Multiple Opportunistic Infections in an 8-month-old Infant: Is it Seronegative HIV Infection?

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Received: 15 May 2017 / Accepted: 23 August 2017 / Published online: 4 November 2017
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Abstract We describe an 8-mo-old boy who presented with acute respiratory failure following a prolonged febrile respiratory illness with features suggesting immunodeficiency.

Keywords HIV infection · Severe immunosuppression · Cytomegalovirus · *Pneumocystis jiroveci* · Opportunistic infections

Clinical Protocol

History An 8-mo-old boy presented with 3 mo history of high grade fever and dry, intermittent, non-paroxysmal cough with weight loss (1.1 kg) requiring hospital admissions. He now presented with persistent fever spikes, worsening cough and respiratory distress associated with poor feeding and lethargy. There was no history of recurrent loose stool, ear discharge, cyanosis, seizure, loss of consciousness or symptoms suggesting cardiovascular involvement. He was admitted to a healthcare facility where his parents tested reactive for human immunodeficiency virus (HIV) following which he was referred to our centre.

He was second born to an unsupervised mother by full term normal vaginal delivery at home with undocumented birth weight. He was exclusively breast fed for initial 2.5 mo

followed by bottle feeds with diluted cow's milk. He had received only Bacillus Calmette Guerin (BCG) and 3 doses each of oral polio vaccine (OPV) and diphtheria-pertussis-tetanus (DPT) vaccine. His father was a daily wage and mother was a housewife with no significant clinical history. His elder sibling had expired at 5 mo of age due to suspected pneumonia.

Clinical Examination Child was grossly emaciated with anthropometry suggesting acute on chronic malnutrition. He was febrile, had respiratory rate 70/min and pulse rate 136/min. He presented in respiratory failure with room air saturation 34% (90% on 40% FiO₂). His circulatory parameters were, however, normal. Gross physical examination revealed pallor, oral thrush and subcentimetric cervical lymph nodes. He had hepatomegaly and bilateral coarse crepitations. Cardiovascular and central nervous system examination were normal.

Investigations Hematological and biochemical investigations are summarized in Table 1.

Microbiological Investigations In view of clinical suspicion of HIV infection and parents being HIV positive, child was worked up for opportunistic infections. Serology for syphilis, toxoplasma, rubella, cytomegalovirus (CMV), hepatitis B and C were negative. Gastric lavage (GL) did not show acid fast bacilli (AFB) or *pneumocystis jiroveci* and mantoux was negative. Blood culture was sterile, fungal serology was negative and stool examination was normal.

Parents tested reactive for HIV by enzyme linked immunosorbent assay (ELISA); it was, however, non-reactive for the child by three HIV test kits (Test I: SD Bioline, Test II: Aidsan Tridot, Test III: Comb Aids). CD4+ T-cell counts of child, father and mother were 458, 203 and 138 cells/ μ l respectively. Ophthalmology evaluation did not reveal retinitis.

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Table 1 Hematological and biochemical investigations in the present case

	02.12.15	05.12.15	07.12.15
Hematological			
Hb (g/dl)	8.1	11.9	9.2
TLC (/mm ³)	10,400	29,700	27,100
DLC % (P/L/M/E)	55/28/14/1	74/16/9/1	76/13/6/1
Platelet (lakh/mm ³)	2.72	-	1.10
Biochemical			
Serum Protein/Albumin	4.8/1.5	5.7/1.8	-
AST	110	224	-
ALT	51	65	-
ALP	134	193	-

ALP Alkaline phosphatase; ALT Alanine aminotransferase; AST Aspartate aminotransferase; DLC Differential leukocyte count; Hb Hemoglobin; P/L/M/E Polymorphonuclear cells/ lymphocytes/monocytes/eosinophils; TLC Total leukocyte count

Imaging Serial chest X-rays revealed bilateral progressive patchy areas of consolidation sparing peripheral one-third of lung parenchyma.

