Features of Alagille Syndrome in 92 Patients: Frequency and Relation to Prognosis

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We have studied 92 patients with Alagille syndrome (AGS) to determine the frequency of clinical manifestations and to correlate the clinical findings with outcome. Liver biopsy specimens showed paucity of the interlobular ducts in 85% of patients. Cholestasis was seen in 96%, cardiac murmur in 97%, butterfly vertebrae in 51%, posterior embryotoxon in 78%, and characteristic facies in 96% of patients. Renal disease was present in 40% and intracranial bleeding or stroke occurred in 14% of patients. The presence of intracardiac congenital heart disease was the only clinical feature statistically associated with increased mortality ($P < .001$). Initial measures of hepatic function in infancy including absence of scintiscan excretion were not predictive of risk for transplantation or increased mortality. The hepatic histology of these AGS patients showed a significant increase in the prevalence of bile duct paucity ($P = .002$) and fibrosis ($P < .001$) with increasing age. Liver transplantation for hepatic decompensation was necessary in 21% (19 of 92) of patients with 79% survival 1-year posttransplantation. Current mortality is 17% (16 of 92). The factors that contributed significantly to mortality were complex congenital heart disease (15%), intracranial bleeding (25%), and hepatic disease or hepatic transplantation (25%). The 20-year predicted life expectancy is 75% for all patients, 80% for those not requiring liver transplantation, and 60% for those who required liver transplantation. (HEPATOLOGY 1999;29:822-829.)

Alagille syndrome (AGS) is an autosomal dominant disorder that involves abnormalities of varying severity in multiple organ systems.[1-6] The diagnosis of AGS traditionally has been based on the finding of paucity of the interlobular bile ducts associated with three to five major features: chronic cholestasis, cardiac disease, skeletal abnormalities, ocular abnormalities, and a characteristic facial phenotype. The phenotypic findings in AGS are highly variable in severity and clinical significance. In a single family, one patient may present with life-threatening congenital heart disease, whereas others may possess only subtle manifestations.[7] The wide range of affected organs coupled with the variable severity of involvement at each site makes overall clinical prognostication difficult. The phenotypic spectrum of clinical AGS has recently been shown to be because of defects in a single gene, *Jagged1*, which encodes a ligand in the developmentally important Notch intercellular signaling pathway.[8,9] The mechanism by which defects in *Jagged1* cause AGS is not currently understood. Preliminary studies have not shown a relationship between genotypes and specific disease phenotypes in AGS.[10]

The goals of this retrospective study of 92 individuals with AGS are: (1) to report the prevalence of the major and minor clinical features of AGS, (2) to describe the findings and discuss the implications of diagnostic testing in infants at initial presentation, (3) to review the initial and subsequent hepatic histology, and (4) to determine if clinical features predict morbidity, transplantation, or death.

PATIENTS AND METHODS

Affected individuals with AGS were identified from the pediatric gastroenterology records at the Children's Hospital of Philadelphia (CHOP; Philadelphia, PA) (n = 36), the Children's Hospital of the University of Chicago (Chicago, IL) (n = 14), or from referrals to the Children's Hospital of Philadelphia by geneticists and pediatric gastroenterologists from 20 different centers (n = 42 patients) in the United States. Medical records and summaries of the patients' clinical courses since diagnosis accompanied referrals. The data were collected over a period of 3 years, which represented records from the years 1974 to 1997. The diagnosis of AGS was based on the presence of three or more of the major clinical features: chronic cholestasis, cardiovascular abnormalities, vertebral anomalies, ocular anomalies, and characteristic facies. The patients were interviewed and examined by the investigators of this report or by another pediatric gastroenterologist. Medical records were reviewed for all laboratory data, test results (*i.e.*, echocardiograms and radiographs), subspecialty consultations (*e.g.*, ophthalmologic examinations), histology reports, surgeries, and family history. Chronic cholestasis was defined as persistent elevation of direct bilirubin (>17 μmol/L or 1.0 mg/dL) beyond 6 months of age.[11] The presence of major and minor features were recorded (Table 1). The diagnosis of AGS was confirmed by a combination of physical examination and chart review, independently verified by both the referring and the consulting gastroenterologists. Family members were assigned

Abbreviations: AGS, Alagille syndrome; PPS, peripheral pulmonic stenosis; TOF, tetralogy of Fallot; PA, pulmonary atresia; VSD, ventricular septal defect; CNS, central nervous system.

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TABLE 1. Syndromic Features of 92 Patients With Alagille Syndrome From This Study and Two Prior Case Series

	Emerick et al 1999	Alagille 1987	Deprettere 1987	Weighted Percent of All Studies
Bile duct paucity	85% (69/81)	100% (80/80)	81% (22/27)	91%
Chronic cholestasis	96% (88/92)	91% (73/80)	93% (25/27)	94%
Cardiac murmur	97% (90/92)	85% (68/80)	96% (26/27)	92%
Vertebral anomalies	51% (37/71)	87% (70/80)	33% (6/18)	67%
Facies	96% (86/92)	95% (76/80)	70% (19/27)	91%
Eye findings	78% (65/83)	88% (55/62)	56% (9/16)	80%
Renal disease	40% (28/69)	73% (17/23)		
Minor features				
Growth retardation	87% (27/31)	50%	73% (16/23)	
Mental retardation	2% (2/92)	16%	0% (0/0)	
Developmental delay	16% (15/92)	—	52% (14/27)	
Pancreatic insufficiency	41% (7/17)[16]			

affected status if they had three or more major features of the syndrome.

