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REVIEW ARTICLE

Guidelines for Screening, Early Diagnosis and Management of Severe Combined Immunodeficiency (SCID) in India

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Abstract Severe combined immunodeficiency (SCID) is one of the most severe and fatal forms of inherited primary immunodeficiency. Early diagnosis of SCID improves the outcome of life before and after hematopoietic stem cell transplant (HSCT). SCID fulfills the internationally-established criteria for a condition to be screened for at birth. T cell receptor excision circle (TREC) assay is commonly used in western countries as part of newborn blood spot screening (NBS) program as the assay has high sensitivity and specificity to identify SCID infants, allowing early intervention and curative bone marrow (BM) transplantation. In India, the blood spot based screening programs are yet to mature into a full-fledged national program. Moreover, TREC assay, a PCR based test, is not widely available and may cost USD 5-7 per test; thus limiting its applicability for screening newborns in Indian scenario. Most of the SCID patients have lymphopenia at birth and routine evaluation for absolute lymphocyte count (ALC) on cord blood samples can help in pre-symptomatic detection and early intervention for neonates with SCID. Although ALC count lacks the sensitivity and specificity of TREC assay; its lower cost and widespread availability makes it an attractive option for identifying newborns with lymphopenia during the post-partum hospital stay. BCG vaccine and other live attenuated vaccines (*e.g.*, oral polio vaccine) should be withheld in lymphopenic infants until SCID is excluded by clinical and/or immunological work-up. A diagnosis of SCID warrants immediate care to prevent and treat infections and wherever feasible, early stem cell transplantation for disease free survival.

Keywords Newborn screening · SCID · TREC assay · CBC

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Introduction

Primary immunodeficiency disorders (PIDs) are a heterogeneous group of inborn errors of immune system. Till date more than 200 distinct PIDs have been described [1]. Majority of these are single gene defects. The estimated combined incidence of these disorders is as high as 1 in 1200 [2]. The common manifestations of PIDs include recurrent, life threatening infections and/or susceptibility to autoimmunity, inflammation and malignancy. Clinical severity varies from mild to life threatening disease depending on the underlying immune defect. PIDs are classified according to underlying immune defect into 9 major categories, as presented in Table 1 [3].

Early diagnosis of PIDs is important to prevent the significant morbidity and mortality associated with these diseases. Treatment consists of antimicrobial therapy to treat and prevent the infections, immunoglobulin supplements for antibody deficiencies and hematopoietic stem cells transplantation (HSCT) for severe forms of PIDs.

Table 1 Classification of primary immunodeficiencies (PIDs)

a) Immunodeficiencies affecting cellular and humoral immunity
b) Combined immunodeficiencies with associated or syndromic features
c) Predominantly antibody deficiencies
d) Diseases of immune dysregulation
e) Congenital defects of phagocyte number, function, or both
f) Defects in Intrinsic and Innate Immunity
g) Autoinflammatory disorders
h) Complement deficiencies

Severe combined immunodeficiency (SCID) is an inherited severe immunodeficiency of T-cell and B-cell functions that occurs in approximately 1: 50,000 live births [4]. Among the affected patients, a male predominance is noted with less than one-third of patients having a positive family history. SCID is not clinically apparent at birth, and the infectious complications which bring them to medical care may not initially be distinguishable from routine childhood infections, thus diagnosis may be significantly delayed. These infants usually die during the first year of life due to complications from a series of infections. Also, these babies are potentially at high-risk of developing Vaccine Associated Paralytic Poliomyelitis (VAPP) and BCGosis due to routine administration of live vaccines like OPV and BCG given at birth in India. The best outcome for SCID is achieved if hematopoietic stem cell transplantation is performed in the first months of life, ideally before clinical presentation with infections and failure to thrive [4]. Screening for SCID at birth would not only prevent children from dying before HSCT can be attempted but also would increase the success of HSCT. Hence, detection of SCID through newborn screening is the ideal approach.

As most infants with SCID have significant lymphopenia at birth, absolute lymphocyte count (ALC) is a promising candidate as a screening test for this syndrome [5]. However, as this test cannot be performed on dried blood spots it did not find favor in countries where newborn blood spot screening (NBS) is widely used for screening for other newborn disorders. The T-cell receptor excision circle (TREC) assay, which is a measure of thymic output of T cells, gives a highly sensitive and specific assessment of T cell function. This assay, which can be performed along with other NBS tests, has transformed the early diagnosis of SCID. It is being implemented in several states across the United States [6] Europe [7] and other countries [8].

