

From chronic cholestasis to liver transplantation

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ABSTRACT

Introduction. Alagille Syndrome is a genetic autosomal dominant disorder with multisystemic manifestations. The diagnosis is suspected in children presenting with jaundice in the first 6 months of life (70%) with or without associated cardiac murmur (17%).

Case presentation. We report the case of a 2 years and 2 months old boy who was first evaluated in our department at 3 days of age for jaundice and meconium ileus. Clinical examination showed abdominal distension and cardiac murmur. Laboratory tests revealed high levels of total and conjugated bilirubin, but normal transaminases, serum proteins and blood coagulation markers. Infectious causes were ruled out – HIV, HBV, HCV, EBV, CMV and syphilis. Echocardiography described large pulmonic stenosis with no hemodynamic implications. Thoraco-lumbar X-ray revealed no anomalies of the spine. Cystic Fibrosis was excluded. Alagille Syndrome was at that point the main diagnostic suspicion, but liver biopsy failed to reveal paucity of bile ducts. Further evaluation was conducted and ophthalmologic examination revealed posterior embryotoxon, one of the classic phenotypic traits of Alagille syndrome. Abdominal CT showed a nodular area in the IVth segment of the liver with no visualization of the bile duct and MRCP showed thin but present intra/extrahepatic biliary ducts and absence of the gallbladder. Genetic testing was not available at the time, but further on it confirmed the diagnosis of Alagille syndrome. The patient associated failure to thrive and signs of chronic cholestasis: severe jaundice, refractory pruritus, acholic stools, hepatosplenomegaly, xanthelasma, persistent hepatic cytolysis, severe dyslipidemia. Due to progressive, chronic hepato-biliary disease and advancing cirrhosis liver transplantation was considered and the patient was referred to a liver transplant facility in Palermo.

Conclusions. Alagille syndrome is not always an easy diagnosis. In certain cases it takes time to reach it and set the course for an optimal therapeutic approach.

Keywords: Alagille syndrome, chronic cholestasis, failure to thrive

Abbreviations

AP – alkaline phosphatase

ASLG – Alagille syndrome

CMV – cytomegalovirus

PFIC – progressive familial intrahepatic cholestasis

CT – computed tomography

GGT – gamma-glutamyl-transferase

EBV – Epstein-Barr virus

HBV – hepatitis B virus

HCV – hepatitis C virus

HIV – human immunodeficiency virus

MRCP – magnetic resonance cholangiopancreatography

INTRODUCTION

Alagille syndrome (ALGS), also known as arterio-hepatic dysplasia, is a genetic multisystemic disorder which involves the liver, heart, brain, eyes and skeleton. The first cases were reported by Alagille in

1969. It is a particularly rare autosomal dominant disease caused by defects in the NOTCH signaling pathway. There are 2 subtypes – mutations or deletions in JAG1 gene are the most frequent and cause ALGS type 1, while ALGS type 2 is less common (< 1%) and occurs due to changes in NOTCH2 gene.

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However, in about half of the cases mutation appears *de novo* (1-3).

CASE PRESENTATION

We present the case of a 2 years and 2 months old boy, who was hospitalized at 3 days of age, in February 2017, for bilious vomiting, meconium ileus and intense jaundice.

Personal history revealed that the patient was prematurely born at 35 weeks of gestation through C-section, with a low APGAR score of 6 and required intensive care treatment. Family medical history includes mother with surgically-resolved coarctation of the aorta and distinctive facial features (Fig. 1), with no other known medical condition. The father was diagnosed with second grade obesity and fatty liver disease.

Clinical examination at the first admission described a newborn with 2,320 g weight, 46 cm length (16th percentile, -1 standard deviations), significantly abdominal distension with no signs of tenderness, absence of meconium passage, cardiac murmur, hypotonia and hypo-reactivity. Laboratory tests showed marked intrahepatic cholestasis (total bilirubin = 11.6 mg/dl, unconjugated bilirubin = 9.4 mg/dl, conjugated bilirubin = 3.3 mg/dl – 28% of total bilirubin, gamma-glutamyl transferase (GGT) 425 U/l). Liver transaminases, serum proteins and blood coagulation tests were within normal limits and inflammatory markers were also normal. Thoraco-lumbar and abdominal X-ray showed no vertebral anomalies, but described a significant small bowel air distension (Fig. 1). Following barium enema the passage of meconium was not obtained, so exploratory laparotomy was performed for de-obstruction. After this surgical procedure the newborn had a slowly ascending weight curve but persistent jaundice associated with dark urine and acholic stools. Levels of total bilirubin continued to be high with the conjugated fraction significantly elevated.



