U.PORTO

Mestrado Integrado em Medicina

Neonatal Screening for Severe Combined Immunodeficiency

Gonçalo Espírito Santo Matos

M

2018

Neonatal Screening for Severe Combined Immunodeficiency

Revisão Bibliográfica

Dissertação da candidatura ao grau de Mestre em Medicina submetida ao Instituto de Ciências Biomédicas Abel Salazar, Universidade do Porto, de

Gonçalo Manuel Espírito Santo da Silva Matos, Nº aluno: 201107459
Mestrado Integrado em Medicina – 6º Ano Profissionalizante
Instituto de Ciências Biomédicas Abel Salazar, Universidade do Porto
Endereço electrónico: goncaloespiritosantomatos@gmail.com

Orientadora:
Dr.ª Laura Elvira Gonçalves Novo da Hora Marques
Especialista em Pediatria
Assistente Graduada do Serviço de Pediatria do Centro Materno-Infantil do Norte
Prof.ª Auxiliar Convidada do Instituto de Ciências Biomédicas Abel Salazar, Universidade do Porto

Porto, Maio de 2018

Aluno: Gonçalo Manuel Espírito Santo da Silva Matos

Gonçalo Espírito Santo Matos

Orientadora: Dr.ª Laura Elvira Gonçalves Novo da Hora Marques

Porto, 24 de Maio de 2018

Abstract

Severe combined immunodeficiency (SCID) is an important health problem and is the most severe form of primary immunodeficiency. This syndrome is caused by genetic mutations that block the development of T cells. As such, serious T cell dysfunction precludes effective humoral immunity, as the B cells require signalization from T cells to generate antibodies. Based on the records from a combined prospective data analysis from the neonatal screening program in the United States of America, the incidence of SCID is approximately 1 in 58.000 infants. The early diagnosis of SCID before any clinical symptom or the occurrence of serious complications is proven to improve the survival rates after the hematopoietic stem cell transplantation, which is the recommended treatment.

This literature review aims at analysing all the advantages and disadvantages of the implementation of SCID testing in all the neonatal screening programs.

The search engine used to conduct this review was PubMed.

In this paper, the presentation of SCID, the available screening methods to detect the disease and the cost-effectiveness related to the screening are presented in order to clarify all the components that need to be taken in consideration when adding a disease to a neonatal screening program.

To conclude, the screening method for SCID that quantifies the T-cell receptor excision circles using reverse transcription for real-time quantitative polymerase chain reaction is proven to be cost-effective has a sensitivity and specificity of nearly 100% and allows to detect the disease at an early stage, while it still has a good prognosis. This method has already been implemented in some countries with good results.

Key-words: Severe Combined Immunodeficiency; Neonatal Screening; Receptors, Antigen, T-Cell; Lymphopenia.

Resumo

Imunodeficiência Combinada Grave (SCID) é um importante problema de saúde pública e a forma mais grave de imunodeficiência primária. Este síndrome é causado por mutações genéticas que impedem o desenvolvimento das células T. Assim sendo, a disfunção grave de células T impede uma resposta humoral efetiva, visto que as células B necessitam de sinalização por parte das células T para desenvolver anticorpos. Tendo por base a análise de dados de um estudo combinado prospectivo do programa de rastreio neonatal nos Estados Unidos da América, estimou-se que a incidência de SCID é 1 em 58.000 recém-nascidos. O diagnóstico precoce de SCID antes de qualquer sintoma clínico ou ocorrência de complicações graves está provado estar associado a melhores taxas de sobrevivência após o tratamento com transplante de células percursoras hematopoiéticas, que é o tratamento recomendado.

Esta revisão bibliográfica analisa as vantagens e desvantagens da implementação do rastreio de SCID em todos os programas de rastreio neonatal.

O motor de busca utilizado para a pesquisa de artigos foi o PubMed.

Neste trabalho, uma apresentação das características da SCID, os métodos de rastreio disponíveis para detectar esta doença e o custo-benefício inerente ao rastreio são apresentados, para clarificar todos os componentes que necessitam de ser considerados para a adição de uma nova doença a um programa de rastreio neonatal.

Concluindo, o método de rastreio para a SCID que quantifica os círculos de excisão do receptor das células T usando uma técnica de reação em cadeia de polimerase de transcrição reversa para quantificação em tempo real tem um bom custo-benefício, tem uma sensibilidade e uma especificidade de quase 100% e permite detectar a doença num estádio precoce, quando ainda tem bom prognóstico. Este método já foi implementado em alguns países e tem demonstrado bons resultados.

List of abbreviations

ADA – Adenosine Deaminase Deficiency

ADA-SCID – Adenosine Deaminase Deficiency-Severe Combined Immunodeficiency

AR – Autosomal Recessive

CHARGE – Coloboma, Heart Anomalies, Choanal Atresia, Retardation of Growth and Development, and Genital and Ear Anomalies

CHH – Cartilage-Hair Hypoplasia

DBS – Dried Blood Samples

EBV – Epstein-Barr Virus

HSCT – Haematopoietic Stem Cell Transplantation

IL-7 – Interleukin-7

IUIS – International Union of Immunological Societies

KREC – Kappa-deleting Recombination Excision Circles

MHC – Major Histocompatibility Complex

NK – Natural Killer

PCR – Polymerase-Chain Reaction

PNP – Purine Nucleoside Phosphorylase

RTqPCR – Reverse Transcription for Real-Time Quantitative Polymerase Chain Reaction

SCID – Severe Combined Immunodeficiency

TCR – T-cell Receptor

TREC – T-cell Receptor Excision Circles

USA – United States of America

WHO – World Health Organization

XL – X-linked

XLA – X-linked Agammaglobulinemia

Index

Introduction 1

Methods 2

Results 2

Neonatal screening 3

Severe Combined Immunodeficiency 5

Screening 8

Available Screening Tests 9

In Portugal 12

Cost-Effectiveness 13

Conclusion 15

Bibliography 16

Introduction

The concept of “primary immunodeficiency” comprises a group of genetic diseases that result from inherited defects of the immune system(1). Combined immunodeficiency syndromes are a heterogeneous group of diseases that result from a disturbance in the development and function of both T and B cells, from the cellular and humoral immunity(2, 3). Furthermore, some of them are termed “severe” because the course of this disease leads to early death from opportunistic infections, normally in the first year of life (if not diagnosed and treated)(4-7).