The liver biopsy specimens were obtained in several different centers and were a combination of percutaneous needle biopsies and surgical wedge specimens. A ratio of interlobular bile ducts to portal areas of less than 0.4 was used to define histological paucity. Adequate estimation of this ratio has been reported to require the evaluation of 10 portal tracts. Needle biopsies rarely contain that number, but the diagnosis can be made with significantly fewer portal tracts in most cases.[12] For this study, an experienced pathologist at the referral center determined paucity based on the available material. A single pathologist reviewed all of the biopsies performed at CHOP. The presence of fibrosis on biopsy was scored semiquantitatively on the basis of reticulum staining. Each biopsy specimen was assigned a value between 0 and 3 to represent degree of fibrosis: 0, no fibrosis; 1, presence of fibrosis (not bridging); 2, bridging fibrosis; and 3, cirrhosis. Initial and follow-up biopsy specimens were compared for progression of fibrosis based on these assigned values.

The information was compiled in a Microsoft Access database (Microsoft Corporation, Seattle, WA). Statistical analysis was performed using SAS for Windows, version 6.12 (SAS Institute, Cary, NC). Comparisons of categorical data were analyzed with the Fisher's exact (2-tailed) test. Differences between continuous data were tested with the nonparametric *t* test approximation. Differences between survival curves were evaluated by the Kaplan-Meier method.

RESULTS

The 92 patients in this series include 83 unrelated probands and 9 significantly affected family members. The 9 family members included here presented with infantile cholestasis (n = 5), heart murmur (n = 3), and adult-onset renal failure and abnormal liver function tests (n = 1). There was a positive family history of features associated with AGS in 30 of 71 families (42%). Minimally affected family members who did not meet inclusion criteria are not reported.

The majority of patients (80 of 92) presented at less than 6 months of age with jaundice and failure to thrive (64 of 92, 69%) or cardiovascular symptoms (16 of 92, 17%). The patients who presented at age greater than 6 months old included family members with heart murmurs (n = 3), an adult with late-onset renal failure (n = 1), and infants whose evaluation for cholestasis (n = 6) and cardiovascular disease (n = 2) occurred between the ages of 6 months and 1 year of age. At the time of the study the age range of the patients was 3.8 months to 40 years: 1%, less than 6 months; 65%, 6 months to 10 years; 23%, 10 years to 20 years; 11%, greater than 20 years.

Bile duct paucity, chronic cholestasis, cardiac murmur, and particular facies were each found in more than 85% of affected individuals (Table 1). Five major syndromic features were present in 23 (25%), four in 49 (53%), and only three in 20 (22%).

Chronic Cholestasis. All infants in whom studies were performed had elevated serum γ-glutamyl transferase, alkaline phosphatase, and total bile acids with normal or mildly elevated aminotransferases. Hypercholesterolemia and hypertriglyceridemia were also observed in the majority of patients. At some point during the clinical course, hepatomegaly was present in 93% and splenomegaly in 70% of patients. Severe pruritus was a cause of significant morbidity (approximately 45% of children) during the first decade of life, but typically improved with time thereafter. Four patients at a median of 3 years of age underwent biliary diversion procedures for intractable pruritus.[13] These patients are presently 4, 8, 8, and 20 years old with a mean time from diversion of 6.5 years old. Three of these patients have had sustained relief from their pruritis and xanthomas since their diversion procedure. Xanthomas were present in 39 of 92 patients (42%) and were most prevalent in the early school age years with gradual improvement thereafter in a moderate number of cases. Vitamin deficiencies were common but were not specifically tabulated. Pathological fracture or rickets was documented in 12 of 92 patients. As these patients were followed over a 20-year period, the degree of surveillance for and supplementation of fat-soluble vitamin deficiency varied highly.

Cardiovascular Abnormalities. An audible heart murmur was present in 90 of 92 patients (97%). A formal cardiology evaluation was performed in 73 patients. The majority (49 of 73, 67%) had stenosis at some level in the pulmonary tree, most commonly peripheral pulmonic stenosis (PPS). Structural intracardiac disease was present in 22 of 92 patients (24%). The lesions included tetralogy of Fallot (TOF) (n = 10, 4 with associated pulmonary atresia [PA]), PA and ventricular septal defect complex (n = 2) (which is similar to TOF with PA), valvular pulmonary stenosis (n = 2), ventricular septal defect (VSD) with pulmonic stenosis (n = 2), and VSD with atrial septal defect (n = 2, 1 with coarctation of the aorta and 1 with pulmonary stenosis), isolated atrial septal defect (n = 3), and isolated VSD (n = 1). Of the 22 patients with structural intracardiac heart disease, 10 also had documented PPS. Patent ductus arteriosus was seen in association with PPS in 4 patients (one of these patients had patent ductus arteriosus ligation as an infant). Cardiac surgery was performed on 10 patients (6 TOF, 2 PA/VSD, and 2 VSD/atrial septal defect), 3 of whom are deceased (two with TOF and one with PA/VSD). Of the 4 TOF patients who did not have surgery, 3 are deceased. The overall mortality for patients with TOF with pulmonary stenosis was 33% (2 of 6) and for patients with TOF and PA was 75% (3 of 4). One of 2 patients with PA complex is deceased. The overall mortality rate for patients who had structural intracardiac lesions is 7 of 22 patients (32%). Associated PPS was present in 4 of 7 of the deceased patients (57%). Cardiovascular failure (associated with multisystem organ failure) was directly implicated as the cause of death in 3 of the 7 patients.

