An Infant with Severe Anemia and Hypoalbuminemia

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We discuss the case of a two-month-old girl admitted with complaints of progressive pallor, generalized body swelling and pale colored stool since the neonatal period. On examination, severe pallor, chubby cheeks and moderate hepatomegaly were noted. Investigations revealed isolated anemia, transaminitis, conjugated hyperbilirubinemia, prolonged prothrombin time and hyperlipidemia. She died due to severe sepsis, shock, and pulmonary hemorrhage. An autopsy revealed characteristic histopathology findings of cystic fibrosis in the liver, lungs, and pancreas. Genetic analysis performed on autopsy tissue was positive for F508del compound heterozygous (WT/F508del) mutation, confirming the diagnosis of cystic fibrosis.

Keywords: *Autopsy, Cholestatic jaundice, Cystic Fibrosis.*

CLINICAL PROTOCOL

History: A two-month-old girl, a product of non-consanguineous marriage, presented with complaints of gradually increasing generalized body swelling starting from the age of 15-20 days. It was associated with progressive pallor, for which she had received one packed red blood cell (PRBC) transfusion at 1½ month of age. Since one month of age, she had passed 8-10 pale-colored semisolid stools per day. She also had fast breathing for 15 days prior to admission. There was no history of jaundice, high colored urine, bleeding from any site, mucus in stool, lethargy, poor feeding, irritability, seizures, and encephalopathy. She was born at term gestation with a birthweight of 2.3 kg and was admitted in the intensive care unit for five days in view of abdominal distension and respiratory distress since birth. She passed meconium at the end of day 2 of life, following which abdominal distension resolved. The baby was exclusively breastfed, immunized for age and developmentally normal for age. The elder sibling had a tracheo-esophageal fistula and had died on day two of life.

Clinical examination: At admission, she was alert and active with a heart rate of 128/min, respiratory rate of 58/min, good volume pulses, normal capillary refill time, and 100% oxygen saturation on room air. She had severe pallor and anasarca. She weighed 3.5 kg (-4.28 *Z*) and the occipitofrontal circumference was 34.1 cm (-4.51 *Z*). The baby had very prominent chubby cheeks. Respiratory system examination showed bilateral basal crepitations. The abdomen was distended with liver being palpable 6 cm below right costal margin and 3 cm below the left costal margin in the midclavicular line (span 9-10 cm), soft-to-firm in consistency, non-tender, with ill-defined borders. The spleen was not palpable. Examination of cardiovascular system and central nervous system was normal. Fundus examination did not show any chorioretinitis or cherry red spot.

Laboratory investigations: She had normocytic normochromic anemia (haemoglobin 6.5 g/dL), leukocytosis (white cell count 34,360/µL, differential counts (53% polymorphs), normal platelet count, transaminitis (alanine aminotransferase – 79 IU/L and aspartate aminotransferase – 367 IU/L), cholestatic jaundice (total bilirubin – 4 mg/dL, direct – 3.3 mg/dL), severe hypoalbuminemia (1.5 g/dL), deranged coagulation profile (prothrombin time – 20.9 seconds, activated thromboplastin time – 44.6 seconds) and hyperlipidemia (total cholesterol – 255 mg/dL, triglyceride – 289 mg/dL) and a high C-reactive protein (33.6 mg/L). Arterial blood gas analysis revealed respiratory alkalosis. She also had persistent hyponatremia (126 mEq/L) and hypochloremia (93 mEq/L). Chest radiograph, stool examination, immunoglobulin profile (IgG- 346 mg/dl, IgA- 51 mg/dL), and T-cell subset assay (CD3+= 55.43%, CD 19+= 32.34%, CD56+=4.84%, CD3+ CD56+= 0.46%) were normal. Blood sugar was 96 mg/dL and serum ammonia was 225.8 µmol/L. Urinary aminoacidogram could not be done. Human immunodeficiency virus, cytomegalovirus, and toxoplasma serology were negative. Ultrasound abdomen showed hepatomegaly with normal liver echotexture. The cranial ultrasound did not show any structural

malformation or calcification. The blood culture sent at presentation was sterile; however, repeat blood culture sent on day 4 grew *Staphylococcus hominis* (sensitive to ciprofloxacin, clindamycin, teicoplanin and vancomycin; oxacillin and erythromycin).