PIDs in India

The true incidence of PIDs in India cannot be known until there is newborn or population screening for these defects. In a study from United States, it is estimated to be 1 in 2000 for children, 1 in 1200 for all persons, and 1 in 600 households [2]. The incidence of PIDs in India is likely to be similar to other parts of the world. However, we expect that the incidence of individual PIDs may be different due to high degree of consanguineous marriages [9]. It is estimated that the number of PID patients in India is at least one million. However, there is very little data available from India largely due to lack of awareness about these disorders and lack of diagnostic facilities [9–15].

However with the efforts of Federation of Primary Immunodeficiency Disorders (FPID), USA and Indian Society for Primary Immunodeficiency Diseases (ISPID), the scenario is gradually changing. The two 'Center of Excellence' for PIDs have been approved by Indian Council of Medical Research (ICMR) at Post Graduate Institute of Medical Education and Research (PGIMER), Chandigarh and National Institute of Immunohematology (NIIH), Mumbai. As the diagnostic facilities and the awareness about PIDs are improving, this is the right time for developing Indian guidelines for newborn screening, early diagnosis and management of these disorders.

Rationale of Screening for SCID

Barrier to Early Diagnosis without Screening

SCID and related conditions are rare. Children appear normal at birth. Gene defect and environmental exposure are required for overt disease and hence presentation is variable. Infections are common in all infants and not just those with SCID. Hence significant crucial time is wasted before one suspects SCID, resulting in significant mortality and morbidity. More than 70 % cases are sporadic without family history. Moreover, family history can be nonspecific and missed.

Importance of Early Identification of SCID

SCID is a pediatric emergency. Establishing diagnosis enables institution of immediate lifesaving treatment. It also avoids administration of live vaccines to the patients which can be harmful to the child. The outcome of HSCT, if done within first few months of life before the child gets any severe infection, is much better compared to the patients who are diagnosed late based on symptoms. It also provides families with genetic diagnosis and advice on reproductive risks. Further, it helps to understand the true incidence and spectrum of SCID and also educates providers and public about SCID.

Thus, SCID being a clinically severe disease; diagnosis not being apparent at birth; availabilty of definitive therapy and significant improvement of quality of life with early intervention makes it an ideal candidate for newborn screening programme.

Methods for Screening

TREC Assay and its Utility as a Screening Test for SCID

SCID is a molecularly heterogeneous condition with a variety of genetic defects leading to the same clinical phenotype. However, majority of these defects are associated with an abnormality of T cell development in the thymus [20]. All the T cells after generation in the bone marrow undergo a process of receptor gene splicing and rearrangement in the thymus. During this process of recombination, the T cell antigen receptor (TCR) genes, which are composed of large numbers of alternate segments, called variable (V), diversity (D) and joining (J) regions, are randomly cut and rejoined to generate a unique rearrangement in each cell. The excised DNA fragments that are not incorporated into the mature TCR locus are joined at their ends to form a great variety of circular DNA by-products, called T cell receptor excision circles, or TRECs [21, 22]. These DNA circles are stable but do not replicate during cell division. They get diluted with T cell division. Since TRECs are expressed only in naïve T cells that have recently left thymus, it provides a surrogate marker for the T cell development in the thymus and is useful as a screening test for SCID [21, 22]. TREC copy number in the peripheral blood varies significantly, depending on the age of the individual. Normal newborns have about 1 TREC per 10 T cells, reflecting high numbers of naïve T cells that have not yet proliferated extensively; whereas older children and adults have about 1 per 100 and 1 per 1000 T cells, respectively, reflecting peripheral expansion [20]. Infants with SCID have very low or undetectable TRECs [21]. Also, the presence of maternal T cells does not influence the TREC count of the infant as maternal cells have few TRECs.

The absolute quantification of TREC by quantitative real-time PCR is performed by generating a standard curve from serial dilutions of TREC signal joint constructs cloned in a bacterial plasmid [23] and extrapolating the TREC value in the dried blood spot (DBS) specimen from the standard curve.

