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LETTER TO EDITOR

Revisiting Fatal Granulomatous Disease of Childhood Through an Autopsy: Still Lethal in the Developing World!

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Abbreviations

CGD	chronic granulomatous disease
NADPH	nicotinamide adenine dinucleotide phosphate
MRSA	methicillin-resistant *Staphylococcus aureus*
CECT	contrast-enhanced computerized tomography
NBT	nitro blue tetrazolium
DHR	dihydrorhodamine

To the editor:

Chronic granulomatous disease (CGD) is an inherited, functional, phagocytic disorder due to a defect in any of the proteins constituting the NADPH oxidase complex. The disease is known to result in recurrent life-threatening bacterial and fungal infections and dysregulated granulomata formation. The age at presentation is variable with cases reported in early infancy and late adulthood. However, the vast majority of cases are diagnosed before the age of 5 years. Children with X-linked CGD, especially when they present early in life tend to have a very severe disease [1], as in the index patient.

A 6-month-old boy, first born to a non-consanguineous couple presented with fever for 2 months and a slowly progressive neck swelling. He also had a history of bilateral purulent ear discharge during this period. He was exclusively breastfed. His birth, development, immunization and family history were unremarkable. Examination revealed tachycardia, tachypnoea, pallor, multiple tender, non-fluctuant, non-matted, cervical, axillary, and inguinal lymph nodes. Bilateral crepitations and hepato-splenomegaly were noted on systemic examination. Laboratory investigations revealed hemoglobin 73 g/L, total leucocyte count 11.3×10^9/L with neutrophilic predominance (80%), platelet count 714×10^9/L, an elevated erythrocyte sedimentation rate (75 mm in the 1st hour), C-reactive protein 120 mg/L, and an elevated procalcitonin (68.97 ng/mL). Fine needle aspiration of lymph node culture grew methicillin-resistant *Staphylococcus aureus* (MRSA). Stain for acid-fast bacilli and periodic acid-Schiff stain did not highlight the presence of any organisms. Blood cultures were sterile on multiple occasions. Work-up for tuberculosis was negative and HIV ELISA was non-reactive. Contrast-enhanced computerized tomography (CECT) of the neck and chest showed necrotic cervical lymphadenopathy, bilateral maxillary sinusitis and left parotitis, and patchy consolidation with few nodules in bilateral lungs with mediastinal lymphadenopathy, likely of infective etiology. Serum immunoglobulin profile showed hypergammaglobulinemia; IgG 18.6 g/L (3.0–9.0 g/L), IgA 0.38 g/L (0.15–0.7 g/L), and IgM 2.75 g/L (0.4–1.6). Nitro blue tetrazolium test (NBT) showed no reduction of dye as compared to control, suggestive of the possibility of CGD. Dihydrorhodamine assay (DHR) showed a stimulation index of 1.35 as compared to a control of 132.31 confirming the diagnosis of CGD. Stimulation index or Neutrophil oxidative index is calculated by dividing the Median fluorescence intensity (MFI) of neutrophils stimulated with PMA by that of unstimulated neutrophils (SI = MFI Stimulated/MFI Unstimulated). DHR assay of the mother showed a double peak suggestive of a carrier state for X-linked CGD. b558 staining by flow cytometry showed absent expression in the patient and mosaic pattern of expression in the mother, consistent with X-linked CGD due to gp91phox defect. Genetic analysis revealed a frame-shift mutation in exon 5 of the *CYBB* gene (c.426delT p.E143fs158X). At admission, the

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child was started on ceftriaxone and cloxacillin along with supportive care. Antimicrobial therapy was changed to vancomycin after the growth of MRSA in lymph node aspirate. However, in view of persistent fever spikes despite intravenous vancomycin and meropenem, he was empirically started on amphotericin. Further, during the course of hospital stay, BAL showed growth of *Acinetobacter baumannii*, for which meropenem was changed to colistin. Work-up for tuberculosis including tuberculin sensitivity test, BAL for acid-fast bacilli, and gene-Xpert were negative. Hence, empirical antitubercular therapy was not considered. He continued to have anemia (requiring transfusions) with leukocytosis. Initial platelet count showed thrombocytosis; however, there was a falling trend over the course of hospital stay. Erythrocyte