Course management: The infant received intravenous cefotaxime, cholestatic regimen [1] and PRBC transfusion. On day 3 of hospital stay, she had one episode of fresh blood in stool along with deranged coagulation profile; therefore, fresh frozen plasma was transfused. However, on the next day she worsened further in the form of tachycardia, poor pulses, and prolonged capillary refill time, for which antibiotics were empirically upgraded to vancomycin, meropenem and amphotericin B. Fluid bolus and inotropic support was given for the shock. On day 5 of hospital stay, she had further cardiorespiratory worsening for which she was intubated and kept on manual intermittent positive pressure respiration. On the same day she developed hypocalcemia and hypokalemia requiring correction. She deteriorated further and received intravenous immunoglobulin, multiple fluid boluses and inotropes (dopamine, dobutamine, and adrenaline). However, she had worsening of shock and hypoxemia followed by massive pulmonary haemorrhage leading to cardiac arrest and death on day 5 of hospital stay.

Unit's final diagnosis: Glycogen storage disorder with refractory septic shock and pulmonary hemorrhage.

DISCUSSION

Clinical discussant: We have a two-month-old girl presenting with severe pallor, anasarca, pale stool, chubby cheeks, failure to thrive, microcephaly, moderate hepatomegaly, transaminitis, cholestatic jaundice, and hyperlipidemia. She had isolated normocytic normochromic anemia, with deranged coagulation profile and required two blood transfusions during the initial two months of life. This case can be analyzed with respect to underlying disease and the pre-terminal events. The primary analysis suggests multi-system disease with predominant hepatic involvement. There are many causes of isolated hepatomegaly with the above findings. Of these, intrauterine infections (TORCH), hemophagocytic syndrome and metabolic/storage disorders can present like this child. Intrauterine infections (particularly CMV and toxoplasma) are unlikely in the absence of splenomegaly, thrombocytopenia, chorioretinitis, hepatic, and cerebral calcification. Moreover, the serology for CMV and toxoplasma was negative. Hemophagocytic syndrome is also unlikely in the absence of fever, splenomegaly, and bicytopenia. The NK cell activity was also normal. The possible storage/metabolic disorders can be glycogen storage disorder (GSD), lipid storage disorder (Gaucher and Niemann-Pick disease), iron storage (neonatal hemochromatosis due to gestational alloimmune liver disease, GALD), alpha-1 antitrypsin deficiency, galactosemia, cystic fibrosis (CF), and citrin deficiency. However, in the absence of splenomegaly, thrombocytopenia, cardiac malformation, and cherry-red spot; lipid storage disorders are less likely. GALD is less likely as it starts from fetal life itself and frequently present in the early neonatal period with prematurity, acute hepatic failure, very high bilirubin, hydrops, and renal failure. Alpha -1 antitrypsin deficiency can have similar presentation, but lack of respiratory symptoms, splenomegaly, and chubby cheeks make it less likely. Presence of chubby cheeks and lack of significant coagulopathy are against galactosemia. The rest of the metabolic disorders (GSD, citrin deficiency and CF) are strong possibilities. Among GSDs; type I, III, VI, and IX have a predominant hepatic presentation with moderate hepatomegaly, but only GSD type I can lead to severe anemia at this age. However, the presence of significant microcephaly, severe hypoalbuminemia, prolonged PT/APTT and lack of hypoglycemia even in extreme sickness is against GSD type I.

