An Infant with Respiratory Distress and Loose Stools

POOJITHA REDDY[1], DEVIKA LAISHRAM[2], ANKUR KUMAR JINDAL[2], KIRTI GUPTA[3] AND AMIT RAWAT[2]

From [1]Department of Pathology, [2]Allergy Immunology Unit, Department of Pediatrics, and [3]Department of Histopathology, Post Graduate Institute of Medical Education and Research, Chandigarh, India.

Correspondence to: Dr Kirti Gupta, Professor, Department of Histopathology, Post Graduate Institute of Medical Education and Research (PGIMER), Chandigarh 160 012, India. kirtigupta10@yahoo.co.in

We present the case of a 3-month-old girl who was admitted with complaints of loose stools and respiratory distress. She also had a history of rash and alopecia. Laboratory investigations revealed lymphopenia with reduced immunoglobulin G and immunoglobulin A. Lymphocyte subset analysis by flow cytometry revealed T-B+NK+ severe combined immunodeficiency (SCID). She died due to severe pneumonia, shock and pulmonary hemorrhage. Autopsy findings revealed disseminated cytomegalovirus infection in the lung, liver, adrenals and heart. Thymus was found to be dysplastic and showed characteristic histopathologic features of SCID.

Keywords: *Alopecia, Cytomegalovirus, Diarrhea, Lymphopenia, Pneumonia, Severe combined immunodeficiency*.

CLINICAL PROTOCOL

History: A 3-month-old girl, product of 3rd degree consanguineous marriage presented with history of generalized, erythematous, maculopapular rash all over body that developed at one month of age. Rash was associated with skin peeling and it resolved spontaneously in one month. She subsequently had progressive loss of scalp hair and eyebrows. She also had bilateral purulent ear discharge since the age of 2 months associated with cough, respiratory distress and loose stools. Child was admitted twice for these complaints in a nearby hospital and was managed with IV antimicrobials. She had received BCG vaccination at 1 month of age, however, she developed no reaction at the BCG site.

Clinical examination: She had tachycardia (heart rate 150/minute), tachypnea (respiratory rate-62/minute), pallor, alopecia with loss of eyebrows (***Fig.* 1**), skin peeling in the left axilla, no BCG scar, coarse crepitation in bilateral chest and hepato-splenomegaly. Initial clinical possibilities were Human Immunodeficiency Virus (HIV) infection, Severe Combined Immunodeficiency (SCID) with maternal engraftment, congenital Cytomegalovirus (CMV) infection and Langerhans cell histiocytosis.

Laboratory investigations: She had anemia (hemoglobin-83 gm/L), lymphopenia (white cell count 16.3×10^9/L, differential counts $P_{79}L_{\mathbf{16}}M_5$, absolute lymphocyte counts 2.6×10^9/L), transaminitis (alanine aminotransferase 94 IU/L and aspartate aminotransferase 303 IU/L), and a high C-reactive protein (39.6 mg/L). Serial arterial blood gas analysis revealed hypoxemia and hypercarbia. Chest *X*-ray revealed bilateral non-homogeneous opacities predominantly distributed in the peri-hilar region. (***Fig.* 1b**). Ultrasound abdomen showed ileo-ileal intussusception. Contrast enhanced Computed Tomography (CECT) chest revealed multiple bilateral pulmonary nodules with consolidations. Human immunodeficiency virus (HIV) serology was non-reactive, Fine needle aspiration cytology from axillary lymph node showed reactive lymphoid hyperplasia, gastric lavage for acid fast bacilli and *pneumocystis jiroveci* stain was negative, serum galactomannan index was elevated (5.8, normal <0.5), blood culture was sterile, IgM Cytomegalovirus (CMV) serology and CMV DNA PCR was positive.

