

Original article

Neonatal screening for absolute lymphopenia

Background: Blood counts with manual differentials could diagnose nearly all cases of severe combined immune deficiency (SCID) at birth. **Objective:** The aim of this study was to outline the prevalence of neonatal lymphopenia among newborns of Obstetrics and Gynecology Hospital, Ain Shams University as an entry to neonatal screening for SCID. **Methods:** Complete blood counting (CBC) with manual differential was performed in the cord blood of 500 newborns. Absolute lymphopenia was considered if the count was less than 2500 lymphocytes/mm³. Parents of lymphopenic infants were advised not to give them any live attenuated vaccines before doing further investigations. The lymphopenic infants were followed up by another CBC after one month. **Results:** In the present study, absolute lymphopenia was found in 8 (1.6%) neonates at delivery. Among our series 44.4% were primigravida and 55.6% were multigravida. Also, 84 (16.8%) experienced pre-mature rupture of membrane, 89 (17.8%) reported maternal diseases and maternal drug intake was reported in 73 (14.6%). Three neonates had congenital anomalies, one only experienced dysmorphic features and 8 (1.6%) had family history of unexplained death but these data could not be linked to the presence of lymphopenia in the studied sample. APGAR scores at 1 and 5 minutes were significantly lower in neonates with lymphopenia ($p = 0.001$). A significant positive correlation was elicited between the absolute lymphocyte count (ALC) and maternal age, total leukocyte count, and HCT ($p = 0.003, 0.001$ and 0.031 respectively). Also a significant negative correlation was found between ALC and gestational age, mean corpuscular hemoglobin and mean corpuscular hemoglobin concentration ($p = 0.013, 0.003$ and < 0.001 respectively). **Conclusion:** Lymphopenia is not an uncommon finding among neonates at screening and is noted to be associated with a lower Apgar score. Serial counting and follow up is needed before considering the diagnosis of SCID.

Keywords: Lymphopenia; neonatal screening; SCID

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INTRODUCTION

SCID is an inherited severe immunodeficiency of T-cell and B-cell functions that occurs in approximately 1: 50,000 live births. About 80% of affected patients are boys, and only approximately one third of patients with SCID have a positive family history. SCID may result from single X-linked or autosomal recessive gene defects or, less commonly, from chromosome abnormalities (Table 1). Peripheral T-cell dysfunction in all forms of SCID is mainly or entirely due to perturbed intrathymic maturation of $\alpha\beta$ T cells (i.e., T cells that express $\alpha\beta$ T-cell receptors [TCRs]) resulting in thymic hypoplasia.¹

The most common type is X-linked SCID, due to mutations in the gene encoding the common γ chain for multiple cytokine receptors; the second most common cause is adenosine deaminase

deficiency (ADA deficiency), and the third most common cause is IL-7R α -chain deficiency. In 25 cases, the molecular defect remains unknown (those in the groups labeled autosomal recessive and unknown). No cases of CD45 deficiency have been seen at this institution.¹

Children who are diagnosed early and receive appropriate management and definitive treatment in the form of a hematopoietic stem-cell transplant (HSCT) have a far better prognosis than those children in whom the diagnosis has been delayed. Prenatal diagnosis is now available for those parents who have a positive family history. However, many children with SCID are born to parents with no family history of the disease. Children with SCID are healthy at birth and have no external characteristics of the condition. The

infectious complications which bring them to medical care may not initially be distinguishable from routine childhood infections, thus diagnosis may be delayed.⁵

Table 1. Classification of severe combined immunodeficiency²

<i>Lymphocyte phenotype</i>		<i>Inheritance</i>	<i>Chromosome</i>
1. T-B-SCID * NK+	RAG 1/2 deficiency	AR	11p13
	DCLRE 1C (Artemis deficiency)	AR	10p13
	* NK-	AR	20q13.11
	Adenosine deaminase deficiency	AR	
2. T-B+SCID * NK+	Reticular dysgenesis	AR	
	IL 7 α deficiency	AR	5p13
	CD45 deficiency	AR	
	CD3 δ /CD3 ϵ /CD3 ζ deficiency	AR	
	* NK-	X-linked	Xq13.1
	JAK3 deficiency	AR	19p13.1

Table 2. Rationale for newborn screening for SCID³

Importance of early identification

- Establish diagnosis and institute immediate lifesaving treatment.
- Avoid inefficient, costly, dangerous ‘diagnostic Odyssey’.
- Provide families with genetic diagnosis and advice on reproductive risks.
- Learn incidence and true spectrum of SCID.
- Educate providers and public about SCID.
- Permit multicenter collaborative trials to determine optimal treatments.