Citrin deficiency has a wide spectrum and can present in infancy as Neonatal intrahepatic cholestasis caused by citrin deficiency (NICCD) and/or Failure to thrive and dyslipidemia caused by citrin deficiency (FTTDCD) [2]. The classical features of citrin deficiency are low birth weight, intrauterine growth restriction, microcephaly, chubby cheeks due to excessive lipid deposition, hepatomegaly, neonatal cholestasis, features of liver failure and hemolytic anemia. They also have mild hyperammonemia, hyperlipidemia, increased alpha-fetoprotein, and fatty liver. Diagnosis is based on abnormal newborn screen (aminoaciduria) followed by genetic analysis. All the features in the index case are consistent with the citrin deficiency except, normocytic anemia, hyponatremia, hypochloremia, and pulmonary symptoms. However, chubby cheeks with predominant hepatic manifestations are classical of GSD and citrin deficiency. Since GSD is less likely in this case, citrin deficiency may be considered as a strong differential diagnosis.

Cystic fibrosis-associated liver disease (CFLD) can present in early infancy with features of hepatomegaly, cholestasis, transaminitis, dyslipidemia, and severe anemia along with hypoalbuminemia. Severe anemia can be the first sign of cystic fibrosis in 7-10 % of infants with cystic fibrosis and the concomitant presence of severe hypoalbuminemia makes it more likely [3]. The anemia of CF is normocytic normochromic and may precede several

months of respiratory symptoms. The index case also has delayed passage of meconium along with hyponatremia and hypochloremia, further favoring the diagnosis of CF. However, the presence of chubby cheeks and normal liver echotexture is not consistent with CF. Overall, citrin deficiency and CF are the most likely clinical differential diagnosis in the index case. The prominence of chubby cheeks strongly favors citrin deficiency. However, disregarding chubby cheeks, the clinical picture is consistent with CF.

In terminal stages, the infant had severe sepsis (most likely of bacterial origin) characterized by increasing CRP, high leukocyte count, and decreasing platelets. She developed refractory septic shock leading to multiorgan dysfunction and subsequently died.

Chairperson: As per the clinical experience of the unit; which is most common among GSD, Citrin deficiency, and CF?

Pediatric gastroenterologist 1: During hospitalisation, GSD was our first possibility. However, on retrospective analysis of the biochemical profile (AST much higher than ALT, dyslipidemia, lack of hypoglycemia and lactic acidosis) in the presence of very prominent chubby cheeks developing over two months, citrin deficiency seems more likely. Citrin deficiency has been described over the last few years, primarily from Japan and South East Asia. It is perceived to be rare, though not uncommon. However, it is unlikely that this case will have a definite histopathological picture of citrin deficiency. In our experience, GSD is the most common clinical condition. However, recently we were able to diagnose a few cases of citrin deficiency too. The children with citrin deficiency generally do well and improve over a period of time. This child died because of sepsis, not of citrin deficiency *per se.*

Pediatric pulmonologist: There was delayed passage of meconium with some component of diarrhea. This child is a classic picture of cystic fibrosis. The chubby cheeks may be due to hypoalbuminemia and edema which may give a false impression of good nourishment.

Neonatologist: In CF, we expect a low level of cholesterol, whereas the cholesterol was significantly elevated in this case.

Pediatric Gastroenterologist 1: Galactosemia is a mimicker of citrin deficiency. However, the chubby cheeks and lack of significant coagulopathy are strongly against it.

Pediatric Neurologist 1: Niemann pick type C can present like hydrops fetalis in early infancy. Tyrosinemia can also have a similar presentation.

Pediatric Gastroenterologist 2: Lack of splenomegaly and thrombocytopenia are against Niemann pick type C. In tyrosinemia, the prominent features are severe coagulopathy and fulminant hepatic failure, which was not seen in this case. I would like to keep the possibility of a congenital disorder of glycosylation (CDG) type Ib/Ih.

Pediatric Neurologist 2: Severe anemia at an early age is not usual in storage disorders. Therefore, it must be due to the marrow involvement and Pearson marrow-pancreas syndrome can be considered as a differential diagnosis. CDG also has similar features along with abnormal fat distribution.

PATHOLOGY PROTOCOL

A complete autopsy was performed in the index case. There was 50 ml straw-coloured fluid in the pleural and peritoneal cavity.