FIGURE 1. Spine X-ray: Small bowel distension

The abdominal ultrasound was normal, but echocardiography described large peripheric pulmonic stenosis with no hemodynamic consequences. At age 9 months an abdominal ultrasound followed by an abdominal CT-scan revealed non-homogenous liver ecostructure, a nodular area (26/18 mm) in the IVth segment of the liver, highly suggestive for benign nodular hyperplasia (Fig. 2) and segmental dilations of the intrahepatic biliary ducts. Hepatocellular carcinoma was excluded based on normal values of alpha-fetoprotein. Magnetic resonance cholangiopancreatography (MRCP) performed at age 11 months described a volume-increased liver with evidence of a well limited nodular area (27/23 mm), thin intra/extrahepatic biliary ducts, absent gallbladder and cirrhotic nodules with minor ascites. Ophthalmologic examination revealed a classic phenotypic trait of Alagille syndrome, posterior embryotoxon (Fig. 3).



FIGURE 2. Nodular hepatic area in segment IV



FIGURE 3. Posterior embryotoxon

Liver biopsy was inconclusive, describing portal fibrosis and biliary stasis with biliary thrombi dwelling in the bile ducts.

Facing a patient with chronic cholestasis and structural cardiac anomaly, multiple diagnostic possibilities presented. Infectious etiologies were excluded – serologies for HIV, hepatitis B virus, hepatitis C

virus, Epstein Barr virus, cytomegalovirus and siphilis were all negative. Cystic fibrosis was ruled out base on normal sweat test values (NaCl 8mmol/L); genetic testing was also negative. Progressive familial intrahepatic cholestasis (PFIC) type 3, which is a genetic autosomal recessive disorders, remains a possibility in this patient, but genetic testing is not an option.

The main differential diagnosis at the time was biliary atresia and Alagille syndrome. Considering the distinctive facial features of both mother and child (triangular facies, pointed chin, deep-set eyes with secondary apparent exophthalmia and hypotelorism) (Fig. 4), together with the large peripheral pulmonic stenosis and posterior embryotoxon – known as a main characteristic of Alagille syndrome, we considered this to be the most likely diagnosis. Genetic testing was not possible at the time, but further on the patient was referred to a liver transplant facility in Palermo, where a heterozygous mutation of JAG1 gene (c.2372+3_2372+6delAAGT variant) was found, thus confirming the diagnosis of Alagille syndrome.



FIGURE 4. Mother with distinctive facial features

Signs of chronic hepatic disease began to surface in time, with clinical features: severe jaundice, refractory pruritus, acholic stools, dark urine, hepatosplenomegaly (Fig. 5), abdominal collateral circulation, xanthelasma (Fig. 6) and laboratory findings: persistent high levels of total and conjugated bilirubin, increasing values for alkaline phosphatase (AP) and gamma-glutamyl-transferase, severe dyslipidemia. Consequences of chronic cholestasis could be observed: malabsorption and failure to thrive with roughly 10 kg weight gain in over 24 month. Liver cytolysis and altered blood coagulation were also associated in time.



FIGURE 5. Significant hepatosplenomegaly



FIGURE 6. Xanthelasma

Due to liver cirrhosis with vascular and parenchymal failure, neof ormation nodules and nutritional deficiencies in a child, liver transplantation was highly recommended, even though PELD (pediatric end-stage liver disease) score was determined and showed promising results (86,8% 1-year survival rate before transplantation) (Fig. 7).



FIGURE 7. Pediatric end-stage liver disease score

Because the procedure cannot be performed in Romania at this point, the patient was referred to a transplantation facility in Palermo. At the Transplantation Centre in Palermo the diagnosis of Alagille syndrome was confirmed and an esophago-gastroduodenoscopy was performed at age 1 year and 7 months which described medium-sized esophageal varices with low-bleeding risk. Abdominal ultrasound showed scratchy ecostructure and neof ormation nodule (4.37 cm) (Figure 8).