Course and Management This 8-mo-old boy, symptomatic for 3 mo with chronic febrile respiratory illness, presented in respiratory failure. He received intravenous ceftriaxone and cloxacillin but continued to have persistent fever spikes and worsening respiratory distress requiring hiking of oxygen support. His antimicrobials were hiked to vancomycin and imipenem along with intravenous amphotericin-B and cotrimoxazole in appropriate dosages for suspected *pneumocystis jiroveci* pneumonia (PJP). On day 4, he developed shock requiring multiple ionotropes. His hemodynamics worsened with refractory shock, and he continued to remain hypoxemic on 100% FiO₂ requiring intubation and manual ventilation. He expired on day 7 of hospital stay following a cardiac arrest.

Unit's Final Diagnosis Retro exposed infant with pneumonia? Bacterial? PJP.

Cause of Death: Respiratory failure with refractory shock.

Discussion (Clinical Discussant) This 8-mo-old boy was symptomatic since 5 mo of age requiring two hospital admissions. There was history of loss of sibling at 5 mo of age due to pneumonia. Child presented with continuous fever, worsening cough and respiratory distress. Examination revealed failure to thrive, pallor, oral thrush, bilateral coarse crepitations and hepatomegaly. Laboratory evaluation showed anemia, leukocytosis with polymorphic predominance, pre-terminal thrombocytopenia, transaminitis, hypoalbuminemia and elevated C-reactive protein (CRP). His parents tested positive for HIV, he was however negative for the same on serology by three HIV ELISA kits. Thus, patient was a retro exposed infant with predominant pulmonary system involvement. Clinical features which favour this child to be HIV

infected include anti retroviral therapy (ART) naive HIV positive parents, presentation with severe pneumonia, failure to thrive, oral thrush and hepatomegaly. Laboratory investigations suggesting HIV infection include anemia, thrombocytopenia and reversal of albumin globulin ratio. His CD4+ T-cell count was low; which, for his age, refers to severe immunosuppression according to World Health Organization (WHO) immunological classification for HIV infection suggesting this child to be HIV infected.

Our centre being accredited to National AIDS Control Organization (NACO), all tests for diagnosis of HIV infection in this child were done as per guidelines of NACO. For diagnosis of HIV infection in children <18 mo of age, test of choice is HIV-deoxyribonucleic acid polymerase chain reaction (DNA PCR) by dried blood spot (DBS). HIV ELISA is not recommended in infants <6 mo of age due to interference by maternal antibodies acquired through transplacental route. However, NACO recommends HIV ELISA as first test of choice for diagnosis of HIV infection in children 6–18 mo of age. If HIV ELISA is negative in this age group, child may be considered uninfected. A positive ELISA, however, has to be confirmed by HIV-DNA PCR by DBS because the result may be false positive due to presence of maternal antibodies. Also, HIV ELISA for a child with HIV positive parents is reported non-reactive on three different test kits while for other children, it is reported non-reactive on a single test kit. Index child was 8-mo-old with negative HIV ELISA on three different test kits. Thus, he may be considered HIV uninfected according to guidelines of NACO. HIV-DNA PCR by DBS could have been confirmatory but was not available in this case.

Negative HIV ELISA in this child may be explained by seronegative HIV infection which is seen in children with advanced or rapidly progressive disease and is usually associated with high viral load. These children are too immunocompromised to produce HIV specific antibodies. Incidence of seronegative HIV infection varies from 2.5 to 10.6% in various studies. Index child was severely immunocompromised

(CD4+ T-cell count <25%) and thus, in all probability, had seronegative HIV infection.

Pneumonia in index child is likely to be polymicrobial. Bacterial infections, being most common, cannot be excluded solely on the basis of negative blood culture. CMV infection can also cause pneumonia along with transaminitis. Child presented in respiratory failure with chest X-ray showing bilateral infiltrates sparing peripheral one-third of parenchyma. In view of severe immunosuppression, these features can be explained by PJP which occurs almost exclusively in immunocompromised host with highest incidence in first year of life. Interstitial edema due to PJP results in progressive hypoxemia and respiratory failure which happened in index child as well. Thus, *Pneumocystis jiroveci* can best explain the pulmonary symptoms in this child, though polymicrobial infection seems to be the most likely etiology for his clinical course. Extrapulmonary manifestations due to *Pneumocystis jiroveci* are rarely seen in children and were absent in our child also.

