patients with and without a history of neonatal cholestasis. Abbreviations: GALA, The Global Alagille Alliance (GALA) Study; *JAG1*, *JAGGED1*; VUS, variant of uncertain significance.

Fig. 2. Cumulative incidence of native liver survival in the presence of competing events (LT or risk of death without LT; n=1184) in children with Alagille syndrome (ALGS) who presented with neonatal cholestasis. At 5, 10, and 18-years, the rate of NLS was 66.8%, 54.4%, and 40.3%. Abbreviation: LT, liver transplantation.

Fig. 3. Patient and graft survival following isolated liver transplantation (LT) in 345 children with Alagille syndrome (ALGS). Patient and graft survival following LT is calculated from the date of LT until retransplantation, death or the date of last known clinical follow-up. (A) After LT, 10- and 20-year patient survival rates were 91.0% and 88.0%, respectively. (B) After LT, 10- and 20-year graft survival rates were 86.3% and 83.4%, respectively. Abbreviation: LT, liver transplantation.

Fig. 4. Time to first adverse liver-related event in 1184 children with Alagille syndrome (ALGS). A Kaplan-Meier analysis revealed by 5, 10, and 18-years, 36.3%, 51.5% and 66.0% of children with ALGS will experience at least one adverse liver-related event.

Fig. 5. Cumulative incidence of clinically evident portal hypertension in an international cohort of children with Alagille syndrome (ALGS). Abbreviation: PLT, platelet count

Fig. 6. Early biochemical predictors for native liver survival in children with Alagille syndrome (ALGS). Abbreviation: AST, aspartate aminotransferase.

Table 1. Baseline clinical features for 1433 children with Alagille syndrome (ALGS) and those who presented with neonatal cholestasis (n=1184).

	All	History of Neonatal Cholestasis ¹	
<i>n</i>	1433	1184	
Male, % (n)	57% (n=823)	59% (n=694)	
Age at first clinical suspicion, % (n)			
0 - 1 years	82% (n=1148/1408)	88% (n=1033/1171)	
<i>de novo</i> , % (n)	54% (n=415/762)	58% (n=348/597)	
Genetically confirmed diagnoses, % (n)	61% (n=878)	60% (n=715)	
Diagnostic criteria, % (n)			
Liver involvement, any ²	95% (n=1321/1387)	100%	
Echo-confirmed cardiac anomaly, any	91% (n=1231/1347)	91% (n=1017/1114)	
Characteristic facies	90% (n=1193/1325)	90% (n=984/1091)	
Posterior embryotoxon	51% (n=605/1179)	51% (n=503/992)	
Butterfly vertebrae	44% (n=549/1262)	44% (n=472/1063)	
Renal anomaly, any	39% (n=500/1275)	39% (n=422/1071)	
Vascular involvement, any	36% (n=189/532)	34% (n=152/448)	
Median laboratory values in the first year of life in children with ALGS who presented with neonatal cholestasis³	≤6 months	>6 and ≤12 months	12 months
Bile acids, μmol/L	139.2 (IQR 98.6 – 205.7) (n=192)	181.8 (IQR 76.4 – 285.8) (n=100)	139.9 (IQR 95.8 – 221.0) (n=247)
Total bilirubin, mg/dL	8.0 (IQR 5.8 – 10.4) (n=497)	6.3 (IQR 1.2 – 11.8) (n=419)	7.9 (IQR 4.1 – 11.0) (n=600)
Conjugated bilirubin, mg/dL	5.5 (IQR 3.5 – 7.5) (n=440)	4.4 (IQR 0.8 – 8.2) (n=338)	5.5 (IQR 2.7 – 7.9) (n=529)
ALT, IU/L	154.0 (IQR 96.0 – 228.0) (n=503)	153.0 (IQR 96.0 – 224.0) (n=439)	153.0 (IQR 99.0 – 222.8) (n=605)
AST, IU/L	178.0 (IQR 116.0 – 263.5) (n=479)	154.0 (IQR 106.0 – 223.0) (n=407)	165.0 (IQR 118.0 – 246.0) (n=577)
GGT, IU/L	510.5 (IQR 258.3 – 830.5) (n=490)	427.5 (IQR 222.0 – 782.0) (n=409)	463.0 (IQR 250.5 – 826.5) (n=599)
Cholesterol, mg/dL	228.0 (IQR 170.0 – 309.0)	340.0 (IQR 224.0 – 619.0)	251.0 (IQR 186.0 – 387.0)