FIGURE 8. Benign nodular hyperplasia

Further evaluation at Palermo transplant facility found the patient in good clinical condition, with ascending weight curve – 2 kg weight gain in 5 months – and preserved liver function.

Evolution of total and direct bilirubin, GGT and AP is presented in Figure 9. Evolution of cholesterol, triglycerides and total lipids is presented in Figure 10.

DISCUSSIONS

One of the most important features of Alagille Syndrome is liver impairment caused by malformed, narrow or absent intrahepatic bile ducts. This leads to a cholestatic syndrome with persistent jaundice, refractory pruritus and xanthomas. Liver disease is progressive, eventually causing cirrhosis and hepatic failure, thus the need of transplantations in approximately 15% of the cases (1,3). Our patient developed early cirrhosis, portal hypertension with F2 esophageal varices and ascites, so liver transplantation is highly necessary in this moment.

Chronic liver disease is the first step of evaluation in a child with ALGS. Persistent cholestasis translates to high levels of total bilirubin with a direct fraction greater than 20%, significantly increased serum bile acids, elevated levels of GGT and AP. Levels of GGT also help differentiate ALGS from types 1 and 2 of PFIC, which go with normal values of these enzymes (4). Our patient had persistent cholestasis with high levels of total bilirubin (4-10 mg/dl),