Conditions Identified by TREC Screening Assay [21]

1. Typical SCID - These forms of SCID are characterized by <300 autologous T cells/μl of blood and <10 % of normal proliferation to mitogens [*e.g.*, phytohemagglutinin (PHA)].
2. Leaky SCID or Omenn syndrome, results from mutations in typical SCID genes that do not completely abolish gene function. These forms of SCID are characterized by 300 to 1500 autologous T cells/μl. Patients with Omenn syndrome may have normal or elevated CD3 T cell counts but they have restricted TCR diversity (oligoclonality) of T cell.
3. Variant SCID with persistently low T-cells but no defect in a known SCID gene.
4. Conditions with primary T cell lymphopenia (CD3 T cells ≤ 1500 cells/uL):

    - Complete DiGeorge syndrome or partial DiGeorge syndrome with low T-cells
    - CHARGE syndrome
    - Jacobsen syndrome
    - Trisomy 21
    - RAC2 dominant interfering mutation
    - DOCK8 deficient hyper-IgE syndrome
    - Cartilage hair hypoplasia

5. Secondary T cell lymphopenia: This is diagnosed in a subset of infants with recognized congenital conditions, such as intestinal lymphangiectasia, hydrops, gastroschisis, a congenital heart defect, chylothorax, or neonatal leukemia. It can also occur due to prenatal administration of glucocorticoids or inflammatory conditions (*e.g.*, sepsis).
6. Premature infants presenting with T cell lymphopenia, T cells ≤1500 cells/ul. However, this resolves with age.

T Cell Defects that cannot be Identified/Detected by the TREC Screening Assay

Diseases in which T-cells develop in the thymus to the point of production of the TRECs but have impaired functions cannot be picked up by the TREC assay. This includes newborns with Zap70 deficiency [24], major histocompatibility complex (MHC) class II deficiency [25], CD40 ligand deficiency, NF-kappa-b essential modulator (NEMO) deficiency and patients with a late-onset of adenosine deaminase (ADA) deficiency.

Interpreting TREC Results

Low or undetectable TREC levels indicate T cell lymphopenia. However, as this assay is a PCR based technique, low number may also arise due to artifacts such as inadequate sample, poor elution of DNA from the DBS or due to presence of PCR inhibitors such as heparin. In order to avoid false

positive results, amplification of a reference gene is carried out. The general strategies employed are [20]:

1. Singleplex TREC assay: This involves parallel amplification of a reference gene and TRECs in samples where the initial TREC qPCR reaction gives result below the predetermined TREC cut off. If the reference gene is not detected, the test is termed inconclusive. If detected, the replicate TREC values are used as the final TREC values.
2. Multiplex Real Time qPCR: This involves the simultaneous amplification of TREC and reference gene. Samples with the abnormal multiplex result are retested using the same multiplex assay. If the reference gene is not detected, the test is termed inconclusive. If detected, the replicate TREC values are used as the final TREC values.

Follow-up Diagnostic Algorithm of Abnormal TREC Results

All the abnormal screening results must be followed up and evaluated further with complete blood count and lymphocyte subset analysis by flow cytometry for enumeration of T, B and NK cells on whole blood sample. Flow chart for screening of SCID by TREC assay is depicted in Fig. 1.

The minimum recommended evaluation includes:

1. Enumeration of absolute counts of lymphocytes: T (including total CD3, CD4 and CD8 T cells), B cells (CD19), and NK cells (CD56/16).
2. Evaluation of memory and naïve cells using CD45RA and CD45RO markers. Additional markers may include CD62L (L-selectin), CD31, CD27 and CCR7 for more specific characterization of naïve T cells.

The specimen of whole blood sample should be fresh and processed as per the guidelines of the immunology laboratory. An individual with expertise in the interpretation of flow cytometry results in children and newborns should evaluate the results and compare them with age related normal references. If flow cytometry evaluation confirms the presence of lymphopenia, the identified newborn should be immediately referred to the specialized center with necessary expertise in the diagnosis and management of PIDs.