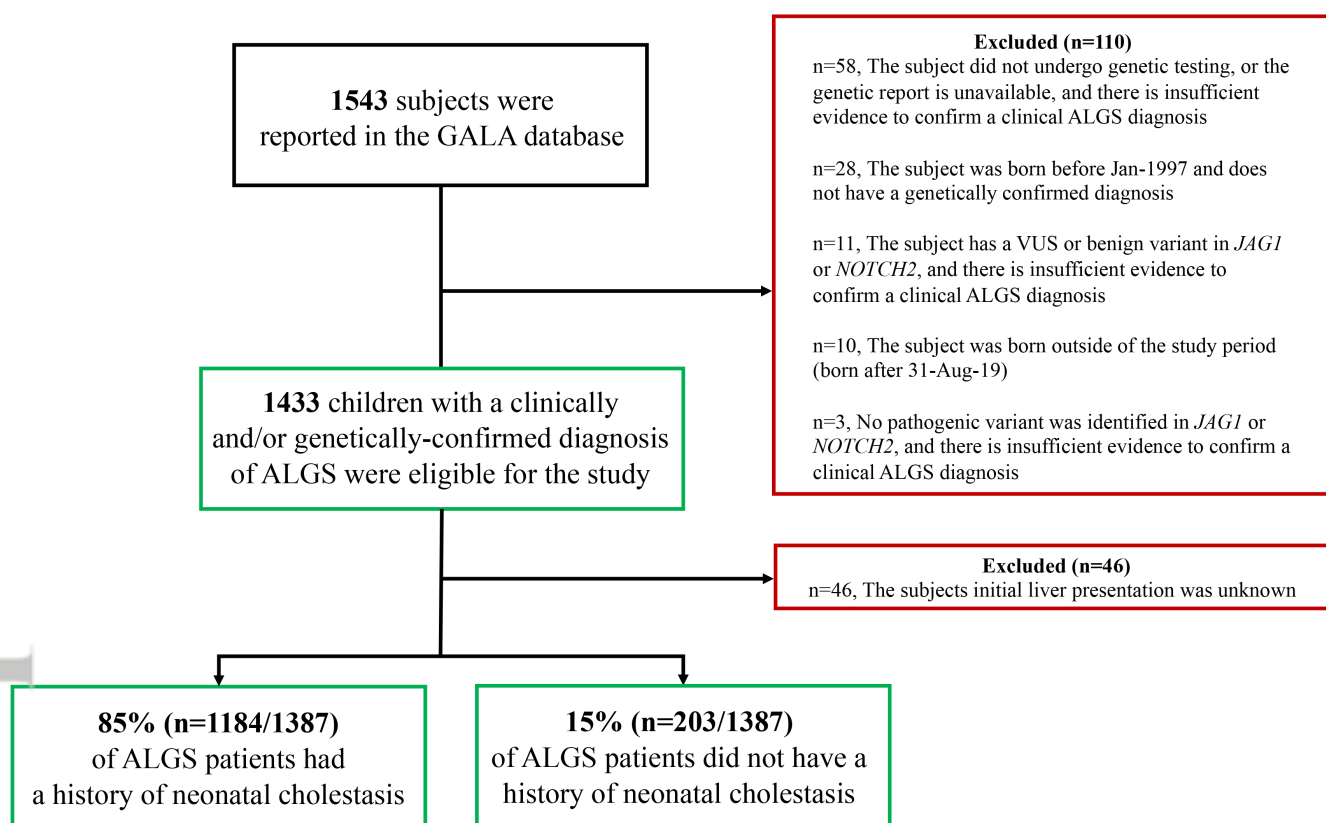
	(n=275)	(n=151)	(n=355)
Triglycerides, mg/dL	381.0 (IQR 274.0 – 531.0) (n=226)	522.0 (IQR 381.0 – 726.0) (n=123)	416.0 (IQR 301.0 – 584.0) (n=298)
Platelet count, 10 ⁹ /L	504.0 (IQR 389.0 – 605.0) (n=451)	386.0 (IQR 312.7 – 482.5) (n=377)	436.5 (IQR 349.8 – 550.3) (n=562)

Abbreviations: echo, echocardiogram; ALT, Alanine transaminase; AST, aspartate aminotransferase; GGT, gamma-glutamyl transferase.