Skeletal Abnormalities. Butterfly vertebrae were present in 37 of 71 of patients studied (51%). Other minor skeletal anomalies involving the spine and hands, such as shortened interpedicular distance, shortened distal phalanges or shortening of the distal ulna and radius, were not systematically evaluated.

Ophthalmologic Abnormalities. Posterior embryotoxon was found in 65 of 83 patients (78%). Of these patients 11 of 83 (13%) also had Axenfeld anomaly (attachment of iris processes to Descemet membrane). Other ocular abnormalities observed included retinitis pigmentosa (n = 1), retinal pigmentary changes/mottling (n = 4; associated with pseudopapilledema in 1) and severe keratoconus (n = 1). Additionally, 2 patients had bilateral hypoplasia of the optic discs with Reiger anomaly. The finding of optic disc drusen, which has recently been reported in AGS,[14] was found in 6 of 7 recently evaluated patients but was not systematically sought in older cases.

Facies. Characteristic facies defined by a prominent forehead, hypertelorism with deep-set eyes, pointed chin, or a straight nose with a bulbous tip were noted in 86 of 92 patients (96%).

Renal Abnormalities. There was evidence for renal disease in 28 of 69 patients evaluated (40%) (Table 2). The most common abnormality was renal tubular acidosis in 10 patients. Renal failure in infancy that resolved in the first 6 months of life occurred in 5 patients.[15] Renal biopsy specimens showed renal lipidosis in a child with proteinuria and cystic tubular dilation in an infant with infantile renal insufficiency and renal tubular acidosis. Renal necropsy specimens from 2 toddlers showed glomeruli filled with foamy macrophages, dilated tubules, and sclerotic glomeruli in one (who did not have clinical renal disease) and dilated tubules with cystic spaces and lipidosis in another who had infantile renal insufficiency and renal tubular acidosis. There were no deaths from renal failure in this series.

Growth Retardation. Birth weights of 38 infants showed that 6 of 38 infants (16%) were small for gestational age (less than 10th percentile). All others were appropriate for gestational age (between the 10th and 90th percentile). Only 1 infant, of a fraternal twin gestation (other twin not affected with AGS), was less than 35 weeks gestation at delivery. Growth retardation, defined as length and weight below the 5th percentile in the first 3 years of life was present in 27 of 31 patients (87%) for whom detailed growth information was available.

TABLE 2. Renal Anomalies and Similar Findings Reported in the Literature

Renal Anomalies	N	Similar Findings Reported in the Literature by Reference Number
Structural		
Small hyperechoic kidney	14	7,17
Ureteropelvic obstruction	4	47
Renal cysts	5	51,52
Duplex collecting system	1	4
Functional		
Renal tubular acidosis	10	49
Recurrent urinary tract infection	4	47
Infantile-onset renal insufficiency	5	49
Adult-onset renal insufficiency	1	7,53
Total	28	

TABLE 3. Intracranial Hemorrhage in 13 Patients

Intracranial Bleeding Events	Number of Bleeds	Number With Trauma	Death as a Result of Intracranial Bleeding
Subarachnoid hemorrhage	3	2	0
Subdural hematoma	3	2	0
Epidural hemorrhage	1	1	1
Massive bleed (undefined)	3	1	3
Cerebrovascular accident	4	0	0
Total bleeds	14	6	4
Total patients with intracranial bleeding		13/92 = 14%	

Developmental Delay. All patients had been evaluated by routine pediatric screening for developmental delay. Mild delays in gross motor skills were present in 15 of 92 patients (16%). In each of these cases, the child had severe morbidity associated with either liver disease or congenital heart disease. Mild mental retardation was documented in only 2 individuals (2%) by psychological testing. Psychological diagnoses were made in 2 other patients after evaluation for abnormal behavior. A female patient with multiple psychiatric admissions for suicide attempts was labeled with major depressive disorder and oppositional defiant personality, and a male with a history of juvenile delinquency was diagnosed with hyperactivity/attention deficit disorder.

Intracranial Bleeding. Intracranial hemorrhage was documented in 13 of 92 patients (14%), and 4 of these 13 (31%) died as a result of the intracranial bleeding (Table 3). The 4 patients who died secondary to intracranial bleeding included 2 toddlers with sudden death after minor head trauma, one toddler who presented with seizures without history of trauma, and a 6-year-old patient posttransplant. The intracranial bleeding presented with headaches (n = 3), seizures (n = 2), stroke-like symptoms (n = 2), catastrophic bleeds in critically ill patients (n = 2), sudden death (n = 2), anemia after birth trauma (n = 1), or as an incidental finding on study magnetic resonance imaging (n = 1). Head trauma was associated with 6 of the hemorrhages. The injury was felt to be disproportionately minor compared with the severity of the resultant bleed in all cases. There was no pattern to the locations of the bleeds.