Barriers to early diagnosis without screening

- SCID and related conditions are rare.
- Infections are common in all infants, not just those with SCID.
- Over 80% of cases are sporadic, with no family history.
- Family history can be missed, or nonspecific.
- SCID infants are protected by maternal IgG for their first months of life.
- Because both a gene defect and environmental exposure are required for overt disease, presentation is variable.

Table 3. Causes of lymphopenia⁴

<p>INFECTIOUS DISEASES</p> <p>AIDS</p> <p>Viral hepatitis</p> <p>Influenza</p> <p>Tuberculosis</p> <p>Typhoid fever</p> <p>Sepsis</p>	<p>IATROGENIC</p> <p>Immunosuppressive therapy</p> <p>Corticosteroids</p> <p>High-dose PUVA therapy</p> <p>Cytotoxic chemotherapy</p> <p>Radiation</p> <p>Thoracic duct drainage</p>
<p>INHERITED CAUSES</p> <p>Aplasia of lymphopoietic stem cells</p> <p>Severe combined immunodeficiency associated with defect in IL-2 receptor γ-chain, deficiency of ADA or PNP, or unknown</p> <p>Ataxia-telangiectasia</p> <p>-Wiskott-Aldrich syndrome</p> <p>Immunodeficiency with thymoma</p> <p>Cartilage-hair hypoplasia</p> <p>Idiopathic CD4 T lymphopenia</p>	<p>SYSTEMIC AND OTHER DISEASES</p> <p>Dietary deficiency</p> <p>Systemic lupus erythematosus</p> <p>Myasthenia gravis</p> <p>Hodgkin disease</p> <p>Protein-losing enteropathy</p> <p>Renal failure</p> <p>Sarcoidosis</p> <p>Thermal injury</p> <p>Aplastic anemia</p>

Early recognition of SCID should be considered a pediatric emergency⁶ because a diagnosis before live vaccines or non-irradiated blood products are given and before the development of infections permits lifesaving unfractionated HLA-identical or T cell depleted haploidentical nonablative hematopoietic stem cell transplantation,⁷ enzyme replacement therapy,⁸ or gene therapy.⁹ However, unlike infants with DiGeorge syndrome, most infants with SCID appear physically normal at birth until they begin to develop infections, where failure to thrive begins. In most cases there is no family history of SCID.⁵

The Incidence of disseminated BCG disease ranged from 0.06 to 3.4 cases per million vaccination, and the mortality rates remained high in immunocompromised patients (60-38%).¹⁰ The disseminated BCG infection may be the first presentation prior to the diagnosis of immunodeficiency.¹¹ The most common symptoms of disseminated BCG infection died even when they were aggressively managed.¹² SCID is not apparent at birth and early recognition is essential for life saving treatment, hence SCID has been recognized as a candidate for newborn screening for many years.¹³

All children with SCID are lymphopenic at birth¹⁴, thus routine blood counts with manual differentials could diagnose nearly all cases of SCID at birth.¹⁵ However there are a high number of both false-positives and -negatives, as not all children who are lymphopenic have SCID and some patients with SCID may have a low-normal absolute lymphocyte count because of the presence of B cells (IL2RG, JAK3 and IL7R gene defects) or maternal lymphocytes. Furthermore this test is potentially labor-intensive since it does not make use of the dried blood spots (DBS) collected routinely from all neonates at birth in the USA. The UK Primary Immunodeficiency Network also uses a low lymphocyte count as an entry into their protocol for screening for SCID. However a recent audit from Birmingham, UK, found that although only 1 out of the 56 cases of lymphopenia was documented by the clinician, there were no clear missed cases of SCID. They therefore thought it would be more cost-effective to discuss cases of lymphopenia with an immunologist before doing further investigations for SCID.¹⁶ Direct detection of gene mutations using re-sequencing microarray chips is also an option. Although this potentially will have a high number of false-negatives, it would also give one an immediate specific gene diagnosis.¹⁷