Immunological investigations (***Table* I**): She had undetectable IgG and IgA and normal IgM; nitroblue tetrazolium (NBT) dye reduction test and

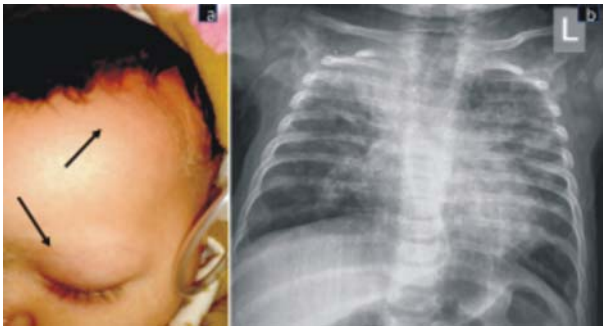


FIG. 1 *Alopecia over left fronto-parietal region and loss of eyebrows (arrows) (a); chest X-ray (anteroposterior view) showing interstitial pattern of infiltrates in both lungs, predominantly perihilar. Homogenous opacity in right upper lung fields and absent thymic shadow (b).*

TABLE I LABORATORY INVESTIGATIONS OF INDEX CASE

Investigation	*Results*	*Normal reference range*
IgG (mg/dL)	<201	240-880
IgA (mg/dL)	<17	10-50
IgM (mg/dL)	90	20-100
CD3+ T cells	32.68%	51-77.6%
CD19+ B cells	29.79%	11-41%
CD16/56+ NK cells	33.41%	3-14%
CD45RA expression on CD3+T cells	2.23%	67.83%
CD45RO expression on CD3+T cells	94.7%	31.18%
CD45RA expression on CD8+T cells	5.18%	74.54%
CD45RO expression on CD8+T cells	93.6%	22.35%
CD-127 (IL-7Rα) expression on CD3+ T lymphocytes	16.5%	53.95% (normal control)

dihydrorhodamine 123 (DHR) assay were normal; lymphocytes subsets showed reduced CD3+ T cells (32% of all lymphocytes), normal CD19+ B cells (29% of all lymphocytes) and normal CD56+ NK cells (39% of all lymphocytes); CD 127 (IL-7Rá) expression was reduced (***Web Fig.*1*a*** and **1*b***); CD45RA expression on CD3+T cells and CD8+T cell was reduced suggestive of low naïve T cells; CD45RO expression on CD3+T cells and CD8+T cells was elevated suggestive of increased memory T cells (likely to be of maternal origin).

Course and management: Child was administered intravenous ceftriaxone and cloxacillin, however, antimicrobials were upgraded to vancomycin, imipenem, cotrimoxazole, amphotericin B and anti-tubercular therapy (ATT). ATT was initiated empirically as the child had received BCG vaccination at birth and pneumonia could be a manifestation of disseminated BCG disease [1]. Oxygen therapy was administered through nasal prong oxygen initially but on 6th day of hospital stay, child was intubated and kept on manual intermittent positive pressure respiration. She was also given 5 grams of intravenous immunoglobulin (IVIG); fluid bolus and inotropes (dopamine and dobutamine) for shock and irradiated packed cell transfusion. She also developed pneumothorax requiring intercostal drainage tube and massive pulmonary hemorrhage leading to cardiac arrest and death.

Unit's final diagnosis: Severe combined immunodeficiency (T-B+NK+) with IL-7R alpha deficiency with maternal engraftment, severe pneumonia, sepsis, septic shock and pulmonary hemorrhage.

Discussion

Clinical discussant: We have a 3-month-old girl with history of consanguinity presenting with rash, alopecia, skin peeling, otitis media, pneumonia, loose stools and intussusception. She had anemia, lymphopenia, transaminitis, elevated galactomannan index, positive CMV DNA PCR, multiple pulmonary nodules on CT chest, low immunoglobulins, T-B+NK+ phenotype on lymphocyte subsets, low naïve T cells and reduced IL-7Rα expression on T cells.

Case analysis can be discussed with respect to the underlying disease; the etiology for pneumonia; the cause for Intussusception; and the terminal events.

The clinical presentation is suggestive of an underlying immune defect. After ruling out HIV infection, the most likely underlying diagnosis is SCID, as she was born to a consanguineously married couple and presented within the first 2 months of age with severe pneumonia, otitis media, alopecia (likely due to maternal engraftment), and absence of BCG scar. Laboratory investigations revealed lymphopenia, hypogammaglobulinemia, low CD3+ T cells, naïve T cells and absence of thymic shadow on chest *X*-ray. She fulfills the European society of immunodeficiency (ESID) registry working definition for clinical diagnosis of SCID.