Severe combined immunodeficiency (SCID) is an important health problem. Based on the records from a combined prospective data analysed from the neonatal screening program in the United States of America (USA), the incidence of SCID is approximately 1 in 58.000 infants(8). The only exception from the data that is available is from the Navajo Nation, whose incidence is 1 in 3.500 infants(9, 10). However, this increased incidence is related to a mutation in the *DCLRE1C* gene that causes SCID, which is frequent in this population(9).

SCID is the most severe form of primary immunodeficiency(11). This syndrome is caused by genetic mutations that block the development of T cells (4). However, these mutations can also affect the B cells lineage. In addition to that, serious T cell dysfunction precludes effective humoral immunity, as B cells require signalization from T cells to generate antibodies(4). Moreover, SCID can affect the natural killer (NK) cells(12).

In order to correct this disease in newborn babies, there must be an early detection and these patients must be referenced to specialized centres. Nowadays, the standard treatment for patients with SCID is:

- Hematopoietic stem cell transplantation (HSCT) HLA-identical or HLA-haploidentical(13-28);
- Enzyme replacement therapy is another option of treatment in some cases of adenosine deaminase deficiency-SCID (ADA-SCID)(4);
- Gene therapy, which is an alternative for some forms of SCID (ADA-SCID and X-linked SCID – common gama-chain deficiency)(29).

Methods

The research was performed on PubMed, using the following formula of MeSH words: ("severe combined immunodeficiency"[MeSH Terms] OR ("severe"[All Fields] AND "combined"[All Fields] AND "immunodeficiency"[All Fields]) OR "severe combined immunodeficiency"[All Fields]) AND ("neonatal screening"[MeSH Terms] OR ("neonatal"[All Fields] AND "screening"[All Fields]) OR "neonatal screening"[All Fields].

Inclusion criteria included studies, review articles or case reports; written in English, Portuguese, Italian or Spanish; published between 1st of January of 2003 and 31th of December of 2017; with full text available; whose articles were related to the analysis of the severe combined immunodeficiency and its neonatal screenings already available. Some opinion articles were excluded. Some articles older than the mentioned timeframe were included because a historic reference was made about neonatal screening and its development.

Results

A total of 149 articles were analysed from which 45 articles that met the inclusion criteria were selected.

Based on the selected articles, the relevant information was collected for the review and organised according to the type of study performed as to compose the body of the review.

Neonatal screening

The neonatal screening of newborn infants started in 1963 according to the paper presented by Robert Guthrie and Ada Susi, for the screening of phenylketonuria(30). They proposed the use of dried blood spots collected from a heel stick, blotted on a filter paper after 2 to 5 days from the date of birth to be analysed by specialized laboratories(30). This was the first step to create a newborn screening. A few years later, in 1968, after a conference organised by the World Health Organization (WHO), Wilson and Jungner proposed 10 criteria to have in consideration when a new disorder to be proposed for population screening(31).

The criteria that Wilson and Jungner proposed initially were:

1. The condition sought should be an important health problem.
2. There should be an accepted treatment for patients with recognized diseases.
3. Facilities for diagnosis an treatment should be available.
4. There should be a recognizable latent or early symptomatic stage.
5. There should be a suitable test or examination.
6. The test should be acceptable to the population.
7. The natural history of the condition, including development from latent to declared disease, should be adequately understood.
8. There should be an agreed policy on whom to treat.
9. The cost of case finding (including diagnosis) should be economically balanced in relation to possible expenditure on medical healthcare as a whole.
10. Case finding should be a continuing process and not a 'once and for all' project.

In 2008, Andermann, Blancquaert, Beauchamp and Dery proposed a review of the criteria because there was a need to adapt them (32). So, the criteria proposed were (32-34):

1. There should be scientific evidence of screening programme effectiveness and the benefits of screening should be shown to outweigh the harm.
2. The test may be multiplexed or overlaid onto an existing structure or system.
3. The 'diagnostic odyssey' for the patient/family may be reduced or eliminated.
4. Adverse outcome(s) are rare with a false-positive test.
5. Treatment costs may be covered by third parties (either private or public).
6. Testing may be declined by parents/guardians.
7. Adequate pretesting information or counselling is available to parents/guardians.
8. Screening in the newborn period is critical for prompt diagnosis and treatment.

9. Public health infrastructure is in place to support all phases of the testing, diagnosis and interventions.
10. If carriers are identified, genetic counselling is provided.
11. Treatment risks and the impact of a false-positive test are explained to parents/guardians.
12. The limitations of screening and risks of a false-negative test are explained to parents/guardians.

Based on these criteria, we are proposing that the severe combined immunodeficiency should be included in most of the Europeans neonatal screenings.

Severe Combined Immunodeficiency

SCID can be classified according to the immunophenotype (for example: $T^-B^+NK^+$ - absence of T cells and presence of B and NK cells; $T^-B^-NK^+$ - absence of T and B cells and presence of NK cells)(4, 35). In fact, T cells are always absent in every type of SCID.

However, nowadays, the appropriate classification of SCID takes in consideration the specific molecular defect that is causing the immunodeficiency. So far, according to the International Union of Immunological Societies (IUIS) there have been discovered 16 genes that can cause SCID and each one of them has different clinical features that can help when the physician is investigating the case(5, 36). In Table I, is presented the classification of the mutation according to the pathogenic mechanism and the genotype and Table II shows the clinical features associated with the genetic defect of some SCID (5, 36).

Table I. Pathogenetic mechanism of SCID

Pathogenetic mechanism	Gene mutated	Immuno-phenotype	Inheritance
Defective survival of haematopoietic precursors	AK2	$T^-B^-NK^-$	AR
Toxic metabolite accumulation	ADA PNP	$T^-B^-NK^-$ $T^-B^+NK^-$	AR AR
Cytokine signaling anomalies	IL2RG JAK3 IL-7RA	$T^-B^+NK^-$ $T^-B^+NK^-$ $T^-B^+NK^+$	XL AR AR
V(D)J recombination and TCR abnormalities	RAG1/RAG2, DCLRE1C, PRKDC, NHEJ1, LIG4	$T^-B^-NK^+$	AR
TCR abnormalities	PTPRC CD3D, CD3E, CD247 CORO1A	$T^-B^+NK^+$ $T^-B^+NK^+$ $T^-B^-NK^+$	AR AR AR
Thymic abnormalities	FOXN1	$T^-B^+NK^+$	AR

AR: autosomal recessive transmission; XL: X-linked transmission

Adapted from Cirillo et al.(5)