Clinical evaluation using checklist (Table 2) must be done and along with initiation treatment/interventions must be

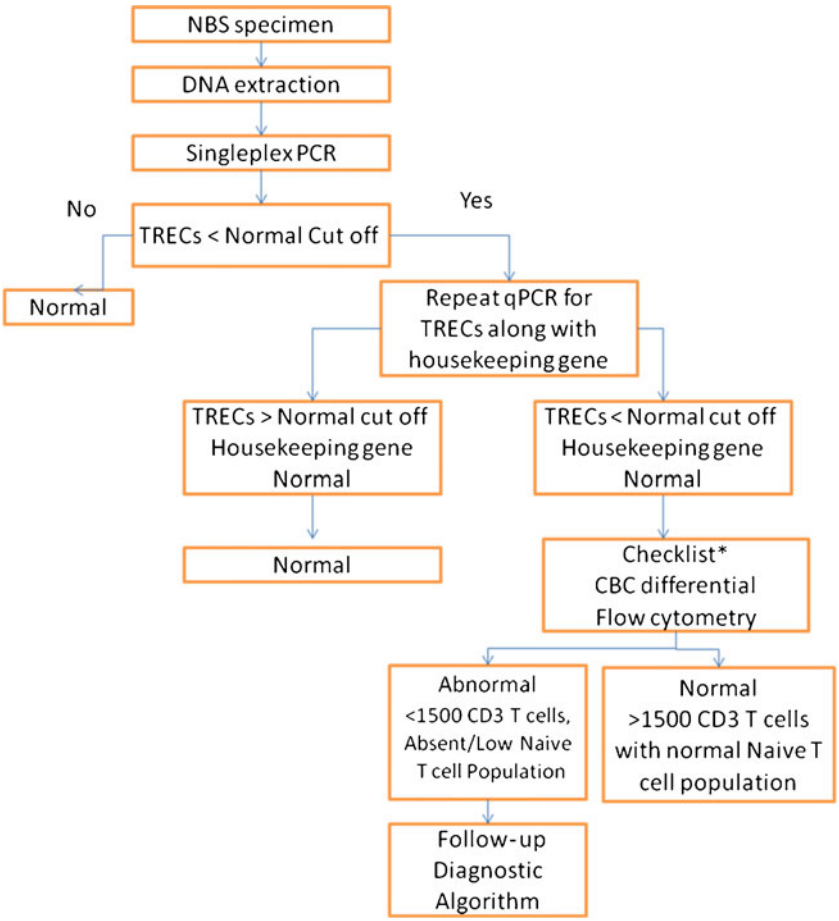

Fig. 1 Flowchart for screening of severe combined immunodeficiency (SCID) by T cell receptor excision circle (TREC) assay

Table 2 Clinical checklist

Physical features s/o SCID (*e.g.*, Skin rash, oral ulcers, midline defects, coarse facial features)
H/o any immunosuppressive to mother
Positive family history in the form of previous siblings with • Infant death • BCGOsis • Severe infections • Diagnosis of PIDs

SCID Severe combined immunodeficiency; *PIDs* Primary immunodeficiency disorders

performed until further evaluation to confirm diagnosis of SCID or other PIDs is performed.

Complete Blood Count as a Screening Test for SCID

Nearly all children with SCID are lymphopenic at birth. It was first suggested by Buckley and Puck in 1997 that all newborns should be screened by a complete blood count and a differential to obtain the absolute lymphocyte count (ALC) per ml of blood [5]. UK Primary Immunodeficiency Network also uses low absolute lymphocyte count as an entry into their protocol for evaluation for SCID [26]. The mean normal cord blood