SCID has four phenotypes based on the presence or absence of B and NK cells (T-B-NK+; T-B-NK-; T-B+NK-; T-B+NK+). Our child had T-B+NK+ phenotype and she also had reduced expression of IL-7Rα.

She had features of maternal engraftment (i.e. presence of maternal T lymphocytes in the circulation leading to manifestations that are like graft versus host disease). Most common manifestation of maternal engraftment is skin involvement with eczema, erythema, alopecia and skin peeling; followed by liver involvement (with mild to moderate elevation of liver enzymes) and gastrointestinal tract involvement (diarrhea). Index child has history of generalized erythema and peeling of skin and alopecia. She also had elevated liver enzymes.

The etiology for pneumonia is likely to be polymicrobial. Invasive fungal infection can be considered in view of positive serum galactomannan index, CMV can be considered in view of positive CMV DNA PCR in the blood and suggestive radiological findings. *Pneumocystis jiroveci* pneumonia can also be considered in view of radiological findings. As she received BCG vaccination, so a possibility of disseminated BCG infection with pneumonia can also be considered.

She also had intussusception as evident on ultrasound of the abdomen. The intussusception in this age group would most likely be related to infections which could be a viral or bacterial infection. In the index child, *Mycobacterium bovis* and CMV can be considered. BCG infection leading to recurrent intussusception has previously been reported in SCID [2].

The cascade of terminal event can be summarized as SCID leading to severe pneumonia and polymicrobial sepsis that has led to multiorgan dysfunction and pulmonary hemorrhage finally causing shock and death.

Therefore, the final diagnosis is severe combined immunodeficiency (T-B+NK+ phenotype with IL-7Rα deficiency) with pneumonia (CMV, Aspergillus, *Pneumocystis jiroveci*), pulmonary hemorrhage and intussusception (*Mycobacterium bovis* or CMV)

Senior resident of the treating unit: The diagnosis of SCID is not in doubt here and other differentials diagnosis that were considered can easily be excluded based on the immunological investigations. I expect severe depletion of T cells in the thymus; changes of GVHD in skin and gut and CMV or *Mycobacterium bovis* in lungs and gastrointestinal tract.

Physician 1: Was fundus checked in the index case to look for evidence of CMV retinitis?

Senior resident of the treating unit: The fundus evaluation was done and there was no evidence of CMV retinitis.

Pediatrician 1: *Mycobacterium bovis* infection secondary to BCG vaccination often produces skin lesions and fine needle aspiration from these skin lesions can yield acid fast bacilli (AFB). However, there were no skin lesion in the index child. Gastric lavage for AFB was negative. However, it is not always necessary to find AFB on gastric lavage. Index child had alopecia and loss of eyebrows that was suggestive of maternal engraftment or Omenn syndrome.

Pediatrician 2: The syndrome of SCID should be suspected if the child presents within the first few months of life with rash and polymicrobial sepsis which is not responding to antimicrobials. If hemogram shows lymphopenia as in the index child, SCID should be considered. The phenotype of SCID can be evaluated with the help of flow cytometry. Index child had an unusual phenotype i.e. T-B+NK+.

PATHOLOGY PROTOCOL

A complete autopsy was performed on this 3-month-old girl. The peritoneal cavity revealed ascites with 270 mL of straw-colored fluid. The other serous cavities were within normal limits.

Thymus weighed 2 g (normal weight for this age is 3 g) and revealed maintained lobular architecture on gross examination. However, on microscopy cortico-medullary distinction was lost and there was absence of Hassall's corpuscle. These features are consistent with thymic dysplasia. CD3 immunostain revealed reduction in T-lymphocytes, however, the thymocytes were preserved as highlighted by pan-cytokeratin (***Web Fig.* 2*a-d***).