Table II. Clinical phenotypes associated with each known genetic defect

Gene defect	Phenotype	Pathogenic mechanism	Reference
AK2	Ommen syndrome	Peripheral expansion of oligoclonal T lymphocytes	(37)
IL2RG (γc) JAK3	Hodgkin like features, invagination and hemophagocytic lymphohistiocytosis Selective $CD4^+$ T lymphopenia	Not clear; maternal graft versus host disease Hypomorphic mutation associated with somatic chimerism	(38-40)
RAG	Granulomatous lesions, EBV-related lymphoma, Idiopatic $CD4^+$ T lymphopenia with extensive chickenpox	Hypomorphic mutations	(41)
CORO1A	EBV B cell lymphoproliferation	Not clear; null and hypomorphic mutations of CORO1A in mice are associated with defects in T cell survival and migration	(42)
FOXN1	Eczematous rash, erythroderma, severe diarrhea and alopecia	Residual T cell development sustained by rudimentary thymus or extrathymic lymphoid sites	(43)

EBV: Epstein-Barr virus

Adapted from Cirillo et al.(5)

In fact, regardless of the immunological phenotype, infants with SCID present similar features as: early-onset and recurrent severe infections, chronic diarrhea and growth impairment(44, 45). Hypermetabolism is more common in SCID patients with growth impairment and may contribute to its development(46). As a matter of fact, all affected infants will have a fatal outcome unless specific measurements to contain the situation are taken, such as: strict isolation and prophylactic antibiotics(44).

To conclude, early diagnosis of SCID before any clinical symptom or serious complication is proven to improve the survival rates after the HSCT(29, 47). The results of these studies show that children who have done the HSCT at the age of 3,5 months or younger and without a history of infection have 90-94% of survival rate, in 5 years; children that have undergone HSCT after 3,5 months of life have 69-90% of survival rate, in 5 years, if they have no history of infection or the infection was resolved by the time of

transplantation; children older than 3,5 months and with an active infection during HSCT have 50% of survival rate, in 5 years(29, 47).

Screening

The importance of screening for SCID is that if the diagnose is not done before the asymptomatic stage, the prognosis is dismal. This way, the screening for SCID can reduce the morbidity and mortality associated with it(48). As presented previously, SCID fulfils the requirements, proposed by WHO, to be a part of a population-based neonatal screening(49).

Effectively, there are already numerous studies showing that the inclusion of SCID in newborn screening programs fills in the requirements by WHO: is asymptomatic at birth; treatment is more effective as soon as precocious as it is possible, preventing the rate of infections and other complications; it helps in genetic counselling and pre-birth diagnose; and, it allows to know the prevalence and the spectrum of SCID(25, 49-52).

In 2007, Puck verified that children with family history of SCID were being diagnosed more precociously (the average age of diagnosis was 2 months) and lead to a survival rate of 100% when compared to the group of children that was diagnosed just after the appearance of the first symptoms related to SCID(53). In this study, from the 32 children that were diagnosed with SCID after the appearance of the first symptoms: 8 died before they received any treatment; from the 24 that received appropriate treatment only 14 survived, presenting a 44% rate of survival amongst the children with SCID and that do not have family history(53).

In 2013, Kwan et al. presented their results after including SCID in the newborn screening program in California State(54). In this study, the authors verified that SCID and other diseases with a number of lymphocytes lower than 1500 T cells/μL had a higher prevalence than half of the diseases that were part of the newborn screening program in USA(54).

After the results presented by Puck and the proposal for the introduction of SCID in the newborn screening in some states from the USA, there were several pilot studies worldwide. In Europe, more specifically, pilot studies were done in the United Kingdom, France, Spain, Sweden, Netherlands, old Serbia-Montenegro and Germany(55-61). Worthy of notice is the fact that in all these studies, they have reached a common conclusion: the sooner the diagnosis of SCID, better the prognosis after applying the appropriate treatment and fewer were the complications after the treatment.

In 2018, the screening of SCID was already included in the newborn screening program in all states of the USA, in the Netherlands, in Norway, in the region of Catalonia, in Israel and in Taiwan (Jeffrey Modell Foundation, http://www.info4pi.org).(8, 62)

Available Screening Tests

Since the scientific community thought of including SCID in the newborn screening, there have already been presented many screening tests: absolute count of lymphocytes(25, 63-65); detection of proteins from T cells CD3 and leukocytes markers CD45 based on the ligation with antibodies(66); an immunoassay with interleukin-7(53, 67); genetic mapping(68); and, the quantification of T-cell receptor excision circles (TREC)(50).

The proposal of using the absolute lymphocyte count considers that typical forms of SCID present lymphopenia due to reduced number of T cells(63-65). In these studies, it was proposed that a value of lymphocytes inferior of 2500 cells/µl would be suspicious of SCID(63, 65). After that, a flow cytometry would be performed in order to determine the presence of T, B and NK cells, the quantity of T cells receptors and the response of the T cells to mitogen or antigen stimulations to confirm the diagnosis(65). Despite the good results from this test, this technique has low sensibility, so it misdiagnoses patients with high levels of B cells and possibly NK cells or patients with residual, autoreactive (e.g. Omen syndrome) or alloreactive (transplacentally acquired maternal cells) T lymphocytes(65). To conclude, this test is not useful to be used as a screening test for SCID, specially, because of the overlap between healthy infants and infants with SCID(44).

In 2010, Janik et al. developed a study to evaluate the applicability of a multiple immunoassay (CD345) as a method of detection of SCID in newborns(66). In this assay, they used dried blood samples (DBS)(66). CD3 was utilized as a marker for T cells and CD45 as a marker for lymphocytes in general(66). The results were positive, however due to the possibility that not all newborns with SCID present lymphopenia, this test could not be used as screening, it could just be used as a complementary test to screen SCID(66).

McGee, Stiehm, Cowan, Krogstad and McCabe, in 2005, studied the use of interleukin-7 (IL-7) as a screening test for SCID, using dried blood samples(67). The IL-7 is a cytokine involved in the development of the T cells. In this study, the sensibility of the IL-7 was estimated to be 85% for this screening method, so this method was cleared as a possibility for a population-based screening for SCID(67).

Another possibility that was studied as a screening method for SCID was genetic mapping using the Guthrie cards. This technique is used in some screenings such as the one for cystic fibrosis, but in terms of SCID, not all the DNA sequence variants are thought to have been discovered(68). Despite that, Lebet et al., in 2008, published a study where a technique of resequencing capable of detecting known and unknown SCID variants (developed by Puck and Warrington) was being used (69). This technique was able to

identify 90% of all known mutations as well as previously unknown ones in prospective SCID cases(69). To conclude, this method would leave a lot of false negative cases and that is why it is not a good screening method(68).