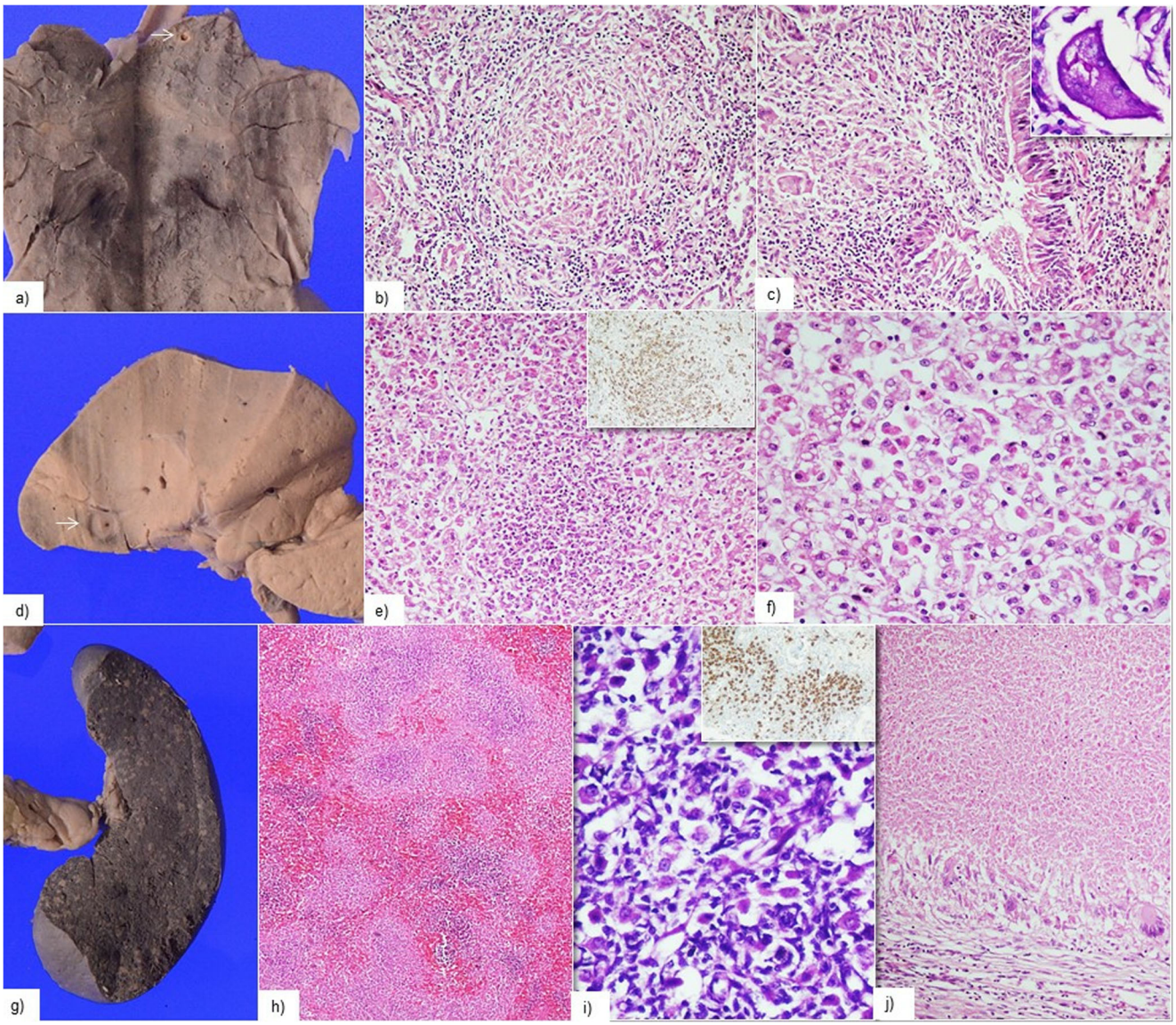

Fig. 1 **a** Cut surface of the lung shows patchy areas of consolidations, with a focus of tiny yellowish areas of abscess (arrow). **b** Microscopic sections from the consolidated areas showed destruction of the alveolar parenchyma and presence of multiple non-caseating epithelioid cell granulomas containing epithelioid cells, macrophages, and multinucleated giant cells (HE, 100×, and 200×). **c** The abscess shows destruction and ulceration of a bronchiole with infiltration of histiocytes and multinucleated giant cells (HE, 200×). Inset: PAS stain highlights fungal hyphae within cytoplasm of a multinucleated giant cell (PAS, 1000×). **d** The liver shows single gray white solid nodule in the right lobe, with the size of 1 cm (arrow). **e** Microscopy of the liver shows multiple foci of hepatocytic loss replaced by granulomas in the form of histiocytic collections admixed with nuclear debris (HE, 100×). Inset: CD68 immunostain highlights nodular collections of histiocytes (IHC, 100×). **f** There were prominent intra-sinusoidal Kupffer cells containing pigments, characteristic of CGD (HE, 400×). **g** Spleen is enlarged and shows multiple tiny gray white nodules (2 to 3 mm). **h** Microscopic section shows multiple granulomas, similar to that of liver lesions (HE, 100×). **i** Microscopic section from thymus shows infiltration of parenchyma by characteristic pigment containing macrophages (HE, 400×). Inset: the same was highlighted in CD68 immunostain. **j** Section from lymph node shows epithelioid cell granuloma with large area of central necrosis (HE, 200×)

sedimentation rate was elevated during the initial phase but declined progressively subsequently. C-reactive protein and serum procalcitonin remained persistently elevated. He had progressively worsening clinical course and later developed seizures, encephalopathy, refractory hypoglycemia, refractory shock and succumbed to his illness.