Table 3 Management of patients diagnosed with SCID

At Point of Care	a) If patient is lymphopenic, hold all live vaccines till final diagnosis is established b) If TBNK/TREC is abnormal, plan for referral. c) Seek and treat infections promptly: i) Antimicrobial therapy may need to be modified in the light of diagnosis of SCID. ii) If SCID is likely and/or lymphopenia is severe, treatment should be initiated whilst awaiting diagnostic results (*e.g.*, CMV/PCP on a bronchoalveolar lavage). iii) Any infants who have received BCG vaccination must be commenced on isoniazid and rifampicin (or other suitable drugs). d) Avoid further infections i) Nurse child in a cubicle with protective isolation measures • Keep cubicle doors closed • Strict hand washing must be adhered to • Visitors must be limited to healthy adults • Staff with infections (including minor respiratory tract infections or cold sores) should not care for the child • If child needs investigations that cannot be undertaken in the cubicle (*e.g.*, radiology), the department must be aware that the child must be seen immediately, and must not sit in a waiting area with other children. If a wait is unavoidable, a separate room must be provided. ii) Give prophylactic co-trimoxazole (Septran) iii) Give prophylactic fluconazole (or other anti-fungal) iv) Start replacement immunoglobulin: (even if IgG normal) consider a loading dose of 1 g/kg All blood products must be irradiated and CMV negative
At Referral Center	a) Confirm and establish specific diagnosis b) Seek and treat infections promptly (as above) c) Avoid further infections (as above) d) Consider definitive treatment i) Stem cell transplantation ii) Replacement therapy (PEG-ADA) iii) Gene therapy trial e) Counseling
Secondary Immunological Evaluation	The secondary immunological evaluation depends on the history, clinical manifestations and results of initial lymphocyte subset analysis. a. Assessment of the proliferative response of T cells by in vitro stimulation including PHA, pokeweed mitogens and/or anti CD3 stimulation to determine the T cell function. b. Maternal engraftment assessment (by sex chromosome analysis for male baby and by STR for female babies). c. HIV testing d. Immunoglobulin (IgG, IgA, IgM and Ig) estimation e. TCR diversity analysis (by flow cytometry using TCRVb spectratyping/immunblot analysis) f. Assessment of specific surface marker expression like expression of cytokine receptors like CD132 (common gamma chain receptor) or CD127 (IL-7 Ra) expression and expression of MHC class I/II molecules (HLA-DR). g. Functional studies for signaling abnormalities like pSTAT5 expression after IL-2 stimulation, h. Enzyme level measurements (for suspected ADA or PNP deficiency) i. Final confirmation by identification of underlying genetic mutation.

TBNK T cell, B cell, NK cell number; *CMV* Cytomegalovirus; *PCP* Pneumocystis carinii pneumonia; *PEG* Polyethylene glycol; *ADA* Adenosine deaminase; *PHA* Phytohemagglutinin; *STR* Short tandem repeat analysis; *HIV* Human immunodeficiency virus; *TCR* T-cell receptor; *TCRVb* T-cell receptor VB repertoire; *MHC* Major histocompatibility complex; *PNP* Purine nucleoside phosphorylase

lymphocyte count is 5500/ul and the lower limit of normal is 2000/ul [27]. While most of SCID patients have low ALC at birth (114–2210/ul reported in 25 children with SCID), some may have a low normal ALC because of the presence of B cells (*IL2RG*, *JAK3* and *IL7R* gene defects) or maternal lymphocytes [26]. Rare infants with Omenn syndrome, characterized by impaired T cell development with immune dysregulation and expansion of oligoclonal T cells may also have a normal to high ALC [20]. Setting the cut-off value for normal ALC at a level to capture all SCID patients would result in a relatively high false positive rate [20]. Shereen et al. reported 1.6 % of 500 newborns to have lymphopenia (ALC < 2500/ul). On further investigation, none of these children eventually had SCID [26].

Despite these limitations, use of CBC to evaluate absolute lymphocyte count as a screening test at birth has several advantages:

a) Its widespread availability with no significant addition to infrastructural cost.
b) Cost per test is approximately 10 times lower than that of TREC.
c) Technically easy and well standardized assay to perform and interpret.
d) CBC is useful not only for calculating ALC useful for identification of SCID but it also can give information about the absolute neutrophil counts (ANC). Very low ANC is suggestive of severe congenital neutropenia and a very high ANC may be suggestive of leukocyte adhesion deficiency (LAD). It also provides information on the other hematological parameters like hemoglobin and platelet count which may be important for management of the newborn.
e) It is performed at point of care and results can be obtained before the newborn is discharged, offering an opportunity for intervention before the infant is lost to follow up. In view of these pros and cons, a screening algorithm with two-tier cut-off of ALC is proposed below (Fig. 2). Lymphopenia noted on CBC and confirmed by Lymphocyte Subset analysis (LSSA) needs clinical evaluation using checklist and appropriate treatment/ intervention while further laboratory investigations are being performed to confirm the diagnosis.

Management

Diagnosis of SCID warrants immediate care and treatment for existing infections and prophylaxis (antimicrobials, antifungals, IVIg) for avoiding further infections. Definitive treatment includes stem cell transplantation, replacement enzyme therapy [PEG-ADA (Polyethylene glycol-Adenosine deaminase)] or selected patients may be offered treatment in a gene therapy trial. Management of SCID patients is depicted in Table 3.

Counseling and Prenatal Diagnosis

Prenatal diagnosis (PND) enables early diagnosis of congenital anomalies and genetic disorders in the unborn fetus [28]. The pediatrician who diagnoses a child with a genetic defect also has a responsibility of counseling the family about the possibility of recurrence of the same or similar problem in future offspring.