All sampled lymph nodes showed depletion of lymphoid population. T cell zones i.e. paracortical and interfollicular regions showed reduction in T lymphocytes as highlighted by CD3. CD20 performed revealed normal population of B lymphocytes (***Web Fig.* 2*e-g***). Peyer's patches were hypertrophic with relative preservation of B cell zone (***Web Fig.* 2*h***). However, there was marked reduction of T cell zone. Spleen weighed 20 g (normal for this age is 25 g). On histology, there was mild congestion of the red pulp with preservation of B cell zone and relative reduction of periarteriolar T cell zone.

Both the lungs were heavy (weight 250 g) with dull pleural surface. Many subpleural nodules were identified. The cut surface showed diffuse areas of hemorrhagic consolidation in all the lobes, bilaterally. Tracheo-bronchial tree was unremarkable. Microscopically, there was interstitial widening with mild lymphomononuclear infiltrate along with many large cells protruding into the alveolar lumen. Many cytomegalovirus inclusions (CMV) were noted within alveolar pneumocytes and endothelial cells. These cells demonstrated nucleomegaly along with presence of basophilic to amphophilic inclusions in the nucleus and a perinuclear halo (***Web Fig.* 2*i***). Immunostaining for CMV revealed the burden of infection. Similar inclusions were also noted in the bronchial epithelial cells and endothelial cells of blood vessels. Adjacent areas of lung parenchyma showed diffuse alveolar hemorrhages and focal hyaline membrane formation. No granulomas were identified and Ziehl-Neelsen stain for acid fast bacilli (AFB) was negative. PAS stain performed did not reveal any fungal hyphae and gram's stain performed did not reveal any micro-organisms.

Adrenals examined showed foci of necrosis with the presence of CMV inclusions within the necrotic foci. Heart (weight 30 g) was unremarkable grossly. However, few endothelial cells with the interstitial vessels in the myocardium showed presence of CMV inclusion which was confirmed on immunohistochemistry. Liver (weight 450 g) was unremarkable on gross examination. Cut

surface was pale yellow to greasy in appearance. On microscopy, diffuse macrovesicular steatosis predominantly involving the zone 1 and zone 2 areas with relative preservation of zone 3 hepatocytes was noted. Few scattered CMV inclusions were also noted in the Kupffer cells of the sinusoids which was confirmed on immunohistochemistry. Rest of the organs including brain, kidneys, bone marrow, stomach, esophagus, skin, thyroid, skeletal muscle were unremarkable grossly and microscopically.

Final autopsy diagnosis:

- Thymic dysplasia
- Disseminated CMV infection – Lung (predominant), Adrenal, Liver, Heart
- Macrovesicular steatosis
- Intussusception of small intestine

Senior resident of the treating unit: There is perfect correlation between flow cytometry reports and pathology findings in lymph nodes. B cells were present in the flow cytometry report and were also found in the lymph nodes. In many types of SCID, the B cells are absent in blood and there we see depletion of B cell zone in the lymph nodes. Was any organism isolated at the site of intussusception?

Pathologist 1: No organism was seen at the site of intussusception. The intussusception was likely due to hyperplasia of Peyer's patches.

Physician 1: Was flow cytometry analysis done for the parent's sample and were parents counselled about future pregnancies.

Senior resident of the treating unit: Flow cytometry analysis of parent's sample is not usually done in cases of SCID. Molecular analysis will be done for both child and parents. Parents have been advised to avoid further pregnancy till the genetic confirmation, following which a prenatal diagnosis will be offered.

Pediatrician 3: This is an autosomal recessive SCID and parents are likely to be the carriers. So, flow cytometry is not useful in diagnosing carrier status which can only be diagnosed with molecular analysis.

Pathologist 2: In the year 1980s, these types of cases did not fit into any clinical diagnosis, these infants will have infections like CMV, PCP with hypogammaglobulinemia and lymphopenia. However, the level of investigations was not as extensive as they are now. One thing that was pursued extensively at that time was the possibility of AIDS. Extensive investigations were done on thymus and lymphoid system. Serial sections of thymus were done which demonstrated isolated Hassall's corpuscles and normal blood vessels. In thymic dysplasia thymus are abnormally small with small blood vessels, lobules are shrunken and no lymphoid tissue in cortex and medulla which are classical of SCID.