Bleeding episodes occurred in the setting of coagulopathy in only two of the children. The first was an 18-month-old child with TOF who presented with acidosis and developed progressive liver dysfunction with a prolonged prothrombin time treated with vitamin K. Postmortem examination showed an organizing subarachnoid hemorrhage with a recent subdural hemorrhage and a congenital absence of the right basilar artery branch. The second was a 6-year-old boy who had a spontaneous fatal intracranial bleed after his second liver transplantation in the setting of mild disseminated intravascular coagulation.

Additional Features. Endocrine abnormalities were seen in 1 patient with hypothyroidism and 1 patient who developed insulin-dependent diabetes mellitus at 14 years of age. Skeletal abnormalities other than butterfly vertebrae include craniosynostosis (n = 1), nonfamilial macrocephaly (n = 1), and spina bifida occulta (n = 2). There is also a single patient with significant large joint arthropathy. One patient has thickened tympanic membranes with conduction deafness without significant history of otitis media. Additional gastro-

intestinal findings include 1 patient with ileal atresia and 1 with asymptomatic malrotation (discovered during fluoroscopic nasoduodenal tube placement). Exocrine pancreatic insufficiency documented by abnormal coefficient of fat absorption or hormone-stimulated pancreatic testing was found in 7 of 17 patients (42%) reported in a separate study.[16] One patient who had severe failure to thrive before his death at 18 months had cystic dilatation of the pancreatic ducts. One adult female had agenesis of a fallopian tube. One patient developed bilateral expanding cystic masses of the mandible, which were explored and excised. The histology of the tissue showed multiple central giant cell granulomas that were nonmalignant. There were no cases of hepatocellular carcinoma.

Transplantation. Liver transplantation was required in 19 of 92 patients (21%), with 15 of 19 long-term survivors (79%) at a mean follow-up of 4.2 years (range, 0.7-12.3 years). The indications for transplantation included progressive synthetic liver dysfunction in the majority, accompanied by failure to thrive, intractable pruritus, osteodystrophy, and massive variceal bleeding in many patients. The median age at transplantation was 72 months (range, 8 months-23 years). Retransplantation for loss of the graft was necessary in 5 patients within 7 months of the initial transplant. Hepatic artery thrombosis was documented as the cause of graft loss in 3 patients (average time after transplant was 2 months).

Mortality. Currently 16 of 92 patients (17%) are deceased. Four deaths occurred after liver transplantation at a mean age of 71 months of age (range, 12-372 months, median 40 months). The causes of death in the 4 transplanted and 12 nontransplanted patients are listed in Table 4. Survival data are shown in Figs. 1 and 2.

Hepatobiliary Evaluation in Infancy. Hepatobiliary scintigraphy was performed as part of the initial evaluation for cholestasis in 36 infants. Uptake of the radiolabeled tracer by the liver was present and adequate to evaluate excretion in all cases, although uptake was mildly abnormal in 6 infants. Phenobarbital and ursodeoxycholic acid were not routinely used for these studies. There was normal excretion of tracer into bowel by 4 hours in 5 of 36 infants (14%). Excretion was delayed but present by 24 hours in 9 of 36 (25%). There was no evident excretion of scintiscan after 24 hours in 22 of 36 (61%).

Cholangiography was performed for clinical suspicion of biliary atresia in 21 infants. Abnormalities of the intrahepatic and extrahepatic biliary tree as assessed by operative cholangiogram were common, and nonvisualization of the intrahepatic tree was the most common important abnormality. Cholangiograms were performed intraoperatively (n = 18) by percutaneous transhepatic approaches (n = 2) or via endoscopic retrograde cholangiopancreatography (n = 1). In both percutaneous transhepatic cholangiograms the intrahepatic ducts were visualized but the extrahepatic biliary tree was not opacified. In the remaining 19 cholangiograms, the distal extrahepatic tree (common bile duct) was normal in 13 of 19 (68%) and small or hypoplastic in 6 of 19 (32%). The proximal extrahepatic tree (hepatic ducts to hilum) was normal in 5 of 19 (26%), small or hypoplastic in 7 of 19 (37%), and not visualized in 7 of 19 (37%). The intrahepatic

TABLE 4. Cause of Death in 16 Patients

Cause of Death	N	Age at Death in Years
Hepatic death (3 after liver transplantation)	4	11.5, 10.1, 5, 3.3
Nontraumatic head bleed (1 after liver transplantation)	2	6, 2
Traumatic head bleed	2	2.5, 1
Multisystem/cardiac failure	3	2, 1.5, 1.2
Squamous cell carcinoma	1	30
Infection	1	10.1
Pneumonia	1	1.5
Unknown	2	1.7