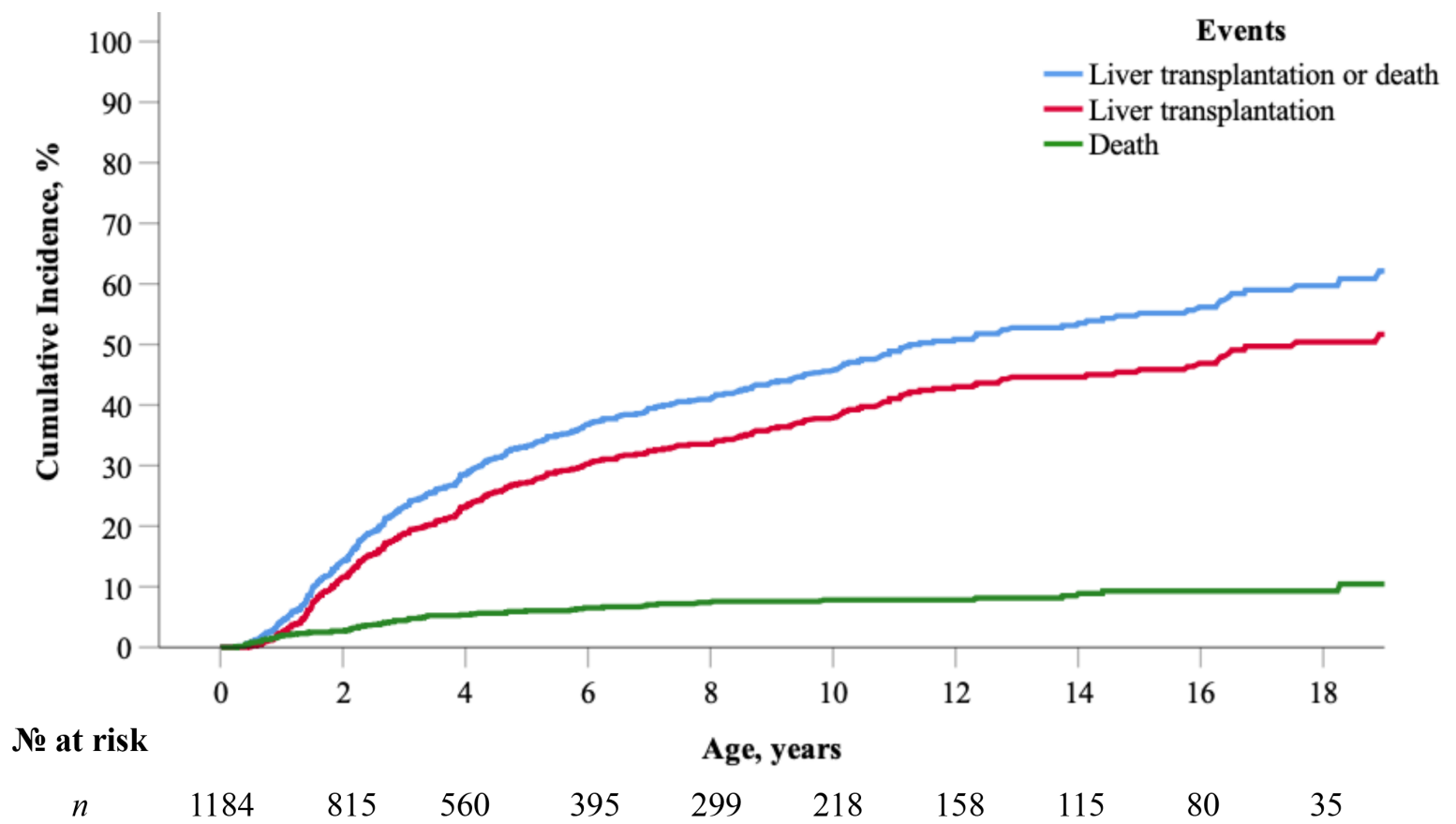
¹Neonatal cholestasis was defined as having at least one of the following features during the first three months of life: (1) direct/conjugated bilirubin greater than >2.0 mg/dL (34.0 µmol/L); (2) serum bile acids or GGT greater than 3 times the upper limit of normal (ULN); (3) fat-soluble vitamin deficiency, otherwise unexplainable.

²Liver involvement at any age was defined as a history of neonatal cholestasis, elevated liver transaminases, histological abnormalities, history of pruritus and/or xanthomas or underwent hepatobiliary surgery.