METHODS

Study Population

This cross sectional study comprised 500 newborn infants delivered either vaginally or by caesarean section at the Obstetrics and Gynecology Hospital, Ain Shams University, Cairo, Egypt. All newborns were included in the study with no exclusion criteria. They were 248 males and 252 females. Their gestational ages ranged from 27 – 42 weeks (mean 37±2.6 wks). Their weight ranged from 0.8- 5 Kg (mean 3.0±0.6 kg). Out of the 500 neonates, only 3 had congenital malformations, while 8 had family history of unexplained sib death. Out of the 500 neonates, 264 were delivered vaginally, while 236 were delivered by caesarean section. Premature rupture of membrane was reported in 84 cases. An informed consent was obtained from the parents or care givers of all children before enrollment. The study protocol was approved by the ethics committee of the Pediatric Department, Ain Shams University.

Study Measurements

Prenatal history was taken from the mother for medical history, fever, chorioamnionitis and premature rupture of membrane >18 hrs. Also family history of previously diagnosed PID sibling or previous death whether due to infections or due to an unknown cause was traced.

Apgar scores were plotted for all neonates at one and 5 minutes. All neonates were subjected to general and systemic clinical examination.

Two ml of cord blood (during time of delivery) were collected from each infant under complete aseptic condition and put in a vacuette tube containing ethylene diamine tetra acetic acid (EDTA) (1.2 multigravida/ml). CBC was performed using coulter counter (Beckman instrumentation) including the red blood cells (RBCs) hemoglobin (HB) hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), WBC and platelets. Manual differential was done.

- Absolute lymphopenia was considered in any cord blood sample if lymphocytic count was less than 2500/mm³.¹⁸
- Parents of lymphopenic newborn infants were told not to give their newborns live attenuated vaccines before doing further investigations.
- Only the lymphopenic newborn infants were followed up by another CBC after one month.

Statistical methods:

The collected data were coded, tabulated, and statistically analyzed using SPSS program (Statistical Package for Social Sciences) software version 18.0.

Inferential analyses were done for normally distributed quantitative variables using independent t-test in cases of two independent groups. Inferential analyses were done for qualitative data using Chi square test for independent variables. Correlations were done using Pearson correlation for numerical parametric data. The level of significance was taken at p value <0.050.

RESULTS

In the present study, absolute lymphopenia was found in 8 (1.6%) neonates at delivery while 492 (98.4%) of them had absolute lymphocytic count above $2.5 \times 10^3/\text{cmm}$. All lymphopenic patients were followed up and advised not to administer BCG vaccine. Another CBC and ALC were done after one month. All of them proved to have normal ALC in the second evaluation after one month.

Analysis of ALC among our patients with normal ALC revealed that, 45 (9%) of our neonates had $ALC \geq 7.5 \times 10^3/\text{cmm}$, 43 (8.6%) had ALC of $6.5-7.5 \times 10^3/\text{cmm}$, 65 (13%) had ALC of $5.5-6.5 \times 10^3/\text{cmm}$, 129 (25.8%) had ALC of $4.5-5.5 \times 10^3/\text{cmm}$, 141 (28.2%) had ALC of $3.5-4.5 \times 10^3/\text{cmm}$ and finally 69 (13.8%) had an ALC of $2.5-3.5 \times 10^3/\text{cmm}$.

Among our study population 222 (44.4%) were primigravida and 278 (55.6%) were multigravida. Also, 84 (16.8%) experienced pre-mature rupture of membrane while maternal diseases were reported in 89 (17.8%) of our mothers.

Three neonates (0.6%) had congenital anomalies, one (0.2%) had dysmorphic features and 8 (1.6%) had family history of unexplained death.