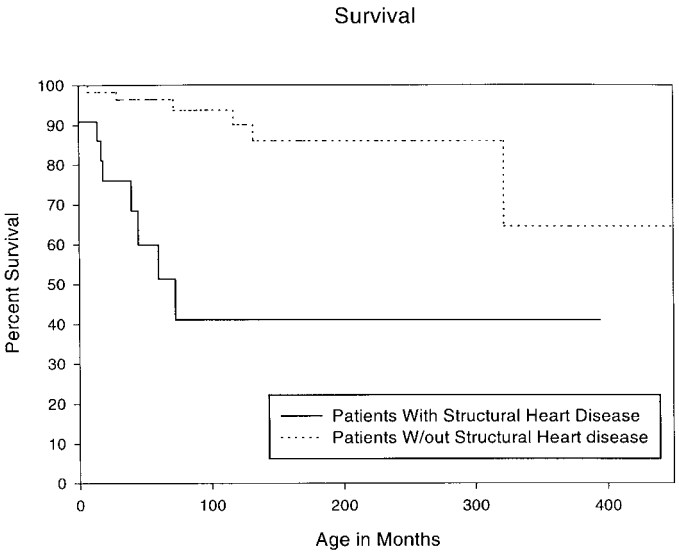

FIG. 1. Kaplan-Meier survival plot comparing survival of patients with intracardiac lesions to those with normal heart structure. The survival of the 22 patients with structural intracardiac lesions are represented by the *dashed line*. The predicted probability of attaining 20 years of age for this group is 40%. The *solid line* represents the 70 patients who did not have structural intracardiac lesions, revealing a predicted 80% probability of attaining 20 years of age.

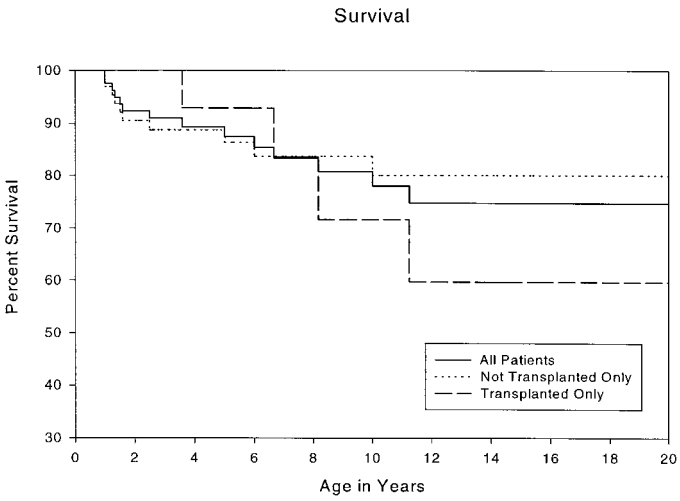

FIG. 2. Kaplan-Meier survival plot on 92 patients comparing transplanted and nontransplanted patients. The *solid line* represents all patients including transplanted patients. The *dotted line* represents all patients who were not transplanted (n = 72) and the *dashed line* represents only transplanted patients (n = 20). The predicted probability of attaining the age of 20 years was 75% for all patients, 60% in the transplanted patients, and 80% in the nontransplanted patients.

ducts were normal in only 2 of 19 (10%), small or hypoplastic in 3 of 19 (16%), and not visualized in 14 of 19 (74%).

Surgery. Seven patients underwent the Kasai procedure (hepatoportoenterostomy or cholecystoportostomy) in infancy for presumed biliary atresia. Factors that suggested biliary atresia included nonexcreting diisopropyl iminodiacetic acid scans and hepatic histology that showed evidence of hepatitis, portal inflammation, or bile duct proliferation (Fig. 3). Each of these infants underwent intraoperative cholangiography that showed drainage from the gallbladder into the duodenum. Each had severely hypoplastic or nonvisualized proximal biliary trees. Liver transplantation was necessary in three of seven of these patients (43%). The other patients are presently greater than 10 years of age without hepatic decompensation.

Hepatic Histopathology. Of 92 AGS patients, 81 had at least one liver biopsy and 69 (85%) had documented bile duct paucity. The remaining 12 patients had biopsy specimens showing cholestasis, portal inflammation, fibrosis, and occasionally bile ductule proliferation. A cross-sectional analysis of prevalence of paucity as it relates to age at biopsy was performed using 88 liver biopsy specimens from 57 individuals. Findings were grouped by age into biopsies performed at less than 6 months (n = 48) or at greater than 6 months of age (n = 40). There was a significant increase in the prevalence of paucity at an older age compared with infancy ($P < .001$). Bile duct paucity was present in only 29 of 48 infants (60%) in comparison with 38 of 40 older patients (95%).

A longitudinal qualitative comparison by the same pathologist in 27 patients of initial versus subsequent liver biopsies showed a significant increase in the presence of bile duct paucity ($P = .002$), prevalence of fibrosis ($P = .001$), and severity of fibrosis ($P < .001$) from initial to repeat biopsy. It is important to note that patients who underwent repeat liver biopsies did so for clinical indications of progressive disease with six of the final biopsies taken from the explant at transplantation. They therefore represent a subset of severely affected individuals in whom the liver disease was clinically progressive. Of the 25 patients with fibrosis on subsequent biopsy, bridging fibrosis was seen in 13 of 27 (48%) and cirrhosis was identified in 7 of 27 (26%). This cirrhosis was shown at transplantation in 5 patients and at postmortem in 2 patients.