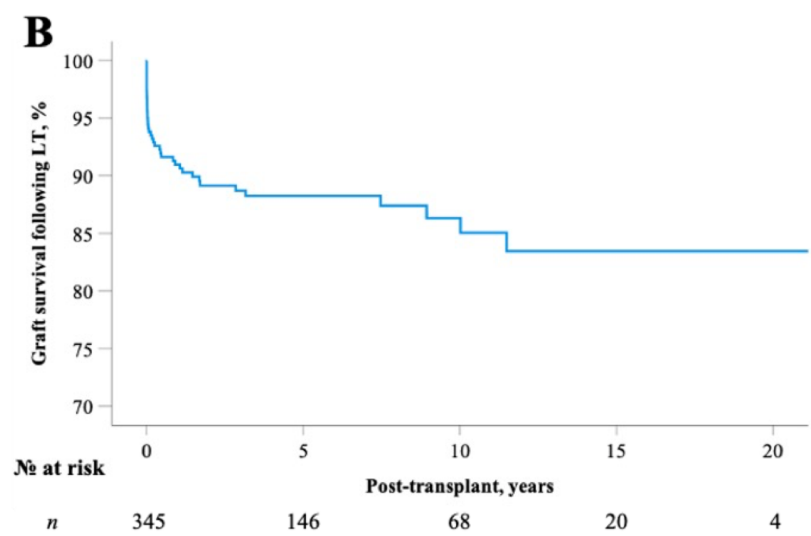
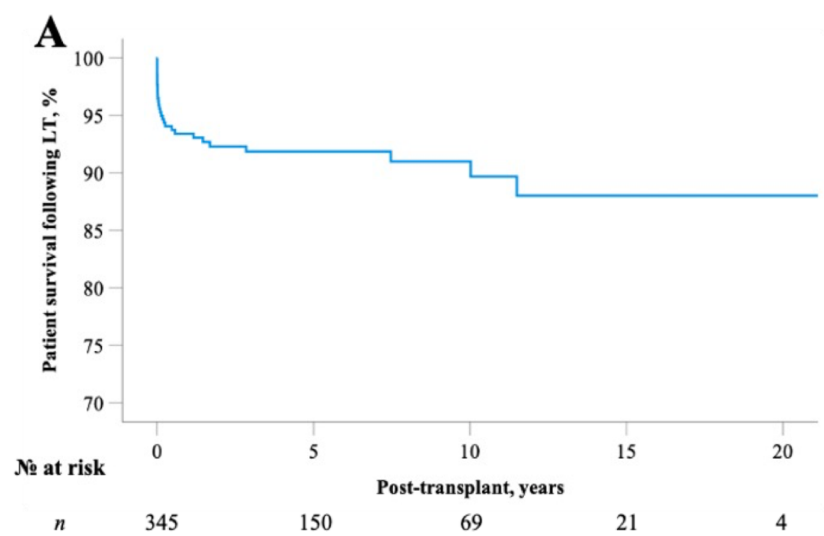
³In children who underwent Kasai portoenterostomy during the first year of life, laboratory data was only reported up until the procedure. Those who underwent LT or died in the first year of life or their follow-up ended before 1 year of age were excluded from the laboratory analysis.