In 2005, Chan and Puck described the utility of DBS (also known as Guthrie cards) for the detection of TREC by quantity polymerase-chain reaction (PCR) techniques as a screening method (50). The TREC molecules are fragments of DNA excised from the T-cell receptor (TCR) gene during the development of mature lymphocytes inside the thymus(44, 68, 70, 71). As all infants with SCID have reduced T lymphocyte development, they have low numbers of T cells derived from the thymus and consequently very low or absent TREC values, compared to the normal infants(70, 72). As TREC are stable in the peripheral circulation, the isolation of DNA by PCR from DBS can be used to quantify TREC in a population-based screening(11, 68, 72). The validity of this test has been demonstrated following the introduction of SCID screening in the USA in 2008(54, 73, 74).

The TREC quantification using reverse transcription for real-time quantitative polymerase chain reaction (RTqPCR) is the one that has been showing better results using peripheral blood(44, 50, 75). Nevertheless, despite the fact that this method requires a blood sample from the infants, the manual extraction of the DNA, the use of the RTqPCR technic, genetic control, the analysis of the data and, initially, the difficulties to implement this test as a screening method(49, 66, 76); the scientific community has accepted it very well as it presents a lot of advantages: the possibility of using the Guthrie cards (already used for the newborn screening of other diseases), the low cost, the elevated efficiency and sensibility to detect the cases of SCID, even in newborn infants that have high levels of maternal T cells in peripheral blood(68, 77). In addition to typical SCID, Omenn Syndrome and non-SCID immunodeficiencies associated with marked T cells lymphopenia may be detected by the TREC screening test including: partial DiGeorge syndrome, CHARGE (coloboma, heart anomalies, choanal atresia, retardation of growth and development, and genital and ear anomalies) syndrome, cartilage–hair hypoplasia (CHH) and ataxia telangiectasia(77-82). On the other hand, the TREC method has one potential limitation: inability to detect some cases of SCID due to combined immunodeficiencies associated with gene defects that affect the development later than the T-cell receptor recombination or associated with impaired T-cell signalling, survival or proliferation(79, 82-84). Examples of this are the adenosine deaminase (ADA) with delayed onset disease, ZAP-70 deficiency and major histocompatibility complex (MHC) class II deficiency (79, 82-84).

Nowadays, in order to detect primary immunodeficiencies, it is possible to add to the TREC essay the analysis of the kappa-deleting recombination excision circles (KREC), which is a sensitive marker of B cells development, increasing the rate of

immunodeficiencies diagnosis that are associated with low numbers of B lymphocytes (such as: late onset ADA deficiency, Nijmegen-breakage syndrome, X-linked agammaglobulinemia (XLA) and purine nucleoside phosphorylase (PNP) deficiency)(85-87). To conclude, the combined assay (TREC and KREC) has been suggested to be included in routine screening for primary immunodeficiency (11, 12, 86, 88).

In practice, the TREC method measures the quantity of TREC available in the blood samples and, also, β-actin, as an internal control for DNA amplification. In cases which β-actin measure is too low (Adams et al. proposed a β-actin cut-off of 35 copies/μL) re-tests must be run to validate the results, independently of the values of TREC in that sample(56). According to a recent systematic review done by van der Speck, Groenwold, van der Burg and van Montfrans, a TREC cut-off of 25 copies/μL may be an appropriate initial value for implementation of routine newborn screening for SCID(7). In addition to this, when analysing the results and the cut-off used in this test, it must be considered that premature infants, infants with trisomy 21 and infants whose mother has taken immunosuppressive agents during pregnancy have lower TREC values compared to term and healthy infants, without needing to have any of the diseases that cause low TREC values (7, 54, 60, 70, 89). To conclude, the possible results from the TREC essay are four: presumptive cases with TREC copies/μL below the cut-off, whose cases need an immediate confirmatory test (flow cytometry is recommended); borderline cases with TREC copies/μL at the cut-off level, for which a second screening test should be performed; normal results with TREC copies/μL higher than the cut-off; and, inconclusive results with an unsatisfactory level of β-actin cut-off lower than the cut-off(90).

This method already allowed to detect more than 100 cases of SCID and, according to all published studies, the screening programmes that use TREC as a measure for identifying the cases of typical and atypical SCID have never left any case undetected or lately detected(7, 8, 79).

According to Chan et al., the sensibility of the TREC test using the RTqPCR technic is estimated to be 99,5%(91); although, there have been no known missed cases(91). Kwan et al., estimated this same test to have a sensibility of 99,97%(54).

In Portugal

In 2016, the Portuguese Group for the Primary Immunodeficiencies presented a national and multicentre retrospective analysis for all know cases of SCID in Portugal since the year 2000(92). This group of researchers concluded that the incidence of SCID is 1 in 48.775 newborn; the average date for the first clinical manifestations is at 4,1 months; the average date between the first clinical manifestations and the diagnose is 2,5 months, which makes the average age of diagnosis 6,6 months; and, the mortality is 60%(92).

The Portuguese Group for Primary Immunodeficiencies presented this data in order to compare with the statistics from countries that have already included SCID screening in their neonatal programs(92). It was concluded that the incidence, despite being superior than most of the published studies, it is under-estimated and that the diagnosis is done so late that 37% of the infants die before the HSCT(92). These conclusions reinforce the need for the implementation of the screening for SCID in most of the neonatal screening programs all over the World.

Cost-Effectiveness

In spite of the efficiency of the tests, the inclusion of a disease in a population-based screening program must be analysed in terms of cost-effectiveness.

In 2016, Ding et al. proposed a model to estimate the cost and the benefits of the newborn screening program using the TREC method to detect SCID(90). For that, the authors modified the model structure from a cost-benefit analysis model developed during 2011-2012 by the Washington Newborn Screening program and used the data available from previous cost-benefit reviews and, also, the cohort from the Washington Newborn Screening program(90).

The incremental costs of adding SCID to a screening program (with the laboratory test and administrative costs), cost of diagnostic test (flow cytometry), costs of the treatment for early-diagnosed or late-diagnosed SCID infants and costs of deaths are specified in Table 3(90). The costs of individual sample collection and transport are not affected by this screening, as this test uses the Guthrie cards already used for other screenings(90). Ding et al. adjusted these values to 2012 USA dollars using the healthcare component of the Personal Consumption Expenditures Price Indexes(90).