Liver weighed 226 g and was slightly enlarged, soft, and bile stained (***Fig.* 1**). Gall bladder was normal in size and the extrahepatic biliary tree was patent. Microscopically, the liver showed expansion of the portal tracts with mild fibrosis and extensive bile ductular proliferation. There was a focal porto-portal bridging, producing focal biliary cirrhosis. There was extensive macrovesicular steatosis, along with intrahepatocytic and intracanalicular cholestasis, producing feathery degeneration of the hepatocytes (***Fig.* 1 b-d**). However, there were no PAS-positive diastase resistant inclusions in the hepatocytes. Larger bile ducts and bile ducts at porta hepatis were markedly dilated and filled with inspissated secretions. These inspissated secretions were Periodic-Acid-Schiff (PAS) positive, diastase-resistant and strongly positive for the alcian blue, indicating mucinous nature. The biliary epithelium showed evidence of mucinous metaplasia. Similar inspissated secretions were also seen in peribiliary glands (***Fig.* 1 e-f**). The pancreas was firm in consistency. It showed marked dilatation of the ducts filled with inspissated secretions. There was marked intra and inter-lobular fibrosis with loss of acini, and focal lymphomononuclear infiltrate (***Fig.* 2 a-c**). Spleen (12 g) showed normal white and red pulp. Stomach, esophagus, small intestine, and large intestine were grossly unremarkable. There were no inspissated secretions. Perl stain did not reveal any evidence of excess iron deposition in liver, spleen or pancreas.

Lung weighed 95 g and the bilateral pleura were dull. Bilateral lower lobes were consolidated. There was extensive hemorrhagic discoloration of both lungs (left > right). Sections of the lungs showed dilated bronchioles, filled with inspissated secretions. Inspissated secretions