Pathologist 1: Lymphoid cells in the thymus were due to maternal engraftment and it is difficult to differentiate lymphoid cells from maternal origin or from the child. CMV infection is most likely acquired as the major bulk of CMV disease was in lung and not in liver.

Discussion

SCID is the most severe form of combined immunodeficiency with an estimated prevalence of 1 in 58,000 live births [3]. It is characterized by a block in T lymphocyte differentiation and variable depletion of B and NK cells depending on the subtype of SCID [4,5]. There are four major subtypes of SCID based on presence or absence of B or NK cells. The index patient had T-B+NK+ SCID. This can be caused by mutation in *IL-7Rα* or CD45 gene. Index patient had reduced expression of *IL-7Rα.* SCID due to mutation in *IL-7Rα* gene accounts for approximately 10% of all cases [6].

Children with SCID often present with recurrent infections with an onset before the age of 6 months. Oral candidiasis, persistent diarrhea, pneumonia and failure to thrive are the most common presenting manifestations. There are no major differences between various subtypes of SCIDs in their initial presentation; however, ADA deficiency SCID tends to have severe lymphopenia ($<0.5\times10^9$/L) and hence very early and severe presentation. Common opportunistic organisms seen in these patients are *Pneumocystis jiroveci*, *Aspergillus* sp. and Cytomegalovirus. Majority of children die within the first 2 years of life if not treated adequately. Respiratory tract infections are common in patients with SCID and CMV pneumonia may be the first presenting manifestation [7]. A persistent CMV respiratory infection may be clinically or radiologically indistinguishable from other respiratory viral infections. Index patient had interstitial infiltrates on chest radiograph and positive CMV DNA PCR in blood and autopsy revealed CMV infection in lung as well as in liver, heart and adrenal glands.

In almost all subtypes of SCID, thymus is usually atrophic [8] and poorly visualized on a chest radiograph [9] as was seen in the index patient also. The histopathology of thymus in SCID demonstrates depletion of lymphocytes and lack of differentiation of thymic epithelium. Poor differentiation of thymic epithelium is responsible for the absence of Hassall's

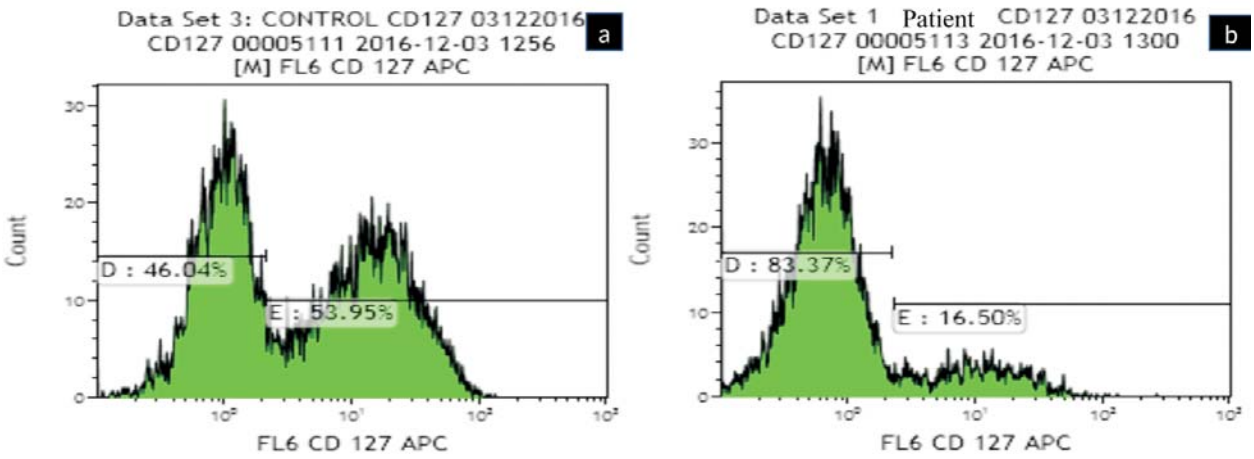
corpuscles and is on one of the most important histopathological feature of SCID [10]. Thymic epithelium has very important role in the normal differentiation of T-cell population and due to thymic dysplasia in SCID, T-cell differentiation is impaired [11].