Hence, index child is a retro exposed infant, seronegative but likely to be HIV infected, with severe immunosuppression and chronic febrile respiratory illness, who presented with acute exacerbation which can be attributed to polymicrobial infection.

Pediatrician 1 This 8-mo-old boy was definitely HIV infected in view of severe immunosuppression and HIV positive parents. Considering his age and clinical and radiological presentation, *Pneumocystis jiroveci* seems to be most likely organism responsible for pulmonary symptoms. Examination of bronchoalveolar lavage (BAL) fluid is recommended for definite demonstration of *Pneumocystis jiroveci*. However, it could not be performed in index case as the child was severely hypoxemic even on 100% FiO₂.

Pediatrician 2 Community acquired bacterial pneumonia is the most common cause of respiratory tract infection in children with HIV (exposed as well as infected). However, PJP is most common in children less than 5 y of age who are severely immunosuppressed. Our yield of *Pneumocystis jiroveci* from GL has been very low and these children are often treated with cotrimoxazole irrespective of result of BAL/GL examination. So, it will not be surprising if both bacteria and fungi are found in this child.

Pediatrician 3 HIV testing by ELISA has good sensitivity. Negative ELISA in a child with strong clinical suspicion of HIV infection could be explained by advanced immunological failure wherein the baby is not producing antibodies. Early treatment with anti retroviral agents (in first 7 d of life) has also been shown to cause failure of immunological response.

Medicine Specialist 1 Chest X-ray alone may not reliably differentiate between a bacterial and *Pneumocystis*

pneumonia and thus, computed tomography (CT) scan of chest is required. Marked leukocytosis with neutrophilic predominance and elevated CRP favours bacterial infection. It would be interesting to see whether the child has PJP, tuberculosis (TB) or a polymicrobial infection.

Pediatrician 4 It is extremely uncommon to make a diagnosis of PJP during life because PCR is not available and conventional stains have very poor sensitivity. Is GL a well recognized specimen for diagnosis of *Pneumocystis jiroveci* or one is supposed to take respiratory secretions exclusively?

Pediatrician 1 GL has low sensitivity and specificity for *Pneumocystis jiroveci*. It is often performed because most patients are not appropriate candidates for BAL. Recommended specimen is BAL fluid and method of choice is PCR.

Microbiologist 1 Recommended sample for *Pneumocystis jiroveci* is either sputum or BAL fluid. GL for *Pneumocystis jiroveci* derives corollary from GL for TB, but it is not the recommended protocol. PCR for *Pneumocystis jiroveci* is available on request.

Pathology Protocol

A complete autopsy was performed on this patient. On opening, both pleural cavities yielded 50 ml fluid each. Peritoneal cavity contained 500 ml straw colored fluid and pericardial cavity had 30 ml fluid.

The lungs weighed 180 g (normal: 96–108 g) with dull pleura and were subcrepitant to feel. Cut surface showed multiple hemorrhagic areas (Fig. 1a). Microscopically, alveolar spaces were filled with fluffy material containing cup shaped structures with minimal inflammation. Periodic Acid Schiff (PAS), Grocott's stain and immunohistochemistry (IHC) confirmed the morphology of these structures to be *Pneumocystis jiroveci* (Fig. 1b, c). In addition, few pneumocytes had nucleomegaly with smudgy nuclei, few having intra-nuclear inclusions. These cells were positive by IHC for CMV (Fig. 1d). Areas of intra-alveolar hemorrhages were seen along with hyaline membrane formation.

Esophageal mucosa revealed gross ulceration which on microscopic examination confirmed an ulcer with neutrophilic infiltrate, granulation tissue and fibrin. The ulcer bed showed many endothelial cells with cyto- and nucleomegaly with intranuclear inclusions indicating CMV esophagitis and ulceration (Fig. 1e–inset shows CMV inclusion in higher magnification). The stomach was unremarkable.