Prognosis. To determine if features of AGS at presentation could predict outcome, transplantation, or mortality, the presence and severity of clinical features at presentation were analyzed with relation to current status: alive with conjugated bilirubin less than 1.0 mg/dL, alive with conjugated bilirubin greater than 1.0 mg/dL, alive posttransplantation, deceased posttransplantation, and deceased without transplantation (Table 4). Complete information about the initial clinical

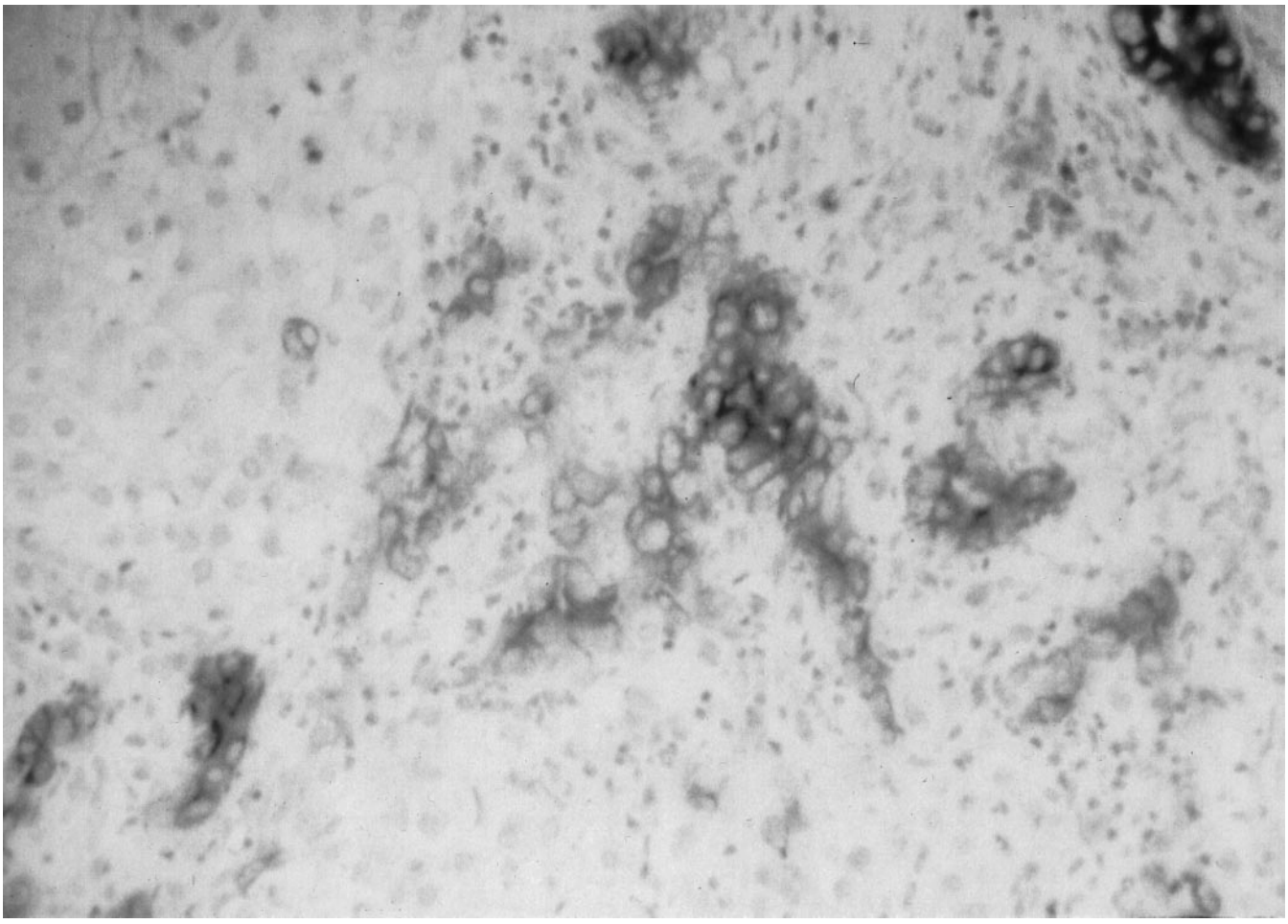

FIG. 3. Liver biopsy from a 4-month-old infant with AGS. Biopsy is stained with cytokeratin AE-1 showing numerous duct elements in the widened portal tracts. This patient underwent the Kasai procedure secondary to suspicion of extrahepatic obstruction. The resected biliary tree was hypoplastic but patent.

presentation was available for 57 patients who were divided by age at presentation into infants (n = 45) versus older (n = 12). At presentation, posterior embryotoxon, vertebral anomalies, renal disease, complex cardiac disease, hepatomegaly, and splenomegaly were each analyzed individually for association with higher mortality or transplantation. Of these, only major structural congenital heart disease in infancy was specifically associated with increased mortality ($P < .001$).