The gestational age, weight and Apgar score at 1 and 5 minutes were significantly lower in neonates with lymphopenia ($p = <0.001, 0.031, <0.001$ and <0.001 respectively). Also our neonates with lymphopenia showed female predominance, M/F ratio was 1:7 in comparison to 247: 245 in the group with normal ALC ($p = 0.034$).

There was no significant difference between those with and those without lymphopenia as regards maternal age, gravida, mode of delivery, pre-mature rupture of membranes presence of maternal diseases and maternal drug intake ($p = 0.35, 0.69, 0.87, 0.74, 0.42$ and 0.06 respectively).

Moreover, the presence of congenital anomalies, dysmorphic features or relevant family history of unexplained death did not vary significantly among those with and those without lymphopenia.

According to our study, a significant positive correlation was found between ALC and maternal age, TLC and HCT ($p = 0.003, <0.001$ and 0.031 respectively). Also a significant negative correlation was found between ALC and gestational age MCH and MCHC ($p = 0.013, 0.003$ and <0.001 respectively). ALC did not vary significantly in relation to Apgar scores at one and 5 minutes ($p = 0.179$).

Studying the combined effect of (child death, pre-mature rupture of membrane gestational age weight, sex, maternal disease, maternal drugs, delivery mode and maternal age) showed a significant correlation with ALC. Neonatal sex and weight bore a significant positive correlation with ALC in the presence of other variables ($p = 0.000$ and 0.003 respectively).

Table 4. Comparison of some data between patients with and without lymphopenia in the current study.

Data	Lymphopenia	No lymphopenia	p value	OR (95% CI)
Gestational age (wk)	34.3 ± 3.8	37.9 ± 2.6	0.001	
Weight (kg)	2.1 ± 1.0	3.1 ± 0.6	0.031	
APGAR (1 min)	4.4 ± 1.0	5.4 ± 0.9	0.001	
APGAR (5 min)	7.6 ± 0.7	8.6 ± 0.6	0.001	
Male	1 (12.5%)	247 (50.2%)	0.034	0.14
Female	7 (87.5%)	245 (49.8%)		

CI: confidence interval; OR: odds ratio

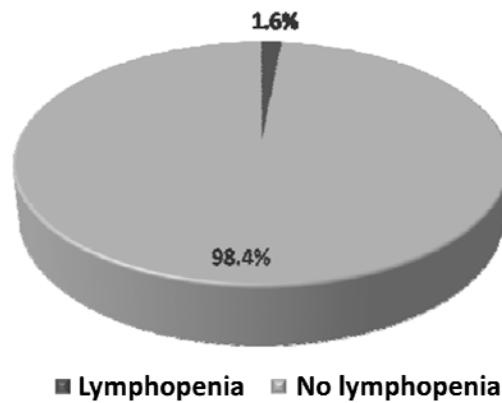


Figure 1. Cord blood lymphopenia in the studied sample

DISCUSSION

Mutations in 13 different genes have been found to cause SCID, which is uniformly fatal in the first two years of life.¹⁹ Disseminated BCG disease is one of the most common causes of death in patients with primary immunodeficiency diseases, particularly SCID.²⁰ Taking an exact family history before vaccination is strongly recommended, as families with low socioeconomic status may not provide information if not asked.²¹

With this as a background we were stimulated to evaluate the incidence of neonatal lymphopenia at birth as an entry to a screening program for SCID as all children with SCID are lymphopenic at birth.¹⁴ Thus, routine blood counts with manual differentials could diagnose nearly all cases of SCID at birth.¹⁵

Our study was performed on 500 neonates. The frequency of lymphopenia ($ALC < 2.5 \times 10^3$) in the study group was (1.6%). One patient was male (12.5%) and 7 were females (87.5%). Thus lymphopenia was more frequent in females than males.

Concerning the ranges of lymphocyte counts in the study group, 1.6% were ($1.5 < ALC < 2.5 \times 10^3$), 13.8% were ($2.5 < ALC < 3.5 \times 10^3$), 28.2% were ($3.5 < ALC < 4.5 \times 10^3$), 25.8% were ($4.5 < ALC < 5.5 \times 10^3$), 13% were ($5.5 < ALC < 6.5 \times 10^3$), 8.6% were ($6.6 < ALC < 7.5 \times 10^3$) and 9% were $\geq 7.5 \times 10^3/\text{mm}^3$. More than half of the cases (54%) had a lymphocytic count in the range of ($3.5 < ALC < 5.5 \times 10^3/\text{mm}^3$).

