Neonatal Screening for Severe Combined Immunodeficiency

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Abstract

Severe Combined Immunodeficiency (SCID) is the most severe form of primary immunodeficiency. This syndrome is caused by genetic mutations that block the development of T cells. 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 analyzing all the advantages and disadvantages of the implementation of SCID testing in the neonatal screening programs. The presentation of SCID, the available screening methods to detect the disease and the cost effectiveness related to the screening are also presented. The search engine used to conduct this review was PubMed. 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.

Keywords: Neonatal screening; Genetic mutations; Stem cell transplantation; Severe combined immunodeficiency; Lymphopenia; Prognosis

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, Genital and Ear Anomalies; CHH-Cartilage-Hair Hypoplasia; DSB-Dried Blood Samples; EBV-Epstein-Barr Virus; HSC/Thematopoietic 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

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 new-born 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 January 2005 and 31st December 2017.

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of January of 2003 and 31st 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 new-born 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 new-born 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 was 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 and 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.

Andermann et al. 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 new-born 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.

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]. 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 [5,35]. In Table 1, is presented the classification of the mutation according to the pathogenetic mechanism and the genotype and Table 2 shows the clinical features associated with the genetic defect of some SCID [5,35-42] (Tables 1 and 2).

In fact, regardless of the immunological phenotype, infants

<table>
<thead>
<tr>
<th>Pathogenetic mechanism</th>
<th>Gene mutated</th>
<th>Immunophenotype</th>
<th>Inheritance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Defective survival of haematopoietic precursors</td>
<td>AK2</td>
<td>T B NK'</td>
<td>AR</td>
</tr>
<tr>
<td>Toxic metabolite accumulation</td>
<td>ADA</td>
<td>T B NK'</td>
<td>AR</td>
</tr>
<tr>
<td>IL2RG</td>
<td>T B NK'</td>
<td>AR</td>
<td></td>
</tr>
<tr>
<td>Cytokine signalling anomalies</td>
<td>IL7R</td>
<td>T B NK'</td>
<td>AR</td>
</tr>
<tr>
<td>V(D)J recombination and TCR abnormalities</td>
<td>RAG1/RAG2, DCLRE1C, PRKDC, NHEJ1, LIG4</td>
<td>T B NK'</td>
<td>AR</td>
</tr>
<tr>
<td>TCR abnormalities</td>
<td>PTPRC CD3D, CD3E, CD247</td>
<td>T B NK'</td>
<td>AR</td>
</tr>
<tr>
<td>Thymic abnormalities</td>
<td>FOXL1</td>
<td>T B NK'</td>
<td>AR</td>
</tr>
</tbody>
</table>

*AR: Autosomal Recessive transmission, XL: X-Linked transmission

Table 1: Pathogenetic mechanism of SCID [5].
with SCID present similar features as: Early-onset and recurrent severe infections, chronic diarrhea and growth impairment [43,44]. Hypermetabolism is more common in SCID patients with growth impairment and may contribute to its development [45]. 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 [43].

To conclude, early diagnosis of SCID before any clinical symptom or serious complication is proven to improve the survival rates after the HSCT [29,46]. The results of these studies show that children who have done the HSCT at the age of 3 mon or younger and without a history of infection have 90-94% of survival rate, in 5 years, children that have undergone HSCT after 3 mon 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 mon and with an active infection during HSCT have 50% of survival rate, in 5 years [29,46].

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 [47]. As presented previously, SCID fulfils the requirements, proposed by WHO, to be a part of a population-based neonatal screening [48].

Effectively, there are already numerous studies showing that the inclusion of SCID in new-born 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 [25,48-51].

Puck verified that children with family history of SCID were being diagnosed more precociously (the average age of diagnosis was 2 mon) 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 [52]. 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 [52].

Kwan et al. presented their results after including SCID in the new-born screening program in California State [53]. 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 new-born screening program in USA [53].

After the results presented by Puck and the proposal for the introduction of SCID in the new-born 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 [54-60]. 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.

The screening of SCID was already included in the new-born screening program in all states of the USA, in the Netherlands, in Norway, in the region of Catalonian, in Israel and in Taiwan (Jeffrey Modell Foundation) [8,61].

Available screening tests

Since the scientific community thought of including SCID in the new-born screening, there have already been presented many screening tests: Absolute count of lymphocytes, detection of proteins from T cells CD3 and leukocytes markers CD45 based on the ligation with antibodies, an immunoassay with interleukin-7, genetic mapping and the quantification of T-cell receptor excision circles (TREC) [25,49,52,62-67].

The proposal of using the absolute lymphocyte count considers that typical forms of SCID present lymphopenia due to reduced number of T cells [62-64]. In these studies, it was proposed that a value of lymphocytes inferior of 2500 cells/μl would be suspicious of SCID [62,64]. 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 [64]. 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, auto-reactive (e.g. Omen syndrome) or allo-reactive (transplacentally acquired maternal cells) T lymphocytes [64]. 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 [43].