The level of conjugated bilirubin at presentation (n = 45) analyzed continuously showed that higher bilirubin at presentation was associated with the outcome of death ($P = .007$). In infants (n = 30) conjugated bilirubin of greater than 5.0 mg/dL at presentation was associated with death (36%), whereas a conjugated bilirubin of less than 5.0 mg/dL was associated with no deaths (0%) ($P = .013$). There was, however, significantly more complex congenital heart disease in the group of patients with a bilirubin greater than 5.0 mg/dL at presentation than those with conjugated bilirubin less than 5.0 mg/dL ($P = .05$). When a further analysis was performed excluding the patients with complex congenital heart disease the association between level of bilirubin and death was no longer significant ($P = .375$).

DISCUSSION

This study was designed to describe this large AGS population and to identify characteristics at presentation that might assist in prognostication. We found that mortality in AGS is not statistically related to any specific features or findings except complex heart disease. Structural congenital heart disease was implicated in 15% of the mortality in this series, which is consistent with previous reports.[17] The presence of intracardiac heart disease does predict higher mortality (Fig. 1). Progressive liver dysfunction resulting in liver transplantation and posttransplant death contributed to 25% of the overall mortality. No other functional or anatomical hepatic feature was predictive of clinical outcome. Intracranial bleeding contributed to 25% of the mortality and was not predictable.

In our AGS population the survival of patients with TOF is 66% and with TOF with PA is 25%, compared with the estimated survival at 10 years old of non-AGS patients with TOF of 77% to 89%, and for TOF with PA of 58%.[18] This suggests that these complex congenital heart lesions in AGS have significantly increased mortality above that seen with the lesion in isolation. This increased mortality may be because of the association of cardiac disease with other systemic manifestations of AGS or may be secondary to the association of the structural heart disease with the pulmonary vascular stenoses that are typical in AGS and found in approximately half of our patients.

Severe hepatic disease is the major cause of morbidity in AGS but a less significant cause of mortality.[2,5] The majority of AGS infants present for evaluation secondary to hepatic dysfunction. Surprisingly, infants with more significant disease as estimated by scintiscan nonexcretion do not have excess transplantation or mortality. From our analysis there seems to be no reliable indicator at presentation that will predict outcome of the hepatic disease of AGS.

Reports in the literature suggest that AGS patients who undergo the Kasai hepatoportoenterostomy or cholecystoportostomy have a worsened outcome, *i.e.*, early transplantation or mortality.[19] We were not able to show increased mortality for the 7 patients in our series who underwent the Kasai procedure. None of our patients developed cholangitis after the procedure. Transplantation, however, was necessary in 3 of 7 patients (43%) post-Kasai compared with only 16 of 85 patients (19%) who did not undergo the Kasai procedure. These results imply that a Kasai procedure predicts future transplantation. This is supported in the literature by reports of high percentages (*e.g.*, 10 of 23 or 43%)[20] of AGS patients who have undergone Kasai portoenterostomy before transplantation. It remains unclear whether AGS patients who undergo the Kasai procedure belong to a subpopulation of patients with more severe liver disease for whom transplantation is likely or whether the progression of their liver disease and need for transplantation develops secondary to the Kasai procedure itself. Because no benefit can be ascribed after surgery, the Kasai procedure should be avoided for patients with AGS.[12,19,21]

Physicians need to consider the range of findings in infantile AGS to avoid unnecessary hepatic surgery. The results of the initial DISIDA scan, cholangiogram, and liver biopsy in AGS infants may mimic those of biliary atresia.[4,19,22,23] Even in the setting of a nonexcreting DISIDA, histological bile duct proliferation and an operative cholangiogram that fails to show the intrahepatic biliary tree, AGS is still a possibility. It is therefore important to identify syndromic features or a family history of AGS.

Unexplained intracranial bleeding is now a recognized complication and cause of mortality in AGS.[11,24,25] We were unable to identify any specific risk factors for intracranial bleeding in our patients. The bleeds varied in location and severity such that no pattern was evident. Vascular abnormalities have been described in various locations in AGS[4,7,26] and, if present in the central nervous system (CNS), could explain these occurrences. None of our AGS patients studied by magnetic resonance imaging (n = 21) had abnormalities that would predict hemorrhage or stroke. Also, histological examination of the CNS vasculature from necropsy specimens of two AGS patients who had parenchymal CNS hemorrhages failed to show any abnormalities.

AGS is caused by mutations in the gene *Jagged1*, which encodes a cell surface ligand for one of the four known human Notch transmembrane receptors. The Notch signaling pathway has been shown to be involved in cell fate determination in a wide variety of tissues in humans and in lower organisms. The Notch signaling proteins are ubiquitously expressed, and their role in human development and disease is poorly understood. Mutations in the gene for the Notch 3 receptor cause adult-onset CADASIL (cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy), which is associated with intracranial bleeding.[27,28] Patients with CADASIL have small deep cerebral infarcts, leukoencephalopathy, and a nonatherosclerotic, nonamyloid angiopathy involving the media of small cerebral arteries. This strongly implicates abnormalities in the Notch pathway as a cause of CNS vascular pathology. Evaluation of the CNS vasculature from necropsy specimens of AGS patients in the future may provide information regarding the vascular histology and the pathogenesis of intracranial bleeding seen in AGS.