Because most infants with SCID lack T cells, the absolute lymphocyte count (ALC) could be used as a screening test for this syndrome. T cells comprise 70% of circulating lymphocytes in healthy human subjects throughout life. Therefore lymphopenia will be present in nearly all infants with SCID from the time of birth as a result of their missing 70% of circulating lymphocytes. However, many infants do not have blood counts performed. Additionally, many physicians are not aware of the usefulness of the ALC, even though they are taught

throughout medical school about the importance of the absolute neutrophil count.²²

Work by Dr. Rebecca Buckley at Duke University compared lymphocyte levels between normal and SCID babies at birth has shown that most SCID patients had low absolute lymphocyte counts at birth (range of absolute lymphocyte counts was 114–2210 lymphocytes/ mm^3 for 25 SCID newborns and 1670–8910 lymphocytes/ mm^3 for 14 normal infants at birth). Patients with SCID often had numerous prior blood counts clearly showing lymphopenia, but the physicians caring for the infants did not appreciate the significance of this.²² However, there is a high number of both false positive and negative results, as not all children who are lymphopenic have SCID and some patients with SCID may have a low normal absolute lymphocytic count because of the presence of B cells (IL2RG, JAK3 and IL7R gene defects) or maternal lymphocytes.²³

The longest pilot study (44 months) was conducted in Wisconsin²⁴ and has reportedly discovered 4 cases of SCID and 7 cases of T-cell lymphopenia that were not related to SCID of a total of 243,707 newborns screened. The initial results of screening for one year have been published.²⁵ The discovered cases had undergone either hematopoietic stem cell transplantation or enzyme replacement therapy and are surviving.

Massachusetts was the second state to initiate a pilot study²⁴ and has screened for 31 months, during which time it has reportedly discovered one case of SCID and 14 cases of T-cell lymphopenia that were not related to SCID of a total of 161,707 newborns screened.²⁶ The infant with SCID had undergone transplantation and is surviving.²⁷

New York followed a screening program²⁴ for a total of 136,635 newborns over 11 months and has reportedly discovered 4 cases of SCID and 12 cases of T-cell lymphopenia that were not related to SCID. All 4 patients with SCID have been treated appropriately and are surviving.

California has been screening for 13 months and has reportedly²⁴ discovered 5 cases of SCID, 6 cases of variant SCID, and 3 cases of T-cell lymphopenia that was not related to SCID of a total of 358,000 newborns screened. All of the infants with SCID have received appropriate therapy and are surviving.

The three pilot studies of Puerto Rico, Louisiana, and the Navajo Nation have reportedly not yet discovered any cases of SCID and only 4 cases of T-cell lymphopenia that were not related to SCID of an aggregate total of just over 60,000 newborns screened. Thus far, newborn screening for SCID with the TREC assay appears to be 100% sensitive because no other cases of SCID have been discovered in the states performing the pilot studies during the time period that the screening has been conducted. However, it is apparent that many other conditions with T-cell lymphopenia that need medical attention are being detected by using this screening method. For the 40 patients without SCID detected in aggregate, approximately 30% had DiGeorge syndrome, 35% had idiopathic T-cell lymphopenia and are being followed closely, 5% had trisomy 21, and 30% had T-cell lymphopenia associated with other genetic diseases or conditions.²⁴

Neonates with lymphopenia were comparable to those with normal lymphocyte counts in terms of variables related to maternal history namely age, gravida, pre-mature rupture of membranes maternal diseases and drug history. Similarly, the presence of congenital diseases, dysmorphic features and history of unexplained sib death did not significantly vary among both groups. This can be attributed to the fact that lymphopenia was transient as evident from the second blood sample.

Weight, gestational age and Apgar score at 1 and 5 minutes were significantly lower in cases with lymphopenia than cases without lymphopenia with female predominance in the lymphopenia group; a finding which can suggest that this transient lymphopenia could be related to transient neonatal infections.