Postmortem Examination

A complete autopsy was performed. Peritoneal cavity revealed 250 mL of straw-colored fluid. The lungs were heavy (weight 167 g) with patchy areas of consolidation and foci of microabscesses (Fig. 1a). The liver and spleen were grossly enlarged. A single focus of abscess (1 cm) was noted in the right lobe of the liver (Fig. 1d). Microscopic sections from the lungs, liver, spleen, and thymus showed granulomatous inflammation admixed with pigmented macrophages, which were consistent with chronic granulomatous disease (Fig. 1b, e–i). Abscesses in the lung showed necrotizing inflammation containing histiocytes, and multinucleated giant cells containing few slender, septate, branching, and fungal hyphae (Aspergillus hyphae) (Fig. 1c). No fungus was seen in the liver abscess. Acid-fast bacilli (AFB), modified AFB, Gram and Gram-Twort stains performed in the lungs, liver, and spleen did not reveal any other organisms. Microscopic sections from the enlarged lymph nodes showed epithelioid cell granulomas with a large area of necrosis (Fig. 1j). Polymerase chain reaction for *Mycobacterium tuberculosis* complex from lymph node aspirate was positive. Granulomas in the lung showed no necrosis.CD20 and CD3 immunostains exhibited preserved B and T cell populations, respectively. There was no evidence of hemophagocytosis. The kidneys showed acute tubular necrosis and hyaline cast. A focus of subarachnoid hemorrhage (2 cm) was noted in the left parietal lobe of the brain. Gross and microscopic sections from the rest of the organs were essentially normal.

CGD is caused by defects in one of the five genes that encode the structural subunits of the NADPH oxidase, the enzyme responsible for the phagocyte respiratory burst and the generation of phagocyte superoxide. Most frequent sites of infection in CGD are the lung, lymph nodes, liver, and skin [2]. Bridges et al. in 1950 reported the first detailed description of 4 male children as a fatal granulomatous disease of childhood. They showed that disease is associated with a generalized granulomatous process, characteristic pigmented macrophages, and hypergammaglobulinemia [1]. The microbiology of the infections of CGD is remarkable for its relative specificity. Patients with CGD are usually predisposed to the catalase-positive organism (bacterial) and fungal infections. Previous studies on infection profile in CGD from India showed that *Staphylococcus* sp. was the most commonly isolated bacterium in CGD followed by *Klebsiella* sp. and *Pseudomonas* sp. Among the fungal infections, *Aspergillus* followed by Candida was seen in patients with CGD. In the index case, FNAC lymph node showed growth of MRSA, BAL showed growth of *Acinetobacter baumannii*. In the index case, lung histopathology revealed septate hyphae that likely are *Aspergillus*. Fungal infections remain a major determinant of survival in patients with CGD [3, 4]. Liver involvement is a significant cause of morbidity in CGD, with abscesses occurring in approximately 30–35% cases in different series [5]. In the index case, the postmortem examination revealed small liver abscess.

Mycobacterium tuberculosis PCR was positive in the autopsy sample by PCR. Although it is a sensitive and quick test for identification of *Mycobacterium tuberculosis*, it is associated with varying rates of false positivity in different studies [6]. Determination of the causal pathogen for infection in patients with CGD is vital for effective management. Polymicrobial infections including bacterial and fungal remain a challenge in patients with CGD. In the index case, multiple attempts were made to obtain an organism; however, fungal infection and *Mycobacterium tuberculosis* were identified only at autopsy.

Granulocyte infusions have been tried and shown to be an effective adjunctive therapy in refractory infections in CGD patients [7]. However, it was not used in the index case.

To conclude, in infants presenting with suppurative lymphadenitis, persistent pneumonia, hepato-splenomegaly, significant neutrophilic leukocytosis, and thrombocytosis CGD should be ruled out even in the absence of family history. CGD patients presenting in early infancy usually have little or no residual NADPH oxidase activity resulting in severe disease with high mortality and require prompt medical attention and management.

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Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

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