Overall survival of patients in this series is 83%, which is similar to previous reports.[2,5,11,17] The predicted probability of survival to 20 years of age in all patients is 75% (Fig. 2). The probability of survival to age 20 is 80% in patients who did not require transplantation and 60% in those who underwent

liver transplantation. These life-curve results represent a large population of affected AGS individuals with a wide spectrum of syndromic features and severity. Our results therefore are more favorable than other studies that included only patients with onset of cholestasis in infancy in which the predicted probability of reaching 19 years old without transplantation was 50%.[11]

In this series 1-year survival after transplantation was 79%, which is similar to the 76% reported in a large pediatric transplantation series over similar years.[29] Variable survival outcome of AGS patients undergoing liver transplantation has been reported. In two previous studies the posttransplant survival in AGS patients was 13 of 23 (57%) in 1993[20] and 5 of 11 (45%) in 1996.[29] Vascular complications and sepsis were responsible for most of the deaths. These results are less favorable than two recent studies that report average to excellent survival in transplanted AGS patients of 11 of 12 patients (92%) in 1995[30] and 8 of 8 (100%) in 1995.[11] These reports suggest that AGS patients can do well after transplantation, although severe cardiopulmonary anomalies place AGS patients at increased risk compared with non-AGS patients.[20]

Analysis of paucity in sequential biopsy specimens proves that paucity does develop with increasing age.[12,24,31-34] There are several theories regarding the pathogenesis of diminished duct number in AGS including a developmental lack of bile ducts versus a loss of previously normal bile ducts.[35-37] The reports of normal bile duct number in AGS infants less than 6 months of age provides evidence against a congenital absence of bile ducts.[5,12,24,31,33,34] Theories of prenatal or postnatal duct loss or destruction have implicated retained toxins, inflammation, or disuse atrophy as mechanisms.[38] We propose that the mechanism of diminishing bile ducts in AGS is related to a lack of postnatal development of the terminal branches of the bile ducts in the growing liver secondary to a defect in *Jagged1*. Landing et al. proposed that the postnatal liver grows through infancy and childhood by increasing the number of lobules, rather than increasing their size, and then maintains the duct supply by increasing the number of duct branchings and terminal ducts.[39,40] This theory is supported by data that the lobular diameter does not increase significantly with age, although the mean liver weight increases eightfold from birth to 12 years of age.[39] Given this model of liver growth and the role of Jagged1-Notch interactions in cell fate determination and commitment,[41] a postnatal lack of development or lack of bile duct terminal branching is more likely than a loss of formed ducts in AGS. This theory may explain the liver biopsy findings in AGS infants in which increasing incidence of bile duct paucity is observed with increasing age.[12,24,31,34] Initial biopsies in young infants representing the most peripherally located lobules may reveal congenitally formed bile ducts whereas a biopsy performed several months later may represent a newly formed lobule that has developed without the simultaneous growth of the ductular system. This theory may also explain the distinct scintigraphic pattern seen in older AGS patients in which there is prolonged retention of tracer in the periphery of the liver but normal tracer clearance in the central portion.[42,43]

Recent data presented here and elsewhere document that renal and pancreatic (exocrine and endocrine) disease should routinely be considered during the evaluation of an AGS patient.[44-49] The presence of renal disease did not correlate with increased mortality in our patients and only rarely is it the cause of death in AGS patients. The diversity of manifestations within each organ system is intriguing. Normal Jagged-Notch interactions appear to be necessary for human cardiac, hepatic, ocular, renal, skeletal, and pancreatic development. Defects in this system, however, do not produce a single pattern of abnormality in any organ system. Further study will be necessary to determine the pathophysiology associated with abnormalities in the Notch signaling pathway. Many patients with AGS will have a documented defect in *Jagged1*, but there are currently no data on whether similar phenotypic patients will have defects in other ligands or in the Notch gene itself.[10] At present, four human *Notch* genes and two *Jagged* genes have been described, but diseases associated with defects in these genes have only been assigned to three of these.[8,27,50]

There is an enormous variability of the clinical expression of AGS. It is clear that family members of affected individuals with AGS have minor forms of the syndrome, some of which do not meet diagnostic criteria. In some of these families we have confirmed mutations of *Jagged1* in the mildly affected individuals.[10] For this study, only patients with symptomatic and well-characterized disease were included. Thus, the frequency of clinical manifestations and the severity of the disease are biased in favor of more severely affected individuals. It is relevant, however, for estimating prognosis of similar patients. Genetic testing and prenatal diagnosis is now easily available, although cumbersome. Further study will identify other family members with less significant or subclinical disease. Inclusion of these patients into life tables and genetic risk assessment will be valuable for genetic counselors and parents assessing risks for affected progeny. Finally, screening patients without AGS who have been ascertained because of isolated features such as congenital heart disease has identified patients with defects in *Jagged1* (Spinner NB, manuscript in preparation). Studies of patients with ocular, renal, and pancreatic disease may produce similar results and will further broaden the spectrum of diseases associated with *Jagged1* defects. The clinical Alagille syndrome will likely be only one of the phenotypes caused by defects in *Jagged1*.

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