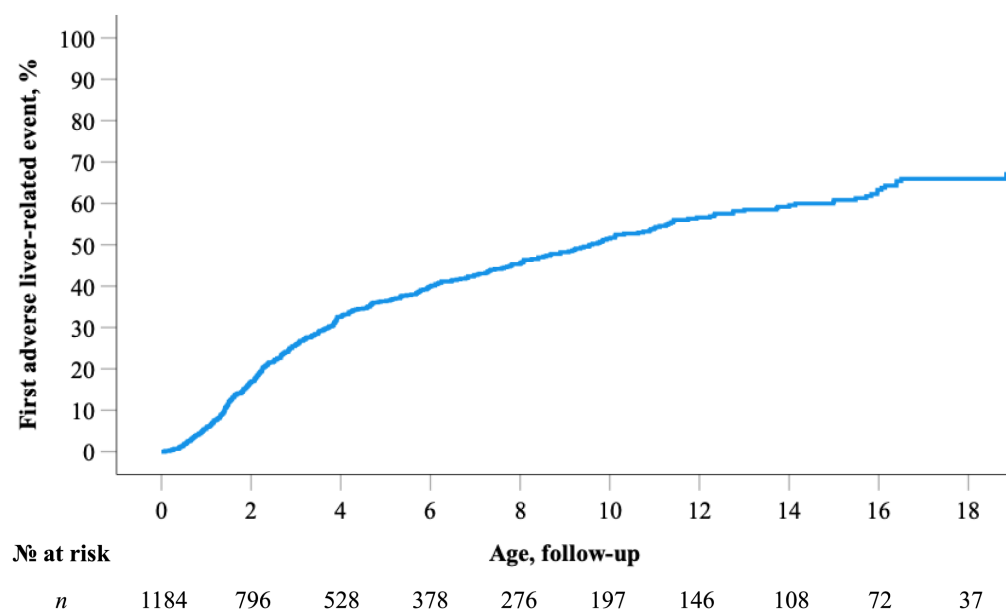
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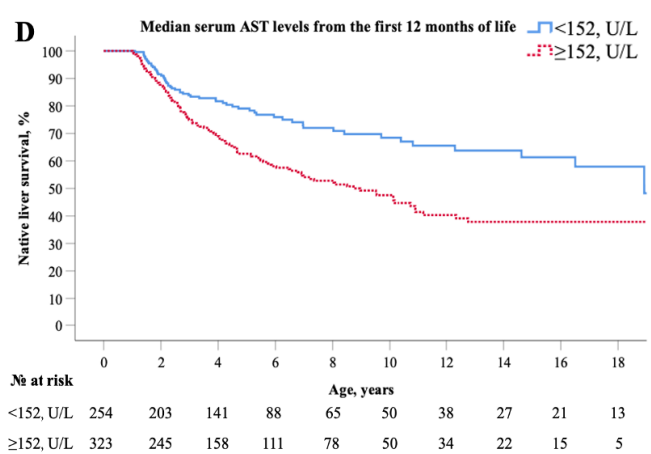
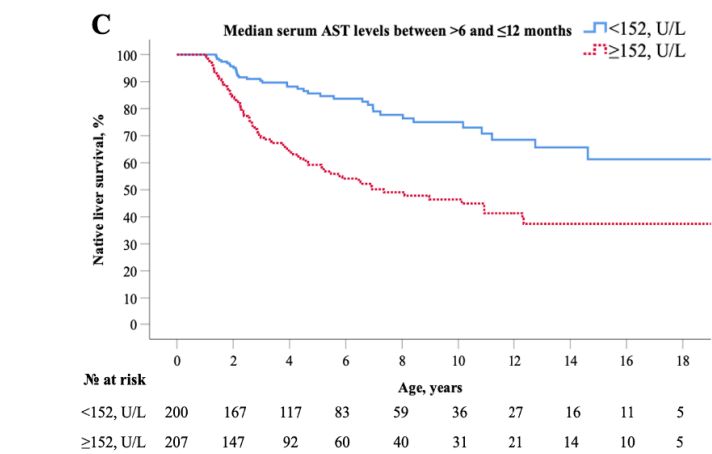
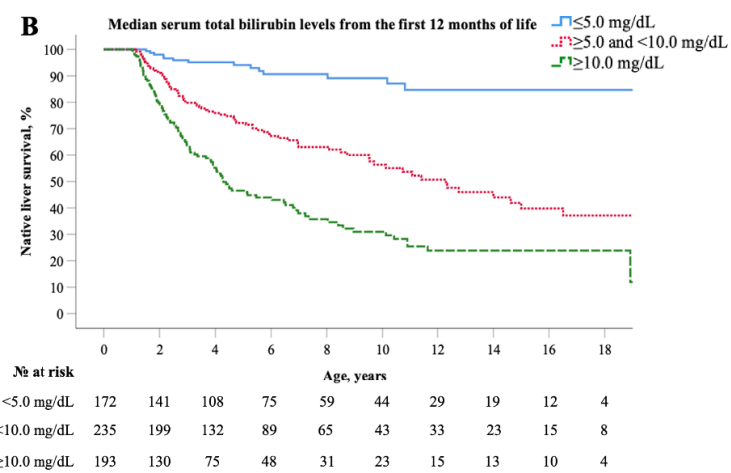
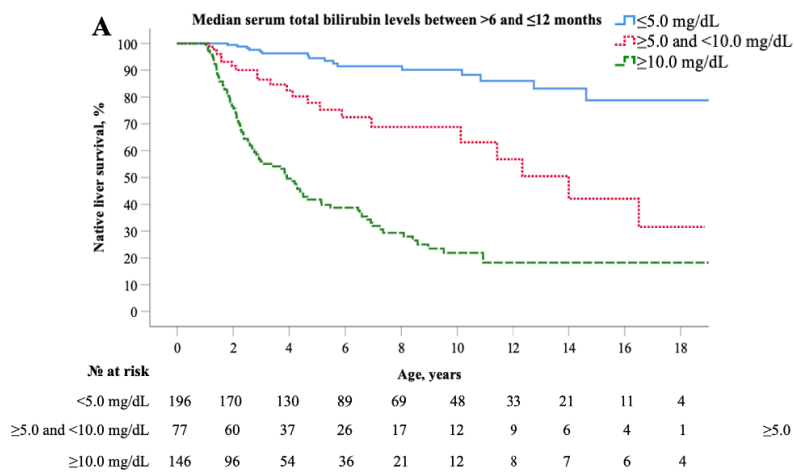