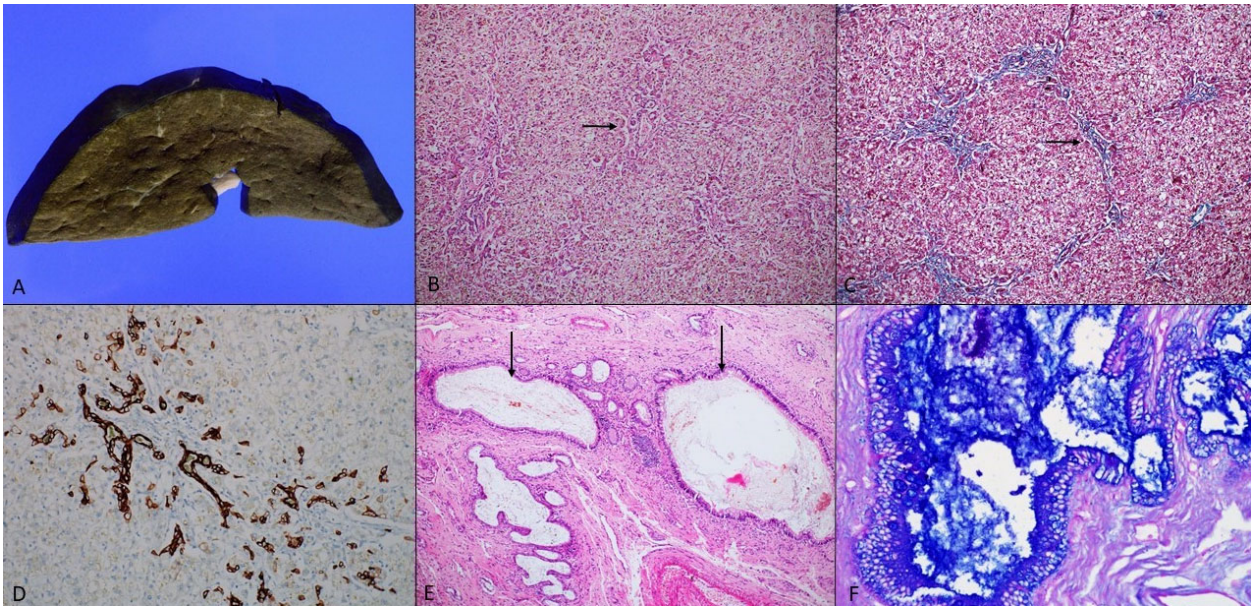

FIG. 1 *(a) Gross photograph of liver shows extensive bile staining.(b) Liver shows irregular portal tracts (black arrow), with macrovesicular steatosis and cholestasis (hematoxylin and eosin, ×100). (c) Masson's trichrome highlights irregular portal fibrosis with occasional porto portal bridging (black arrow) (×100). (d) Bile ductular proliferation is highlighted by cytokeratin 7 (immunohistochemistry, ×200). (e) Section from porta of liver shows marked dilatation of larger bile ducts, filled with inspissated secretions (black arrow) (hematoxylin and eosin, ×100). (f) Alcian blue- Periodic acid Schiff stain highlights the inspissated secretions and mucinous metaplasia of the biliary epithelium (×400).*

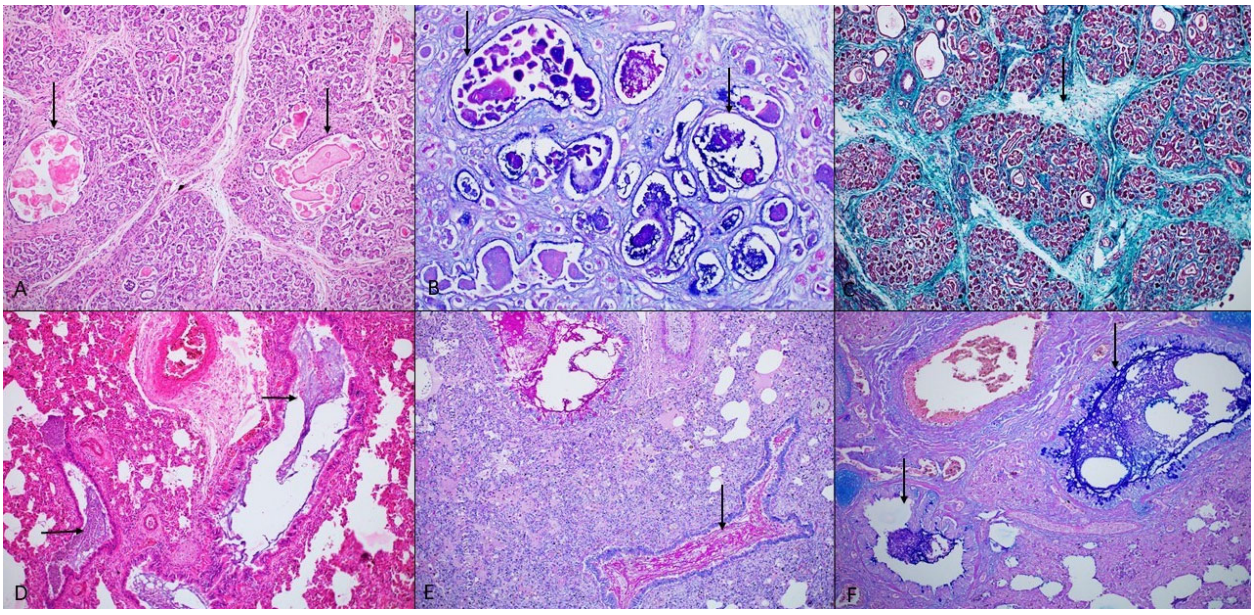

FIG. 2 *(a) Pancreas shows dilated ducts with inspissated secretions (black arrows) and parenchymal fibrosis (hematoxylin and eosin, ×100). (b) Alcian blue- Periodic acid Schiff stain highlights the inspissated secretions in the pancreatic ducts (black arrows) (×100). (c) Masson's trichrome stain highlights inter and intralobular fibrosis in pancreas (black arrows) (×100). (d) Section from lung shows dilated bronchioles filled with inspissated secretions (black arrows) (hematoxylin and eosin, x100), which is highlighted by (e) periodic acid Schiff (×100) and (f) Alcian blue stain (×100).*

were also seen in the main bronchus (***Fig*. 2 d-f**). The subepithelial glands were hypertrophied and showed inspissated secretions. In addition, lungs showed exuberant capillary proliferation in the alveolar septa, with the capillaries infiltrating the wall of the pulmonary arteries, producing pulmonary capillary hemangiomatosis. Extensive fresh pulmonary hemorrhage was noted. Also, there was evidence of bronchopneumonia with diffuse alveolar damage.