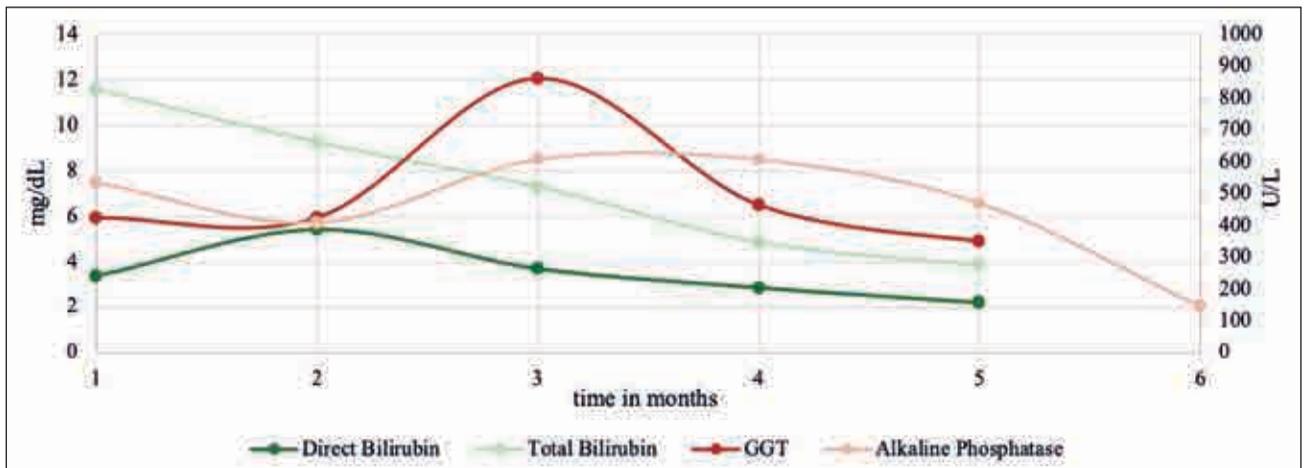


FIGURE 9. Evolution of cholestatic syndrome

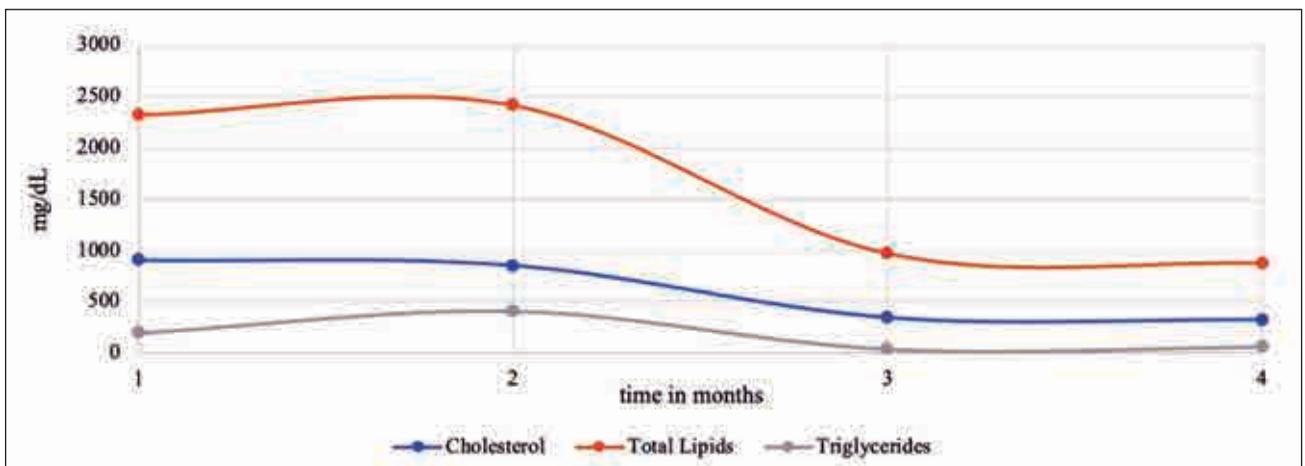


FIGURE 10. Evolution of dyslipidemia

conjugated bilirubin (4,5-9 mg/dl), AP and GGT (400-600 U/l).

Chronic cholestasis also leads to fats and fat-soluble vitamin deficiencies, so a careful assay of fat-metabolism, 25-hydroxi D3 and vitamins A, K, E is essential (4). Patients with Alagille Syndrome have increased serum levels of total cholesterol, triglycerides and low-density lipoprotein cholesterol (LDL-C) as a result of chronic cholestasis. Due to an enzymatic deficiency (lecithin-cholesterol acyltransferase) the hypercholesterolemia seen in ALGS is mostly unesterified. The free/esterified cholesterol ratio can be used as a confirmation analysis (2). Our patient had severe combined dyslipidemia with persistent high levels of cholesterol and triglycerides (total cholesterol levels » 300-900 mg/dl, triglycerides » 170-400 mg/dl).

Coagulation parameters such as prothrombin time and activated partial thromboplastin time are frequently prolonged. In our patient dynamic testing of blood coagulation was normal or slightly altered.

Patients with Alagille Syndrome may develop in time hepato-carcinoma, that is why ultrasound screening and alpha-fetoprotein levels are essential if changes in the liver structural architecture emerge (5). In our case, cirrhosis with neoformation nodules was described on imaging studies at age 9 months, but alpha-fetoprotein level was normal, so the diagnosis established was benign nodular hyperplasia.

Children with ALGS develop failure to thrive due to the inability of digestion and absorption of fats and fat-soluble vitamins. Also, trace elements deficiencies such as calcium and zinc are commonly associated with ALGS. These nutritional issues may lead to poor growth and delayed puberty (3). In our case, the child associated poor weight and height gain, with weight=10 kg (percentile 2, -2 standard deviations), height=82 cm (percentile 0.6, -2,5 standard deviations) at 2 years 2 months.

Alagille syndrome can also cause pancreatic insufficiency, therefore fat malabsorption can aggravate, playing an important role in the poor nutritional status of these patients (3). Our patient didn't show clinical or laboratory signs of pancreatic involvement.

All patients with ALGS should benefit from a detailed cardiac assessment to determine possible structural or electric anomalies. The most common heart malformation associated with Alagille Syndrome is peripheric pulmonic stenosis. But there are other structural abnormalities which may occur in these patients, such as atrial septal defect, ventricular septal defect, persistent ductus arteriosus or Tetralogy of Fallot. Furthermore, recent studies have reported a subset of patients with ALGS and Wolf-Parkinson-White

syndrome (1,4). Fortunately, echocardiography described in our patient the most frequent cardiac abnormality associated with ALGS – large peripheral pulmonic stenosis with no hemodynamic implications.