Intussusception can have variable etiology (*i.e.* viral, bacterial or protozoal infections and benign or malignant tumors). As compared to adults in whom a definite lead point is often found, it is rather uncommon to find a cause in children. Hyperplasia of Peyer's patches may be seen in upto a third cases of idiopathic intussusception [12]. Index patient also had similar pathology at the site of intussusception.

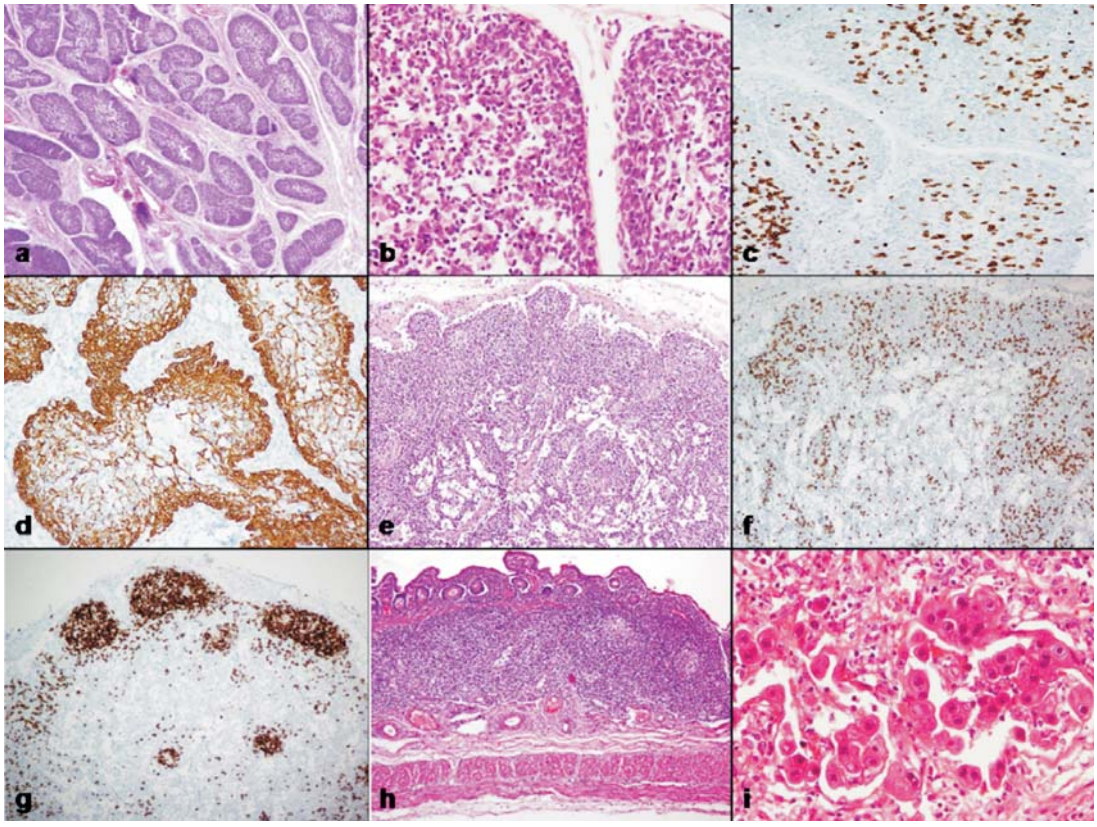
Contributors: PR: pathology protocol presentation, writing of manuscript, review of literature; DL: clinical protocol presentation, writing of manuscript, review of literature; AKJ: clinical protocol presentation, writing of manuscript, editing of manuscript, review of literature, patient management; KG: pathology protocol presentation, review of literature, editing and final approval of manuscript; AR: laboratory investigations, review of literature, editing and final approval of manuscript.
Discussants: Clinical discussant and Pediatrician 1: Devika Laishram; Senior resident of treating unit: Ankur Kumar Jindal; Pathologist 1: Poojitha Reddy; Pathologist 2: Kirti Gupta; Pediatrician 2: Amit Rawat; Pediatrician 3: Surjit Singh.
Funding: None; *Competing interest*: None stated.