Table 3. Model variables and ranges

Variables	**Base-case**	**Range/Alternative**	**References**
Birth prevalence of SCID	1/58.000	1/46.000-1/80.000	(8)
Proportion of SCID cases detected without the screening	0,203		(25, 93)
Sensitivity of the TREC method	99,50%	99,00%-100,00%	(8, 91)
Specificity of the TREC method	99,97%	99,92%-99,98%	(54, 94)
Survival rate			
For early-identified SCID (pretreatment)	94%		(8)
For early-identified SCID (posttreatment)	94%		(8, 25, 95)
For late-identified SCID (pretreatment)	78%		(91, 93)
For late-identified SCID (posttreatment)	69%		(47, 72)
Costs of screening and diagnosis			
Lab tests for TREC assay sample	$4,04	$3,00-$6,00	(90)
Short-term follow-up per positive case	$50,00		(90)
Flow cytometry per case	$250,0		(90, 91)
Costs of treatment			
Average cost per infant with SCID who die before definitive treatment	$300.000		(90)
Average cost per infant with ADA-SCID who do not undergo early HSCT	$450.000	$200.000-$750.000	(90)
Average cost for infants with SCID who			

receive HSCT as first-line therapy			
Per early-idenitifed baby	$100.000	$80.000-$120.000	(72, 91, 96)
Per late-identified baby	$450.000	$300-000-$1.200.000	(72, 90, 96)

Adapted from Ding et al. (90)

Conclusion

SCID is a primary immunodeficiency that brings enormous implications to the quality of life from the affected infants and their life expectancy. As referred, if the diagnosis of the syndrome is not done before the first symptoms, life expectancy diminishes considerably when compared to patients that are diagnosed in the first three months of life. In addition to this, the TREC test using the RTqPCR technic has already been proven to be a very sensible and specific test, without excluding any case of SCID. That is why this test is already used in some countries as a population-based screening. Furthermore, the TREC test has proven to have good cost-effectiveness.

To conclude, this review brings up the important facts related to the screening of SCID, supporting the idea that screening SCID using the TREC technique in the newborn programs should be included worldwide, as it improves the quality of life, expectancy of life and diminish the expenses in health.

Bibliography

1. Al-Herz W, Bousfiha A, Casanova JL, Chatila T, Conley ME, Cunningham-Rundles C, et al. Primary immunodeficiency diseases: an update on the classification from the international union of immunological societies expert committee for primary immunodeficiency. Frontiers in immunology. 2014;5:162.
2. Picard C, Al-Herz W, Bousfiha A, Casanova JL, Chatila T, Conley ME, et al. Primary Immunodeficiency Diseases: an Update on the Classification from the International Union of Immunological Societies Expert Committee for Primary Immunodeficiency 2015. Journal of clinical immunology. 2015;35(8):696-726.
3. Bonilla FA, Khan DA, Ballas ZK, Chinen J, Frank MM, Hsu JT, et al. Practice parameter for the diagnosis and management of primary immunodeficiency. The Journal of allergy and clinical immunology. 2015;136(5):1186-205.e1-78.
4. Bonilla FA. Severe combined immunodeficiency (SCID): An overview. 2017 October 12, 2017. In: UpToDate [Internet]. Available from: https://www.uptodate.com/contents/severe-combined-immunodeficiency-scid-an-overview?search=severe+combined+immunodeficiency&source=search_result&selectedTitle=1%7E127.
5. Cirillo E, Giardino G, Gallo V, D'Assante R, Grasso F, Romano R, et al. Severe combined immunodeficiency--an update. Annals of the New York Academy of Sciences. 2015;1356:90-106.
6. Griffith LM, Cowan MJ, Notarangelo LD, Kohn DB, Puck JM, Shearer WT, et al. Primary Immune Deficiency Treatment Consortium (PIDTC) update. The Journal of allergy and clinical immunology. 2016;138(2):375-85.
7. van der Spek J, Groenwold RH, van der Burg M, van Montfrans JM. TREC Based Newborn Screening for Severe Combined Immunodeficiency Disease: A Systematic Review. Journal of clinical immunology. 2015;35(4):416-30.
8. 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(7):729-38.
9. Li L, Moshous D, Zhou Y, Wang J, Xie G, Salido E, et al. A founder mutation in Artemis, an SNM1-like protein, causes SCID in Athabascan-speaking Native Americans. Journal of immunology (Baltimore, Md : 1950). 2002;168(12):6323-9.
10. Kwan A, Hu D, Song M, Gomes H, Brown DR, Bourque T, et al. Successful newborn screening for SCID in the Navajo Nation. Clinical immunology (Orlando, Fla). 2015;158(1):29-34.
11. Gaspar HB, Hammarstrom L, Mahlaoui N, Borte M, Borte S. The case for mandatory newborn screening for severe combined immunodeficiency (SCID). Journal of clinical immunology. 2014;34(4):393-7.
12. Buelow BJ, Routes JM, Verbsky JW. Newborn screening for SCID: where are we now? Expert review of clinical immunology. 2014;10(12):1649-57.
13. Reisner Y, Kapoor N, Kirkpatrick D, Pollack MS, Cunningham-Rundles S, Dupont B, et al. Transplantation for severe combined immunodeficiency with HLA-A,B,D,DR incompatible parental marrow cells fractionated by soybean agglutinin and sheep red blood cells. Blood. 1983;61(2):341-8.
14. Friedrich W, Goldmann SF, Ebell W, Blutters-Sawatzki R, Gaedicke G, Raghavachar A, et al. Severe combined immunodeficiency: treatment by bone marrow transplantation in 15 infants using HLA-haploidentical donors. European journal of pediatrics. 1985;144(2):125-30.
15. Buckley RH, Schiff SE, Sampson HA, Schiff RI, Markert ML, Knutsen AP, et al. Development of immunity in human severe primary T cell deficiency following haploidentical bone marrow stem cell transplantation. Journal of immunology (Baltimore, Md : 1950). 1986;136(7):2398-407.
16. O'Reilly RJ, Brochstein J, Collins N, Keever C, Kapoor N, Kirkpatrick D, et al. Evaluation of HLA-haplotype disparate parental marrow grafts depleted of T lymphocytes by differential agglutination with a soybean lectin and E-rosette depletion