Facial features are an important distinctive characteristic in people with ALGS, even if they do not seem so obvious in the first years of life. "Cholestatic facies" includes a broadened forehead, an elongated nose with bulbous tip, deep-set eyes and a small, pointed chin (1,3). In our patient we could describe all these distinguishing features. Same facial features could be observed in the mother.

In terms of neurological damage, children may present with mild developmental delays or mental retardation (3). Evaluation of our patient showed a normal neuro-psycho-motor development according to his age.

Emerick et al. reported that posterior embryotoxon, which is a "circular faded line" on the surface of the eye or outskirts of the iris, is the most common ocular abnormality (>75% of the cases). But it has been described in 15% of the population without Alagille Syndrome (1,3,4). In our case, posterior embryotoxon was a major finding that sustained the diagnosis of ALGS. Other findings include Axenfeld anomaly, retinitis pigmentosa and optic disc anomalies (4). Recent studies by Nischal et al. reported the importance of ocular ultrasound which describes a new sign associated with Alagille Syndrome – optic disc drusen (6).

Children with ALGS may have an increased risk of bone fractures or isolated skeletal anomalies such as shorter radius, ulna and phalanges due to vitamin D and calcium deficiencies. Butterfly shaped vertebrae is described in more than 50% of patients with ALGS; fortunately it almost never causes functional problems of the nerves in the spinal cord (3,4). In our patient spinal X-ray did not describe vertebrae or other skeletal anomalies.

Vascular lesions, stenosis or aneurysms of the head and neck vessels represent a life-threatening anomaly associated with ALGS (3). Kamath et al. reported that 6% of patients with confirmed Alagille Syndrome have vascular anomalies which include middle cerebral artery and basilar artery aneurysm, Moyamoya disease or internal carotid artery anomalies (7). These malformations have not been described in our patient.

JAG1 and NOTCH2 genes are involved in the proximal nephron structures and podocytes. That is why mutations in these genes cause a renal dysplasia and proteinuria particular for Alagille Syndrome. Also, because of the expression of JAG1 gene in renal collecting ducts, children with ALGS may

develop renal tubular acidosis (4). Abdominal ultrasound and urodynamic analysis excluded renal involvement in our patient.

Before genetic testing, diagnosis of Alagille syndrome can be sustained based on the presence of at least 3 of 5 major clinical criteria (Table 1) alongside the specific histological trait – paucity of intrahepatic biliary ducts (8).

TABLE 1. Diagnostic criteria (8)

Diagnostic criteria	Our patient
Positive liver biopsy + 3 of 5 major criteria	Inconclusive liver biopsy + 4 of 5 major criteria
MAJOR CRITERIA	
1. cholestasis 2. Cardiac disease with peripheric pulmonic stenosis 3. Skeletal anomalies („butterfly-shaped” vertebrae) 4. Posterior embryotoxon (slit lamp ophthalmological examination) 5. Distinctive facial features: triangular facies, broadened forehead, deep-set eyes, elongated nose with bulbous tip, small, pointed chin	1. Intrahepatic cholestasis 2. Large peripheral pulmonic stenosis 3. Posterior embryotoxon 4. Distinctive facial features

Distinguishing Alagille syndrome from biliary atresia (BA) has a major impact on the further management, because patients with extrahepatic biliary atresia undergo a surgical procedure – Kasai portoenterostomy - with good hepatic outcome, whereas in ALGS this procedure leads to higher rates of liver transplantation and mortality (9).

The analysis which confirms Alagille syndrome and distinguish it from other cholestatic liver disorders (such as progressive familial intrahepatic cholestasis) remains chromosomal testing of JAG1 gene (20p12) (1,10). Due to financial issues, genetic testing was not initially available for our patient, but later on it confirmed the diagnosis.

Liver biopsy is not considered mandatory to establish diagnosis (2). In our case, liver biopsy failed to reveal paucity of bile ducts (this may have been the result of already-installed liver cirrhosis and intrahepatic cholestasis).