Jank et al. developed a study to evaluate the applicability of a multiple immunoassay (CD345) as a method of detection of SCID in new-born [65]. In this assay, they used dried blood samples (DBS) [65]. CD3 was utilized as a marker for T cells and CD45 as a marker for lymphocytes in general [65]. The results were positive, however due to the possibility that not all new-born s with SCID present lymphopenia, this test could not be used as screening, it could just be used as a complementary test to screen SCID [65].
McGee et al. studied the use of interleukin-7 (IL-7) as a screening test for SCID, using dried blood samples [66]. The IL-7 is a cytokine involved in the development of the T cells. In this study, the sensitivity 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 [66].

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 [67]. Despite that, Lebet et al. published a study where a technique of re-sequencing capable of detecting known and unknown SCID variants (developed by Puck and Warrington) was being used [68]. This technique was able to identify 90% of all known mutations as well as previously unknown ones in prospective SCID cases [68]. To conclude, this method would leave a lot of false negative cases and that is why it is not a good screening method [68].

Chan et al. 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 [49]. The TREC molecules are fragments of DNA excised from the T-cell receptor (TCR) gene during the development of mature lymphocytes inside the thymus [43,67,69,70]. 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 [69,71]. 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 [11,67,71]. The validity of this test has been demonstrated following the introduction of SCID screening in the USA in 2008 [53,72,73].

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 [43,49,74]. 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, the scientific community has accepted it very well as it presents a lot of advantages: The possibility of using the Guthrie cards (already initially, the difficulties to implement this test as a screening method, the scientific community has accepted it very well as it presents a lot of advantages: The possibility of using the Guthrie cards (already

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The Portuguese Group for the Primary Immunodeficiencies presented a national and multicentre retrospective analysis for all known cases of SCID in Portugal since the year 2000 [91]. This group of researchers concluded that the incidence of SCID is 1 in 48,775 new-born, the average date for the first clinical manifestations is at 4 mon, the average date between the first clinical manifestations and the diagnose is 2 mon, which makes the average age of diagnosis 6 mon and the mortality is 60% [91].

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 [91]. 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 [91]. 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.
Ding et al. proposed a model to estimate the cost and the benefits of the new-born screening program using the TREC method to detect SCID [89]. For that, the authors modified the model structure from a cost-benefit analysis model developed during 2011-2012 by the Washington new-born screening program and used the data available from previous cost-benefit reviews and also, the cohort from the Washington New-born 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 [89]. 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 [89]. Ding et al. adjusted these values to 2012 USA dollars using the healthcare component of the Personal Consumption Expenditures Price Indexes [89] (Table 3).

### 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 sensitive 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 new-born programs should be included worldwide, as it improves the quality of life, expectancy of life and diminishes the expenses in health.

### Tables

**Table 3: Model variables and ranges [89]**

<table>
<thead>
<tr>
<th>Variables</th>
<th>Base-case</th>
<th>Range/Alternative</th>
</tr>
</thead>
<tbody>
<tr>
<td>Birth prevalence of SCID</td>
<td>1/58,000</td>
<td>1/46,000-1/80,000</td>
</tr>
<tr>
<td>Proportion of SCID cases detected without the screening</td>
<td>0.203</td>
<td></td>
</tr>
<tr>
<td>Sensitivity of the TREC method</td>
<td>99.50%</td>
<td>99.00%-100.00%</td>
</tr>
<tr>
<td>Specificity of the TREC method</td>
<td>99.97%</td>
<td>99.92%-99.98%</td>
</tr>
<tr>
<td>Survival rate</td>
<td></td>
<td></td>
</tr>
<tr>
<td>• For early-identified SCID (pre-treatment)</td>
<td>94%</td>
<td></td>
</tr>
<tr>
<td>• For early-identified SCID (post-treatment)</td>
<td>94%</td>
<td></td>
</tr>
<tr>
<td>• For late-identified SCID (pre-treatment)</td>
<td>78%</td>
<td></td>
</tr>
<tr>
<td>• For late-identified SCID (post-treatment)</td>
<td>69%</td>
<td></td>
</tr>
<tr>
<td>Costs of screening and diagnosis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Lab tests for TREC assay sample</td>
<td>$4.04</td>
<td>$3.00-$6.00</td>
</tr>
<tr>
<td>• Short-term follow-up per positive case</td>
<td>$50.00</td>
<td></td>
</tr>
<tr>
<td>• Flow cytometry per case</td>
<td>$290.0</td>
<td></td>
</tr>
<tr>
<td>Costs of treatment</td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Average cost per infant with SCID who die before definitive treatment</td>
<td>$300,000</td>
<td></td>
</tr>
<tr>
<td>• Average cost per infant with ADA-SCID who do not undergo early HSCT</td>
<td>$450,000</td>
<td>$200,000-$750,000</td>
</tr>
<tr>
<td>• Average cost for infants with SCID who receive HSCT as first-line therapy</td>
<td>$100,000</td>
<td>$80,000-$120,000</td>
</tr>
<tr>
<td>• Per early-identified baby</td>
<td>$450,000</td>
<td>$300,000-$1,200,000</td>
</tr>
<tr>
<td>• Per late-identified baby</td>
<td></td>
<td></td>
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</table>


References