for the treatment of severe combined immunodeficiency. Vox sanguinis. 1986;51 Suppl 2:81-6.
17. Moen RC, Horowitz SD, Sondel PM, Borcherding WR, Trigg ME, Billing R, et al. Immunologic reconstitution after haploidentical bone marrow transplantation for immune deficiency disorders: treatment of bone marrow cells with monoclonal antibody CT-2 and complement. Blood. 1987;70(3):664-9.
18. O'Reilly RJ, Keever CA, Small TN, Brochstein J. The use of HLA-non-identical T-cell-depleted marrow transplants for correction of severe combined immunodeficiency disease. Immunodeficiency reviews. 1989;1(4):273-309.
19. Wijnaendts L, Le Deist F, Griscelli C, Fischer A. Development of immunologic functions after bone marrow transplantation in 33 patients with severe combined immunodeficiency. Blood. 1989;74(6):2212-9.
20. Fischer A, Landais P, Friedrich W, Morgan G, Gerritsen B, Fasth A, et al. European experience of bone-marrow transplantation for severe combined immunodeficiency. Lancet (London, England). 1990;336(8719):850-4.
21. Dror Y, Gallagher R, Wara DW, Colombe BW, Merino A, Benkerrou M, et al. Immune reconstitution in severe combined immunodeficiency disease after lectin-treated, T-cell-depleted haplocompatible bone marrow transplantation. Blood. 1993;81(8):2021-30.
22. 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. The Journal of pediatrics. 1993;123(4):564-72.
23. Giri N, Vowels M, Ziegler JB, Ford D, Lam-Po-Tang R. HLA non-identical T-cell-depleted bone marrow transplantation for primary immunodeficiency diseases. Australian and New Zealand journal of medicine. 1994;24(1):26-30.
24. Buckley RH, Schiff SE, Schiff RI, Markert L, Williams LW, Roberts JL, et al. Hematopoietic stem-cell transplantation for the treatment of severe combined immunodeficiency. The New England journal of medicine. 1999;340(7):508-16.
25. 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.
26. Antoine C, Muller S, Cant A, Cavazzana-Calvo M, Veys P, Vossen J, et al. Long-term survival and transplantation of haemopoietic stem cells for immunodeficiencies: report of the European experience 1968-99. Lancet (London, England). 2003;361(9357):553-60.
27. Buckley RH. Molecular Defects in Human Severe Combined Immunodeficiency and Approaches to Immune Reconstitution. Annual Review of Immunology. 2004;22(1):625-55.
28. Buckley R, Fischer A. Bone marrow transplantation for primary immunodeficiency diseases. Primary immunodeficiency diseases: a molecular and genetic approach. 1999;2:669-83.
29. Buckley RH. Transplantation of hematopoietic stem cells in human severe combined immunodeficiency: longterm outcomes. Immunologic research. 2011;49(1-3):25-43.
30. Guthrie R, Susi A. A Simple Phenylalanine Method for Detecting Phenylketonuria in Large Populations of Newborn Infants. Pediatrics. 1963;32:338-43.
31. Wilson JM, Jungner YG. [Principles and practice of mass screening for disease]. Boletin de la Oficina Sanitaria Panamericana Pan American Sanitary Bureau. 1968;65(4):281-393.
32. Andermann A, Blancquaert I, Beauchamp S, Dery V. Revisiting Wilson and Jungner in the genomic age: a review of screening criteria over the past 40 years. Bulletin of the World Health Organization. 2008;86(4):317-9.
33. Burgard P, Rupp K, Lindner M, Haege G, Rigter T, Weinreich SS, et al. Newborn screening programmes in Europe; arguments and efforts regarding harmonization. Part 2. From screening laboratory results to treatment, follow-up and quality assurance. Journal of inherited metabolic disease. 2012;35(4):613-25.

34. Petros M. Revisiting the Wilson-Jungner criteria: how can supplemental criteria guide public health in the era of genetic screening? Genetics in medicine : official journal of the American College of Medical Genetics. 2012;14(1):129-34.
35. Bonilla FA. Severe combined immunodeficiency (SCID): Specific defects. 2017 October 12, 2017. In: UpToDate [Internet]. Available from: https://www.uptodate.com/contents/severe-combined-immunodeficiency-scid-an-overview?search=severe+combined+immunodeficiency&source=search_result&selectedTitle=1%7E127.
36. Bousfiha A, Jeddane L, Picard C, Ailal F, Bobby Gaspar H, Al-Herz W, et al. The 2017 IUIS Phenotypic Classification for Primary Immunodeficiencies. Journal of clinical immunology. 2018;38(1):129-43.
37. Henderson LA, Frugoni F, Hopkins G, Al-Herz W, Weinacht K, Comeau AM, et al. First reported case of Omenn syndrome in a patient with reticular dysgenesis. The Journal of allergy and clinical immunology. 2013;131(4):1227-30, 30.e1-3.
38. Ban SA, Salzer E, Eibl MM, Linder A, Geier CB, Santos-Valente E, et al. Combined immunodeficiency evolving into predominant CD4+ lymphopenia caused by somatic chimerism in JAK3. Journal of clinical immunology. 2014;34(8):941-53.
39. Patiroglu T, Haluk Akar H, van den Burg M, Unal E, Akyildiz BN, Tekerek NU, et al. X-linked severe combined immunodeficiency due to a novel mutation complicated with hemophagocytic lymphohistiocytosis and presented with invagination: A case report. European journal of microbiology & immunology. 2014;4(3):174-6.
40. Slatter MA, Angus B, Windebank K, Taylor A, Meaney C, Lester T, et al. Polymorphous lymphoproliferative disorder with Hodgkin-like features in common gamma-chain-deficient severe combined immunodeficiency. The Journal of allergy and clinical immunology. 2011;127(2):533-5.
41. Schuetz C, Huck K, Gudowius S, Megahed M, Feyen O, Hubner B, et al. An immunodeficiency disease with RAG mutations and granulomas. The New England journal of medicine. 2008;358(19):2030-8.
42. Moshous D, Martin E, Carpentier W, Lim A, Callebaut I, Canioni D, et al. Whole-exome sequencing identifies Coronin-1A deficiency in 3 siblings with immunodeficiency and EBV-associated B-cell lymphoproliferation. The Journal of allergy and clinical immunology. 2013;131(6):1594-603.
43. Pignata C, D'Agostino A, Finelli P, Fiore M, Scotese I, Cosentini E, et al. Progressive deficiencies in blood T cells associated with a 10p12-13 interstitial deletion. Clinical immunology and immunopathology. 1996;80(1):9-15.
44. Somech R, Etzioni A. A call to include severe combined immunodeficiency in newborn screening program. Rambam Maimonides medical journal. 2014;5(1):e0001.
45. Griffith LM, Cowan MJ, Notarangelo LD, Puck JM, Buckley RH, Candotti F, et al. Improving cellular therapy for primary immune deficiency diseases: recognition, diagnosis, and management. The Journal of allergy and clinical immunology. 2009;124(6):1152-60.e12.
46. Barron MA, Makhija M, Hagen LE, Pencharz P, Grunebaum E, Roifman CM. Increased resting energy expenditure is associated with failure to thrive in infants with severe combined immunodeficiency. The Journal of pediatrics. 2011;159(4):628-32.e1.
47. Pai SY, Logan BR, Griffith LM, Buckley RH, Parrott RE, Dvorak CC, et al. Transplantation outcomes for severe combined immunodeficiency, 2000-2009. The New England journal of medicine. 2014;371(5):434-46.
48. Barbouche MR, Galal N, Ben-Mustapha I, Jeddane L, Mellouli F, Ailal F, et al. Primary immunodeficiencies in highly consanguineous North African populations. Annals of the New York Academy of Sciences. 2011;1238:42-52.
49. Puck JM. The case for newborn screening for severe combined immunodeficiency and related disorders. Annals of the New York Academy of Sciences. 2011;1246:108-17.
50. Chan K, Puck JM. Development of population-based newborn screening for severe combined immunodeficiency. The Journal of allergy and clinical immunology. 2005;115(2):391-8.