REFERENCES

1. Marciano BE, Huang C-Y, Joshi G, Rezaei N, Carvalho BC, Allwood Z, *et al*. BCG vaccination in patients with severe combined immunodeficiency: Complications, risks, and vaccination policies. J Allergy Clin Immunol. 2014;133:1134-41.
2. Venaille A, Viola S, Peycelon M, Grand d'Esnon A, Fasola S, Audry G, *et al*. [Recurrent small-bowel intussusceptions revealing a disseminated BCG infection related to severe combined immunodeficiency]. Arch Pediatr. 2014;21: 879-81.
3. Kwan A, Abraham RS, Currier R, Brower A, Andruszewski K, Abbott JK, *et al*. Newborn screening for severe combined immunodeficiency in 11 screening programs in the United States. JAMA. 2014;312:729-38.
4. Rivers L, Gaspar HB. Severe combined immunodeficiency: recent developments and guidance on clinical management. Arch Dis Child. 2015;100:667-72.
5. Chinn IK, Shearer WT. Severe combined immunodeficiency Disorders. Immunol Allergy Clin North Am. 2015;35:671-94.
6. Cirillo E, Giardino G, Gallo V, D'Assante R, Grasso F, Romano R, *et al*. Severe combined immunodeficiency—an update. Ann N Y Acad Sci. 2015;1356:90-106.
7. Szczawiñska-Pop³onyk A, Joñczyk-Potoczna K, Ossowska L, Brêborowicz A, Bartkowska-Œniatkowska A, Wachowiak J. Cytomegalovirus pneumonia as the first manifestation of severe combined immunodeficiency. Cent-Eur J Immunol. 2014;39:392-5.
8. Notarangelo L. Primary immunodeficiencies. *In*: Male D, Brostoff J, Roth DB, Roitt IM, editors. Immunology. 8th *ed*. Amsterdam: Elsevier; 2013. p. 263-4.
9. Nickels AS, Boyce T, Joshi A, Hagan J. Absence of the thymic shadow in a neonate suspected of primary immunodeficiency: Not a straightforward clinical sign of immunodeficiency. J Pediatr. 2015;166:203.
10. Gupta K, Rawat A, Agrawal P, Jindal A, Nada R, Saikia B, *et al*. Infectious and non-infectious complications in primary immunodeficiency disorders: An autopsy study from North India. J Clin Pathol. 2018;71:425-35.
11. Nezelof C. Thymic pathology in primary and secondary immunodeficiencies. Histopathology. 1992;21:499-511.
12. Pang LC. Intussusception revisited: Clinicopathologic analysis of 261 cases, with emphasis on pathogenesis. South Med J. 1989;82:215-28.



WEB FIG. 1 *Flow cytometry for study of expression of CD127 (IL7R) on gated CD3+ T lymphocytes reveals 53.95% cells showing expression of CD127 in control (a) and only 16.50 % cells showing expression in patient (b) thus suggestive of reduced CD127 expression in patient as compared to control.*



WEB FIG. 2 *(a): Low power magnification of thymus depicting loss of corticomedullary distinction (H&E × 100); (b): Epithelial cells of thymus present in diffuse sheets with absent Hassall's corpuscles (H&E × 400); (c): CD3 immunostain highlights depleted T cell population (immunoperoxidase × 400); (d): Pan-cytokeratin stain highlights preserved thymocytes (immunoperoxidase × 400); (e-f): Microscopic picture of lymph node showing well maintained B cell zone (H&E × 100); (f): CD3 immunostain depicting reduction in interfollicular and paracortical T-cells (immunoperoxidase × 400); (g): CD20 immunostain depicting preserved B cell (immunoperoxidase × 400); (h): Microscopic picture of small intestine showing hyperplasia of Peyer's patches (H&E × 200); (i): Numerous CMV inclusions lining the alveolar epithelial cells (H&E × 400).*