Thymus showed lymphodepletion and relative accentuation of epithelial components confirmed by IHC for CD20 and CD3. The lymph nodes also revealed lymphodepletion with folliculolysis (Fig. 2a). There were

Fig. 1 **a** Cut surface of lungs showing multiple hemorrhagic infarcts and consolidation; Microphotographs showing **b** Fluffy frothy exudates within alveolar spaces, with mild inflammation, H&Ex20X; **c** *Pneumocystis jiroveci* confirmed on immunohistochemistry, HCx10X;

d CMV inclusions in pneumocytes, H&Ex20X; **e** Microphotograph of esophagus showing ulcerated mucosa with moderate inflammation, H&Ex10X; with CMV inclusion in inset

depleted peyer's patches with small lymphoid aggregates (Fig. 2b) in small intestine. The lymphoid depletion and folliculolysis suggested acquired immunodeficiency related changes. Bone marrow revealed erythroid hyperplasia with adequate representation of granulocytic and megakaryocytic series of cells.

Both kidneys weighed 45 g (normal: 54–60 g) with distinct cortico-medullary junction and medullary congestion. Microscopically, glomerular capillaries showed endothelial cell swelling and sub-endothelial space expansion with fluffy bloodless appearance indicating thrombotic microangiopathy.

The brain weighed 750 g (normal: 770–774 g) with hemorrhages in cerebrum and cerebellum. Microscopically, hemorrhagic areas revealed microthrombi in meningeal and parenchymal blood vessels and the fibrin was highlighted on Martius Scarlet blue stain.

Liver weighed 240 g (normal: 280–290 g) with hemorrhagic areas alternating with grey-white areas giving a nutmeg appearance. Microscopically, centrilobular hemorrhagic necrosis and periportal macrovesicular steatosis was noted. No CMV inclusion or any other organism or granulomas were noted.

Spleen and pancreas were unremarkable and so was the heart.

Final Autopsy Diagnosis

- *Pneumocystis jiroveci* pneumonia, CMV pneumonitis with focal acute respiratory distress syndrome
- CMV esophagitis
- Lymphodepletion in lymph nodes, thymus and small intestines (Peyer's patches)
- Folliculolysis in lymph nodes

Fig. 2 Microphotographs of **a** Lymph node with lymphodepletion and folliculolysis, H&Ex10X; **b** Depleted peyer's patches with remnant lymphoid aggregates in small intestine, H&Ex10X

- Thrombotic microangiopathy in kidneys
- Microthrombi in cerebrum and fresh hemorrhages in cerebellum secondary to disseminated intravascular coagulation (DIC) due to septicemia.
- Macrovesicular steatosis and centrilobular hemorrhagic necrosis in liver

In view of acquired immunodeficiency syndrome (AIDS) defining illnesses, the child appears to have HIV infection with acquired immunodeficiency.

Open Forum

Pediatrician 5 Thrombotic microangiopathy could be due to DIC. Can immunostain for p24 antigen be done in tissues to demonstrate HIV?

Pathologist 1 Whole cortex of brain is necrotic because of extreme hypoxia and global ischemia. There was also some evidence of DIC. p24 antigen is not available.

Pediatrician 2 Could negative HIV ELISA be due to lymphodepletion and folliculolysis that have been demonstrated? Negative HIV ELISA in a symptomatic child is uncommon, though seronegative HIV infection has been described in case reports. This might be our first patient with seronegative HIV infection.

Discussion

Since the epidemic of HIV infection in 1981, it has spread to involve more than 36 million people worldwide in 2015 [1]. More than 2 million people were newly infected with HIV in 2015, of which children less than 15 y constituted 1.5 lakh [1]. Global ART coverage has increased from 3% in 2000 to 46% in 2015 and more than 8 lakh children were receiving ART by the end of 2014 [2]. Despite an increase in ART coverage and increased diagnostic facilities, HIV is still a major cause of morbidity and mortality in children, especially in infants. HIV poses several challenges in infants because clinical manifestations often mimic other viral or bacterial infections resulting in significant delays in diagnoses. HIV infection progresses more rapidly in children as compared to adults. There are two broad categories of children who are diagnosed with HIV infection – asymptomatic children born to HIV positive parents diagnosed on screening, and symptomatic children who manifest clinical signs and symptoms suggesting acute HIV infection.