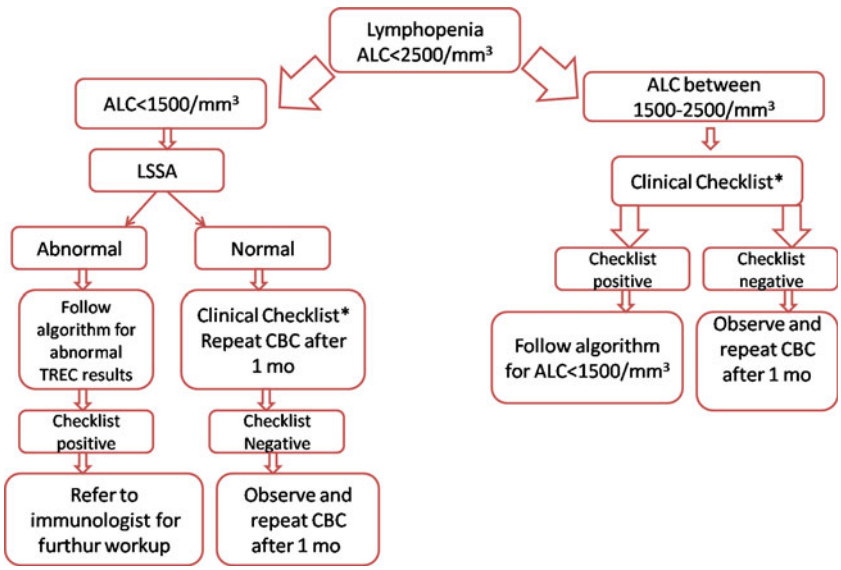

Fig. 2 Screening algorithm of severe combined immunodeficiency with two-tier cutoff of absolute lymphocyte count (ALC). *LSSA* Lymphocyte subset analysis

PND Approach for SCID

If the genetic defect leading to SCID is known in the index case then molecular diagnosis is used to detect the same mutation in the fetal sample obtained either by chorionic villous sampling (CVS) or amniocentesis. However, sometimes though a phenotypic diagnosis in the index case is clear (like abnormal lymphocyte subsets), underlying genetic defect may not been identified. In such cases, phenotypic diagnosis using flowcytometry can be performed on fetal blood sample obtained by cordocentesis [29].

Management of SCID is far from satisfactory in India and results in significant financial and emotional burden on the family and the society. Hence, genetic counseling becomes very important in families affected with SCID.

Conclusions

SCID is an ideal candidate for newborn screening. Many states in the west have implemented the TREC assay for newborn screening of SCID. This assay has been proven to be highly sensitive and specific to identify infants affected with SCID.

The decision to implement TREC assay in India is heavily limited by the cost effectiveness of the screening test, cost ratio of the intervention and availability of transplantation facility. Presently, the transplant protocols in India are not yet optimized to support a newborn screened positive in a TREC assay. Without a backbone of efficient transplant services for management of these patients, implementation of this assay would be a costly affair. Further, performing this assay as a centralized screening service on a Guthrie's card would delay the availability of results by 4–6 wk which, in an Indian setting, could risk losing the child to follow-up and would not be effective in controlling live vaccine related complications.

Notably, a simple Complete Blood Count on cord blood samples can serve as a guide to identify a lymphopenic patient. Not only does the ALC helps picking up lymphopenia cases, CBC also gives a measure of the ANC and other blood indices like Hb, platelet counts which are helpful in management of newborns. Though CBC also has a drawback of giving false negative and false positive results, it is more widely available, easy to perform, results are available immediately and this test is routinely performed in most hospitals. Thus, if CBC is looked at more carefully especially the ALC before giving live vaccines, it may prevent vaccine associated complications and also help in early diagnosis of SCID.

The authors, hence put forth the proposition that a CBC on cord blood samples should be performed on a routine basis. Lymphopenia noted on CBC should be strongly considered as an indicator of SCID and needs an immediate measure of avoiding administration of live vaccines till confirmation of the final diagnosis. The CBC should be repeated after a month and be followed by flow cytometric evaluation for confirmation of SCID. TREC, as a screening assay, can be considered once the transplantation scenario improves in our country and a robust infrastructure is in place for timely and reliable screening and reporting of results.

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Compliance with Ethical Standards

Conflict of Interest None.

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