51. Champi C. Primary immunodeficiency disorders in children: prompt diagnosis can lead to lifesaving treatment. Journal of pediatric health care : official publication of National Association of Pediatric Nurse Associates & Practitioners. 2002;16(1):16-21.
52. Newborn screening: toward a uniform screening panel and system--executive summary. Pediatrics. 2006;117(5 Pt 2):S296-307.
53. Puck JM. Population-based newborn screening for severe combined immunodeficiency: steps toward implementation. The Journal of allergy and clinical immunology. 2007;120(4):760-8.
54. Kwan A, Church JA, Cowan MJ, Agarwal R, Kapoor N, Kohn DB, et al. Newborn screening for severe combined immunodeficiency and T-cell lymphopenia in California: results of the first 2 years. The Journal of allergy and clinical immunology. 2013;132(1):140-50.
55. de Pagter AP, Bredius RG, Kuijpers TW, Tramper J, van der Burg M, van Montfrans J, et al. Overview of 15-year severe combined immunodeficiency in the Netherlands: towards newborn blood spot screening. European journal of pediatrics. 2015;174(9):1183-8.
56. Adams SP, Rashid S, Premachandra T, Harvey K, Ifederu A, Wilson MC, et al. Screening of neonatal UK dried blood spots using a duplex TREC screening assay. Journal of clinical immunology. 2014;34(3):323-30.
57. Audrain M, Thomas C, Mirallie S, Bourgeois N, Sebille V, Rabetrano H, et al. Evaluation of the T-cell receptor excision circle assay performances for severe combined immunodeficiency neonatal screening on Guthrie cards in a French single centre study. Clinical immunology (Orlando, Fla). 2014;150(2):137-9.
58. Pasic S, Vujic D, Veljkovic D, Slavkovic B, Mostarica-Stojkovic M, Minic P, et al. Severe combined immunodeficiency in Serbia and Montenegro between years 1986 and 2010: a single-center experience. Journal of clinical immunology. 2014;34(3):304-8.
59. Tagliaferri L, Kunz JB, Happich M, Esposito S, Bruckner T, Hubschmann D, et al. Newborn screening for severe combined immunodeficiency using a novel and simplified method to measure T-cell excision circles (TREC). Clinical immunology (Orlando, Fla). 2017;175:51-5.
60. Barbaro M, Ohlsson A, Borte S, Jonsson S, Zetterstrom RH, King J, et al. Newborn Screening for Severe Primary Immunodeficiency Diseases in Sweden-a 2-Year Pilot TREC and KREC Screening Study. Journal of clinical immunology. 2017;37(1):51-60.
61. de Felipe B, Olbrich P, Lucenas JM, Delgado-Pecellin C, Pavon-Delgado A, Marquez J, et al. Prospective neonatal screening for severe T- and B-lymphocyte deficiencies in Seville. Pediatric allergy and immunology : official publication of the European Society of Pediatric Allergy and Immunology. 2016;27(1):70-7.
62. Blom M, Pico-Knijnenburg I, Sijne-van Veen M, Boelen A, Bredius RGM, van der Burg M, et al. An evaluation of the TREC assay with regard to the integration of SCID screening into the Dutch newborn screening program. Clinical immunology (Orlando, Fla). 2017;180:106-10.
63. 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. The Journal of pediatrics. 1997;130(3):378-87.
64. Gossage DL, Buckley RH. Prevalence of lymphocytopenia in severe combined immunodeficiency. The New England journal of medicine. 1990;323(20):1422-3.
65. Amariglio N, Lev A, Simon A, Rosenthal E, Spirer Z, Efrati O, et al. Molecular assessment of thymus capabilities in the evaluation of T-cell immunodeficiency. Pediatric research. 2010;67(2):211-6.
66. Janik DK, Lindau-Shepard B, Comeau AM, Pass KA. A multiplex immunoassay using the Guthrie specimen to detect T-cell deficiencies including severe combined immunodeficiency disease. Clinical chemistry. 2010;56(9):1460-5.
67. McGhee SA, Stiehm ER, Cowan M, Krogstad P, McCabe ER. Two-tiered universal newborn screening strategy for severe combined immunodeficiency. Molecular genetics and metabolism. 2005;86(4):427-30.