A significant positive correlation was found between (maternal age, WBC, HCT) and ALC. Moreover, no significant correlation between APGAR score 1 and 5 and ALC could be plotted, a finding which might be due to the small sample size.

Neonatal sex and weight bore a significant positive correlation with ALC in the presence of other variables namely previous child death, pre-mature rupture of membranes, gestational age, weight, sex, maternal diseases and drugs, delivery

mode and maternal age ($p=0.000$ and 0.003 respectively); finding which can suggest the multifactorial impact on absolute lymphocytic count.

In conclusion, lymphopenia is not an uncommon finding among neonates at screening and is noted to be associated with a lower Apgar score. We recommend introduction of a neonatal absolute lymphocytic count evaluation as a part of neonatal screening program for pre-symptomatic detection and early intervention for neonates with SCID. Also, BCG vaccine and other live attenuated vaccines should be withheld until SCID is excluded.

REFERENCES

1. **BUCKLEY RH.** Molecular defects in human severe combined immunodeficiency and approaches to immune reconstitution. *Annu Rev Immunol* 2004;22:625-55.
2. **STEPHAN JL, VLEKOVA V, LE DEIST F, BLANCHE S, DONADIEU J, DE SAINT-BASILE G, ET AL.** Severe combined immunodeficiency: a retrospective single-center study of clinical presentation and outcome in 117 patients. *Pediatr* 1993;123:564-72.
3. **PUCK JM.** Neonatal screening for severe combined immunodeficiency. *Curr opin Pediatr* 2011;23(6):667-73.
4. **BOXER LA.** Approach to the patient with leucopenia. In: Humes HD editor. *Kelly's Textbook of internal medicine* 4th ed. Philadelphia: Lippincott Williams and Wilkins 2000.p.1580.
5. **MYERS LA, PATEL DD, PUCK JM, BUCKLEY RH.** Hematopoietic stem cell transplantation for severe combined immunodeficiency in the neonatal period leads to superior thymic output and improved survival. *Blood* 2002;99(3):872-8.
6. **BUCKLEY RH, SCHIFF RI, SCHIFF SE, MARKERT ML, WILLIAMS LW, HARVILLE TO, ET AL:** Human severe combined immunodeficiency: genetic, phenotypic, and functional diversity in one hundred eight infants. *J Pediatr* 1997;130(3):378-87.
7. **BUCKLEY RH.** Transplantation of hematopoietic stem cells in human severe combined immunodeficiency: long term outcomes. *Immunol Res* 2011;49(1-3):25-43.
8. **HERSHFIELD MS, BUCKLEY RH, GREENBERG ML, MELTON AL, SCHIFF RI, HATEM C, ET AL.** Treatment of adenosine deaminase deficiency with polyethylene glycol-modified adenosine deaminase. *N Engl J Med* 1987;316(10):589-96.
9. **GASPAR HB, COORAY S, GILMOUR KC, PARSLEY KL, ADAMS S, HOWE SJ, ET AL.** Longterm persistence of a polyclonal T cell repertoire after gene therapy for x-linked severe combined immunodeficiency. *Sci Transl Med* 2011;3(97) :79-97.