Abdominal ultrasound is considered to be the first step in imaging evaluation of a patient with cholestasis. It grossly assesses the hepatobiliary tree, liver parenchyma and vascular failure, also excludes renal anomalies (4). In our patient, first ultrasound assessment didn't encountered hepato-biliary tree anomalies. Ultrasound is required not only for diagnosis but for surveillance, as patients with Alagille Syndrome are at high risk of developing cirrhosis and hepato-

carcinoma. In a study by Rapp et al. is mentioned that there is limited literature regarding liver masses in ALGS (11). Large regenerative nodules can occur in patients with underlying cirrhosis (approximately 30% of the cases), more likely as a functional adaptation to vascular changes rather than a malignant process (5,12).

MRCP is the next non-invasive method of evaluation. However, this procedure has its pitfalls: false positive diagnosis due to poor bile production in the patent biliary tree. Previous studies showed that gallbladder abnormalities such as small length or oddly-shaped have a high incidence among patients with Alagille syndrome (9).

Medical care of a child with Alagille syndrome is a rather challenging process, as it requires a multidisciplinary team (2). Children with this pathology need a special nutritional assessment which includes a diet based on carbohydrates and medium-chain triglycerides (MCT). Our patient benefited from special nutritional formulas enriched with MCT and branched-chain amino-acids. Correction of fat-soluble vitamins and zinc deficiencies must be made with optimal oral dosage to ensure a proper development of these children (3,4). Our patient received oral supplements with vitamin K, vitamin D and calcium, vitamin E.

Altered metabolism pathways for cholesterol and biliary acids are described in patients with ALGS; they must receive medication which decreases liver production and intestinal absorption of cholesterol, such as ursodeoxycholic acid. Pruritus often does not respond to medication and has a major impact on the quality of life. There are studies which describe the efficiency of Cholestyramine or Rifampin in reducing bile-acid induced pruritus (2,4).

Standard immunization among children with ALGS is encouraged. Patients with liver manifestations should receive hepatitis A vaccine and if they develop ascites, the multivalent pneumococcal vaccine must be administered to prevent spontaneous bacterial peritonitis (4). Our patient was vaccinated against hepatitis B, tuberculosis, polio, rubella, measles, mumps, diphtheria, tetanus, pertussis and Hemophilus B.

Surgical assessment of children with Alagille Syndrome includes a palliative procedure – partial external biliary diversion and liver transplantation. In a study by Emerick et al. ALGS patients who underwent external biliary drainage had milder cholestasis without changes in bile composition, because this procedure diverges bile away from the enterohepatic circulation but doesn't adjust the hepatobiliary function (13).

Liver transplantation is considered in case of progressive hepatic dysfunction, severe portal hyperten-

sion, resistant pruritus or osteodystrophy (4). A pre-transplant assessment must take into account the Pediatric End-Stage Liver Disease Score, which includes several clinical and laboratory parameters – gender, age, weight, height, albumin and bilirubin levels, INR. This score is accurate for children under 12 years old and correlates with post-transplant outcome (14). Our patient had a promising PELD score with a 1-year survival rate without liver transplantation of 86,8% and 1-year posttransplant survival rate of 93,6%.

The overall survival rate is approximately 80% at 5 years and 60% at 20 years post liver transplantation, although survival rate is significantly lower in patients with Alagille syndrome than in those with biliary atresia (4).

Another issue that surfaces is whether grafts from close family relatives should be accepted or not. Chi-Ning Lee et al. reported a case of aborted liver graft due to a liver biopsy which described paucity of bile ducts during the operation (15). Our patient is awaiting liver transplantation from father donor. Taking into account the distinguishing facial features and

structural cardiac malformation of the mother, the child is more likely to have inherited the gene from her. But, father with significant liver steatosis remains a rather spiky issue.

If serious cardiovascular anomalies such as Fallot tetralogy are associated, cardiac surgery is mandatory, with a 20-year predicted survival rate of 40% (4). In our case, the child presented a large pulmonic stenosis that didn't required surgical assessment.

CONCLUSIONS

Alagille syndrome can sometimes be a tricky diagnosis keeping in mind the variable phenotypes of the disease and limited possibilities for genetic testing. However, diagnosis should not be missed when the classic criteria is met.

In terms of treatment and management, ALGS patients require attention from a multidisciplinary team which must monitor the child's nutritional status, cardiovascular activity, manage the chronic liver disease and indicate liver transplantation when appropriate.

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