68. Puck JM. Laboratory technology for population-based screening for severe combined immunodeficiency in neonates: the winner is T-cell receptor excision circles. The Journal of allergy and clinical immunology. 2012;129(3):607-16.
69. Lebet T, Chiles R, Hsu AP, Mansfield ES, Warrington JA, Puck JM. Mutations causing severe combined immunodeficiency: detection with a custom resequencing microarray. Genetics in medicine : official journal of the American College of Medical Genetics. 2008;10(8):575-85.
70. Puck JM. Neonatal screening for severe combined immunodeficiency. Current opinion in pediatrics. 2011;23(6):667-73.
71. Somech R. T-cell receptor excision circles in primary immunodeficiencies and other T-cell immune disorders. Current opinion in allergy and clinical immunology. 2011;11(6):517-24.
72. Buckley RH. The long quest for neonatal screening for severe combined immunodeficiency. The Journal of allergy and clinical immunology. 2012;129(3):597-604; quiz 5-6.
73. Vogel BH, Bonagura V, Weinberg GA, Ballow M, Isabelle J, DiAntonio L, et al. Newborn screening for SCID in New York State: experience from the first two years. Journal of clinical immunology. 2014;34(3):289-303.
74. Baker MW, Laessig RH, Katcher ML, Routes JM, Grossman WJ, Verbsky J, et al. Implementing routine testing for severe combined immunodeficiency within Wisconsin's newborn screening program. Public health reports (Washington, DC : 1974). 2010;125 Suppl 2:88-95.
75. Baker MW, Grossman WJ, Laessig RH, Hoffman GL, Brokopp CD, Kurtycz DF, et al. Development of a routine newborn screening protocol for severe combined immunodeficiency. The Journal of allergy and clinical immunology. 2009;124(3):522-7.
76. Clement MC, Mahlaoui N, Mignot C, Le Bihan C, Rabetrano H, Hoang L, et al. Systematic neonatal screening for severe combined immunodeficiency and severe T-cell lymphopenia: Analysis of cost-effectiveness based on French real field data. The Journal of allergy and clinical immunology. 2015;135(6):1589-93.
77. Morinishi Y, Imai K, Nakagawa N, Sato H, Horiuchi K, Ohtsuka Y, et al. Identification of severe combined immunodeficiency by T-cell receptor excision circles quantification using neonatal guthrie cards. The Journal of pediatrics. 2009;155(6):829-33.
78. Villa A, Notarangelo LD, Roifman CM. Omenn syndrome: inflammation in leaky severe combined immunodeficiency. The Journal of allergy and clinical immunology. 2008;122(6):1082-6.
79. Kwan A, Puck JM. History and current status of newborn screening for severe combined immunodeficiency. Seminars in perinatology. 2015;39(3):194-205.
80. Lingman Framme J, Borte S, von Dobeln U, Hammarstrom L, Oskarsdottir S. Retrospective analysis of TREC based newborn screening results and clinical phenotypes in infants with the 22q11 deletion syndrome. Journal of clinical immunology. 2014;34(4):514-9.
81. Mallott J, Kwan A, Church J, Gonzalez-Espinosa D, Lorey F, Tang LF, et al. Newborn screening for SCID identifies patients with ataxia telangiectasia. Journal of clinical immunology. 2013;33(3):540-9.
82. Verbsky J, Routes J. Screening for and treatments of congenital immunodeficiency diseases. Clinics in perinatology. 2014;41(4):1001-15.
83. Grazioli S, Bennett M, Hildebrand KJ, Vallance H, Turvey SE, Junker AK. Limitation of TREC-based newborn screening for ZAP70 Severe Combined Immunodeficiency. Clinical immunology (Orlando, Fla). 2014;153(1):209-10.
84. Kuo CY, Chase J, Garcia Lloret M, Stiehm ER, Moore T, Aguilera MJ, et al. Newborn screening for severe combined immunodeficiency does not identify bare lymphocyte syndrome. The Journal of allergy and clinical immunology. 2013;131(6):1693-5.

85. Speckmann C, Neumann C, Borte S, la Marca G, Sass JO, Wiech E, et al. Delayed-onset adenosine deaminase deficiency: strategies for an early diagnosis. The Journal of allergy and clinical immunology. 2012;130(4):991-4.
86. Borte S, von Dobeln U, Fasth A, Wang N, Janzi M, Winiarski J, et al. Neonatal screening for severe primary immunodeficiency diseases using high-throughput triplex real-time PCR. Blood. 2012;119(11):2552-5.
87. la Marca G, Canessa C, Giocaliere E, Romano F, Malvagia S, Funghini S, et al. Diagnosis of immunodeficiency caused by a purine nucleoside phosphorylase defect by using tandem mass spectrometry on dried blood spots. The Journal of allergy and clinical immunology. 2014;134(1):155-9.
88. Chiarini M, Zanotti C, Serana F, Sottini A, Bertoli D, Caimi L, et al. T-cell Receptor and K-deleting Recombination Excision Circles in Newborn Screening of T- and B-cell Defects: Review of the Literature and Future Challenges. Journal of public health research. 2013;2(1):9-16.
89. Verstegen RH, Borte S, Bok LA, van Zwieten PH, von Dobeln U, Hammarstrom L, et al. Impact of Down syndrome on the performance of neonatal screening assays for severe primary immunodeficiency diseases. The Journal of allergy and clinical immunology. 2014;133(4):1208-11.
90. Ding Y, Thompson JD, Kobrynski L, Ojodu J, Zarbalian G, Grosse SD. Cost-Effectiveness/Cost-Benefit Analysis of Newborn Screening for Severe Combined Immune Deficiency in Washington State. The Journal of pediatrics. 2016;172:127-35.
91. Chan K, Davis J, Pai SY, Bonilla FA, Puck JM, Apkon M. A Markov model to analyze cost-effectiveness of screening for severe combined immunodeficiency (SCID). Molecular genetics and metabolism. 2011;104(3):383-9.
92. Esteves I, Pinto S, Cordeiro A, Caetano A, Figueiredo A, Vitor AB, et al. Casuística Nacional. Meeting. VII Reunião de Imunodeficienências Primárias: GPIP; 2016 26 of February from 2016.
93. Chan A, Scalchunes C, Boyle M, Puck JM. Early vs. delayed diagnosis of severe combined immunodeficiency: a family perspective survey. Clinical immunology (Orlando, Fla). 2011;138(1):3-8.
94. 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. The Journal of allergy and clinical immunology. 2010;126(5):1073-4.
95. Brown L, Xu-Bayford J, Allwood Z, Slatter M, Cant A, Davies EG, et al. Neonatal diagnosis of severe combined immunodeficiency leads to significantly improved survival outcome: the case for newborn screening. Blood. 2011;117(11):3243-6.
96. Kubiak C, Jyonouchi S, Kuo C, Garcia-Lloret M, Dorsey MJ, Sleasman J, et al. Fiscal implications of newborn screening in the diagnosis of severe combined immunodeficiency. The journal of allergy and clinical immunology In practice. 2014;2(6):697-702.