10. **BERNATOWSKA EA, WOLSKA - KUSNIERZ B, PAC M, KURENKO-DEPTUCH M, ZWOLSKA Z, CASANOVA JL ET AL.** Disseminated bacillus Calmette- Guérin infection and immunodeficiency. *Emerg Infect Dis* 2007;13 (5):799-801.
11. **HUANG LH, SHYUR SD, WENG JD, SHIN-GHI, TZEN CY, HUANG FY.** Disseminated Bacille Calmette-Guérin disease as the initial presentation of X-linked severe combined immunodeficiency - a case report. *Asian Pac J Allergy Immunol* 2005;23(4):221-6.
12. **AFSHAR PAIMAN S, SIADATI A, MAMISHI S, TABATABAIE P, KHOTAAE G.** Disseminated Mycobacterium bovis infection after BCG vaccination. *Iran J Allergy Asthma Immunol* 2006;5(3):133-7.
13. **MCGHEE SA, STIEHM ER, MCCABE ER.** Potential costs and benefits of newborn screening for severe combined immunodeficiency. *J Pediatr* 2005;147(5):603-8.
14. **LINDEGREN ML, KOBRYNSKI L, RASMUSSEN SA, MOORE CA, GROSSE SD, VANDERFORD ML, ET AL.** Applying public health strategies to primary immunodeficiency diseases: a potential approach to genetic disorders. *MMWR Recomm Rep* 2004;53(RR-1):1-29.
15. **BUCKLEY RH.** Primary immunodeficiency diseases: dissectors of the Immune system. *Immunol Rev* 2002;185:206-19.
16. **KRISHNA MT, TARRANT JL, GHEADLE EA, NOORANI S, HACKETT S, HUISOON AP.** An audit of lymphopenia infants under 3 months of age. *Arch Dis Child* 2008;93(1):90-1.
17. **PUCK JM.** Neonatal screening for severe combined immune deficiency. *Curr Opin Allergy Clin Immunol* 2007;7:522-7.
18. **BUCKLEY RH.** Primary combined antibody and cellular immunodeficiencies. In: Behrman RE, Kliegman RM, Jenson HB, editors. *Nelson textbook of pediatrics*. 19th ed. Philadelphia: WB Saunders; 2012. p.731-40.
19. **ROBERTS JL, LAURITSEN JP, COONEY M, PARROTT RE, SAJAROFF EO, WIN CM, ET AL.** T-B+NK+ severe combined immunodeficiency caused by complete deficiency of the CD3 ζ subunit of the T cell antigen receptor complex. *Blood* 2007; 109 (8) :3198-206.
20. **REZAEI N, POURPAK Z, AGHAMOHAMMADI A, FARHOUDI A, MOVAHEDI M, GHARAGOZLOU M, ET AL.** Consanguinity in primary immunodeficiency disorders; the report from Iranian Primary Immunodeficiency Registry. *Am J Reprod Immunol* 2006; 56:145-51.
21. **SADEGHI-SHABESTARI M, VESAL S, JABBARPOUR-BONYADI M, DE VILLATARY JP, FISCHER A, REZAEI N.** Novel RAG2 mutation in a patient with T-B-severe combined immunodeficiency and disseminated BCG disease. *J Investig Allergol Clin Immunol*. 2009;19(6):494-6.
22. **KALMAN L, LINDEGREN ML, KOBRYNSKI L, VOGT R, HANNON H, HOWARD JT, ET AL.** Mutations in selected genes required for T cell development: IL7R, CD45, IL2R gamma chain, JAK3, RAG1, RAG2, ARTEMIS and ADA and severe combined immunodeficiency. *Genet Med* 2004;6:16-26.
23. **FINLAYSON H.** Severe combined immunodeficiency (SCID) – advances in molecular diagnosis, neonatal screening and long term management. *Curr Allerg Clin Immunol* 2008;21:20-4.
24. **MICHELE GAGGANA.** National SCID pilot study. Available from: <http://www.hrsa.gov/advisorycommittees/mchbavisory/heritable/advisorycommittees/mchbavisory/heritabledisorders/reports/CommitteeSCIDReport.pdf>. Accessed on May 15, 2012.
25. **ROUTES JM, GROSSMAN WJ, VERBSKY J, LAESSIG RH, HOFFMAN GL, BROKOPP CD, ET AL.** Statewide newborn screening for severe T-cell lymphopenia. *JAMA* 2009; 302 (22) :2465-70.
26. **COMEAU AM, HALE JE, PAI SY, BONILLA FA, NOTARANGELO LD, PASTERNAK MS, ET AL.** Guidelines for implementation of population-based newborn screening for severe combined immunodeficiency. *J Inherit Metab Dis* 2010;33(suppl 2):S273-81.
27. **HALE JE, BONILLA FA, PAI SY, GERSTEL-THOMPSON JL, NOTARANGELO LD, EATON RB, ET AL.** Identification of an infant with severe combined immunodeficiency by newborn screening. *J Allergy Clin Immunol* 2010;126(5):1073-4.