CD4 + T lymphocytes are the primary targets of HIV. Hence, CD4 + T-cell count is used as a marker of degree of immunosuppression in patients with HIV infection. Absolute

CD4+ T-cell count might be influenced by an increase or decrease in total lymphocyte count. Children usually have relative lymphocytosis and a variable absolute CD4+ T-cell count. Hence, CD4 + T cell percentage is an important parameter for following children up to 5 y of age as it varies less with age [3]. Enumeration of CD4+ T-cell count is also important because development of an opportunistic infection coincides with drop in CD4+ T-cell count, *e.g.*, infection with *Mycobacterium avium* complex, cryptosporidium, CMV and *pneumocystis jiroveci* are usually seen in HIV infected children with CD4+ T-cell count <200cell/μl (severe immunosuppression).

CMV and PJP are important opportunistic infections in children and are considered pediatric AIDS defining illnesses [4]. PJP occurs almost exclusively in immunocompromised hosts. Highest incidence is seen in first year of life with peak at 3–6 mo [4]. Onset is usually acute in infants with fever, cough, dyspnea and tachypnea. *Pneumocystis jiroveci* causes interstitial edema which results in progressive hypoxemia and respiratory failure. Chest X-ray picture may be variable ranging from normal (at onset) to mild infiltrates to classical bilateral diffuse parenchymal infiltrates (ground glass appearance) spreading from perihilar area to apical portions of the lungs. Pneumocystis cannot be cultured and definitive diagnosis relies on demonstration of organism in respiratory secretions [5]. BAL specimen combined with PCR has high sensitivity for diagnosis of PJP in HIV infected children [5, 6].

CMV infection in infants may be acquired through vertical transmission or during postnatal period. Most infants are asymptomatic at birth [4]. Clinical features include microcephaly, intracranial calcifications and chorioretinitis. Children with systemic disease present with fever, hepatosplenomegaly, generalized lymphadenopathy and respiratory symptoms. Diagnosis in infants is difficult because of transplacental passage of maternal antibodies. Definitive diagnosis depends on PCR or viral culture from body fluids and tissues; and demonstration of characteristic ‘owl’s eye’, intranuclear inclusion bodies in biopsy specimens [4]. CMV is an important cause of mortality in immunocompromised infants, especially HIV infected.

Diagnosis of HIV infection depends on demonstration of HIV antibodies through ELISA which has a sensitivity and specificity of more than 99% [7]. Children infected with HIV develop antibodies within weeks of infection. However, not all HIV positive patients develop antibodies despite clinical manifestations suggesting HIV infection and demonstrable p24 antigen positivity [8–10].

False negative HIV ELISA may be seen during window period, due to infection with HIV-2 or non-clade B HIV-1, in patients with immunoglobulin deficiency or in infants due to transient hypogammaglobulinemia [11, 12]. Studies have shown absence of a full antibody response

against HIV-1 in patients where ART is initiated early, possibly due to suppression of the virus by ART [13]. Another important reason for negative ELISA is presence of severe immunosuppression wherein patients are too immunocompromised to produce HIV specific antibodies. Seronegative HIV infection is usually seen in patients with advanced or rapidly progressive disease and is usually associated with high viral load and significant mortality. Whether clinical presentation of these patients with severe disease is a cause or effect of deficient antibody response remains unknown. Exact etiology of seronegative HIV infection is unclear, though, post-mortem histopathologic findings in lymph nodes and thymus of HIV infected pediatric patients have shown extensive lymphoid depletion [14]. However, return of HIV-1 specific antibodies after initiation of ART in some patients [15] signifies that seronegative status could be due to ineffective cellular immunity which causes uncontrolled viral replication resulting in rapid depletion of CD4+ T-cells which are required by B-cells for activation and antibody production during acute infection. These patients represent an important subset of HIV infected children because they are prone to rapid disease progression and thus have important therapeutic implications.

Contributions UNS prepared the pathology protocol for the manuscript and provided histopathology images; AG reviewed the literature on the subject, prepared the manuscript and was involved in patient care; AK prepared the pathology protocol for the manuscript; SG reviewed the literature on the subject. Dr. Surjit Singh, Prof and Head, Advanced Pediatrics Centre, PGIMER, Chandigarh, will act as guarantor for this paper.

Compliance with Ethical Standards

Conflict of Interest None.

Source of Funding None.

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