Heart (25 g) showed normal chambers and valves. Kidneys (46 g) showed normal fetal lobulations. Microscopic examination did not reveal any pathology. Brain (491 g) showed normal sulci and gyri. No gross or microscopic pathology was seen. Bone marrow was normocellular for age and showed marked erythroid hyperplasia. Other hematopoietic lineages were adequately represented. Thymus showed extensive cystic degeneration of the Hassel corpuscles likely due to stress-induced involution.

Genetic mutations in the CFTR gene: Peripheral blood was collected at autopsy and was subjected to *CFTR* gene mutation by the mass array. A limited *CFTR* gene panel was examined and showed F508del compound heterozygous (WT/F508del) mutation. Mass array performed for *FIC 2* and *3* genes did not reveal any mutation.

Final autopsy diagnosis:

- Cystic fibrosis involving lung, pancreas, and liver (F508del compound heterozygous)
- Bronchopneumonia with diffuse alveolar damage
- Pulmonary capillary hemangiomatosis
- Pulmonary hemorrhage
- Erythroid hyperplasia of bone marrow

Pediatric Pulmonologist: The histopathology shown here is the classical book picture of cystic fibrosis. This mutation is likely a compound heterozygote and they do not have a very good phenotypic-genotypic correlation. The parents should also be evaluated for the mutation. If we would have suspected in the antemortem period, the course would have been different and treatment can be offered in earlier stages.

Pediatric Gastroenterologist 1: The clinical picture was not very classical of CF. Even if we would have done an antemortem percutaneous liver biopsy, the conclusive diagnosis was unlikely. The focal biliary cirrhosis is a very nonspecific finding and is seen in myriad conditions. Even sweat chloride testing is not feasible at this age. Therefore, it is very difficult to make an antemortem diagnosis of CF at such an early age. The classical features appear during adolescence only.

Pathologist 1: The pulmonary capillary hyperplasia shown in histopathology is a reactive finding and is commonly observed during infancy. It should not be confused with the pulmonary capillary hemangiomatosis (PCH), which is a diagnosis of exclusion. PCH should show infiltration of pleura, septa, veins, and the vessel wall.Moreover, in PCH, the capillaries form a nodule and show multiple layers. Therefore, here it was just a reactive pulmonary capillary hyperplasia.

Pediatric Pulmonologist: PCH is also known to occur in CF when they develop pulmonary artery hypertension. There is hypersecretion of VEGF that leads to this finding in CF.

Clinical discussant: Everything was consistent with CF, but the chubby cheeks took us away from it; likely these were a manifestation of severe hypoalbuminemia.

Pathology discussant: The histopathology shown here is very characteristic of reactive pulmonary capillary hemangiomatosis, which can be associated with cystic fibrosis and various metabolic liver diseases.

Pathologist 2: Even if we would have done an antemortem liver biopsy, the diagnosis was unlikely. Here we have a whole liver, so we could show classical findings. In suspected CF, we should always look for changes in Brunner gland in the duodenum.

Pathologist 3: Liver biopsy plays an important role in the work-up of infantile cholestasis. Although, it may not be diagnostic in all cases, it provides important information to exclude other conditions presenting as infantile cholestasis such as congenital hepatic fibrosis, extrahepatic biliary atresia, progressive familial intrahepatic cholestasis or paucity of intrahepatic bile duct.

DISCUSSION

Cystic fibrosis is one of the commonest life-limiting autosomal recessive monogenic disorders. Initially thought to be affecting the Caucasians only, its presence is pan-ethnic [4]. It is caused by mutations in the *CFTR* (*cystic fibrosis transmembrane conductance regulator*) gene. Till now, more than 1500 mutations have been described in CF, of which the deletion of phenylalanine at codon 508 (δF508) is the commonest. Different mutations have varying genotypic effects on *CFTR* function and can result in different phenotypic expression of the disease [5,6]. The manifestations of CF may begin in early infancy itself in the form of delayed passage of meconium, meconium ileus, recurrent loose stool, malabsorption, cholestatic jaundice and failure to

thrive. Later they have recurrent respiratory infections, features of malabsorption and involvement of many other organ systems, namely endocrine, hepatobiliary and reproductive system [7].