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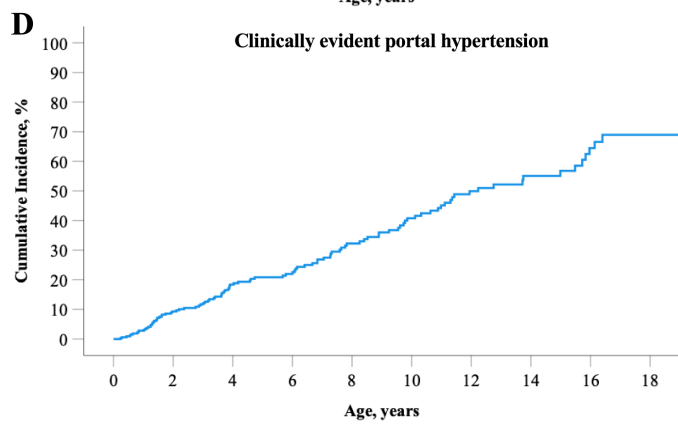
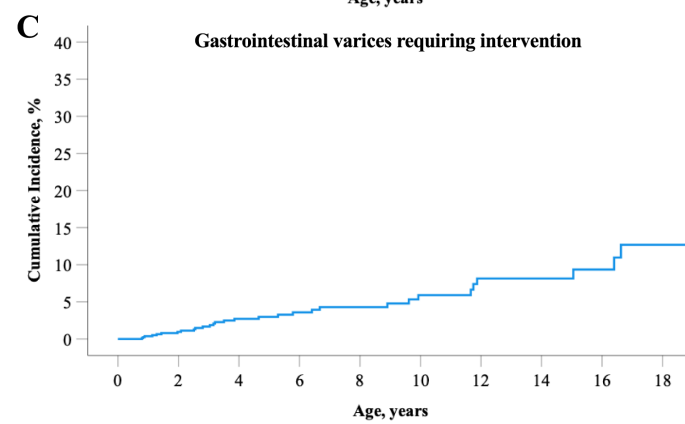
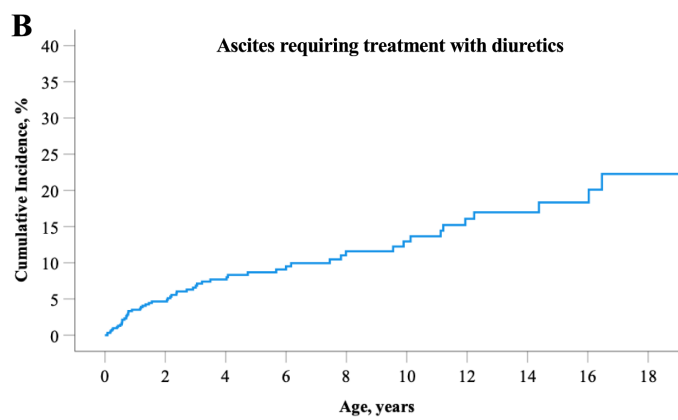
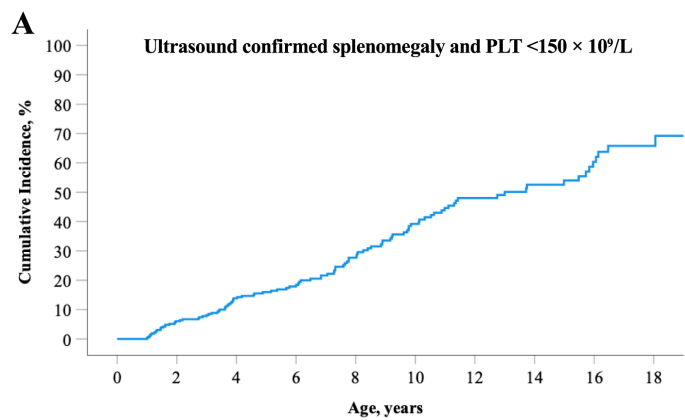
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