CFLD, the liver involvement in cystic fibrosis can be found in infancy in 13%-17% cases of cystic fibrosis [8,9]. The presentation of CFLD may vary from asymptomatic transaminitis to prolonged cholestasis, hepatomegaly, and severe liver dysfunction [8]. The diagnostic criteria of CFLD comprises either a positive histopathological test (focal or multilobular biliary cirrhosis) or presence of at least two of the following criteria, evaluated at least twice a year: (*i*) Hepatomegaly (>2 cm below the costal margin in the midclavicular line) confirmed by ultrasound test; (*ii*) abnormal elevation of liver enzymes; and (iii) positive ultrasound findings (increased echogenicity of liver parenchyma, tuberosities, irregular edges and splenomegaly) [8,10]. Pulmonary complications are the predominant cause of morbidity and mortality in CF. CFLD is an evolving paradigm and is believed to the third commonest cause of mortality in patients with CF [8].

The symptoms evolve over time, and in early infancy, anemia may be the first clinical presentation of CF [3]. These infants typically have normocytic normochromic anemia secondary to multiple etiologies like iron deficiency, chronic inflammation, vitamin E deficiency, ineffective erythropoiesis, and ongoing micro-bleeding. The anemia is often accompanied by hypoalbuminemia [11]. The severity of hypoalbuminemia can be used as a marker of severity of respiratory morbidities in later life [12]. These two manifestations can precede respiratory symptoms for many months [3]. Therefore, the concomitant presence of severe anemia and hypoalbuminemia in early infancy should raise the possibility of CF.

To establish the diagnosis of CF, sweat chloride estimation is the first test to be done, followed by *CFTR* genetic analysis, and CFTR physiologic tests. All individuals diagnosed with CF should have a sweat test and a *CFTR* genetic analysis performed [13]. However, in neonates and early infancy, the sweat chloride test is difficult to perform due to logistic issues; therefore the reliance is more towards the genetic analysis (*CFTR* mutation panel).

F508del is the commonest *CFTR* gene mutation in the Western population, up to the tune of 80% of all tested alleles. However, this mutation is much less commonly observed in Asian and Indian patients [14,15]. Thus, a limited mutation analysis may not be able to provide a genetic diagnosis of CF, and we may need complete *CF* gene sequencing for the confirmation of the diagnosis.

In the index case, the antemortem diagnosis could not be made, but post-mortem histopathology along with positive mutation is diagnostic of cystic fibrosis.

Contributors: JK: clinical protocol discussant, reviewed the literature and drafted the manuscript; DC: pathology protocol discussant, reviewed the literature and edited the manuscript; SL: treating unit consultant, provided critical inputs in the draft of the manuscript, and edited the manuscript; PK: substantial inputs in analysis of the case, critically reviewed and edited the manuscript. All the authors approved the final version of the manuscript.

Funding: None; *Competing interests:* None stated.

Ethics statement: The authors certify that the family was informed about the final diagnosis, and appropriate counselling provided.

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Annexure I

Name of Discussants (In the sequence they appear in the manuscript)

Clinical Discussant : Jogender Kumar

Chairperson : KL Gupta

Pediatric Gastroenterologist 1 : Sadhna B Lal

Pediatric Pulmonologist : Meenu Singh

Neonatologist : Sourabh Dutta

Pediatric Neurologist 1 : Renu Suthar

Pediatric Gastroenterologist 2 : G. Vybhav Venkatesh

Pediatric Neurologist 2 : Arundhati B Mukherjee

Pathologist 1 : Kirti Gupta

Pathology discussant : Debajyoti Chatterjee

Pathologist 2 : Kim Vaiphei

Pathologist 3 : Uma Nahar