J Pediatr (Rio J). 2016;92(4):374–380

Jornal de Pediatria
www.jped.com.br

ORIGINAL ARTICLE

Neonatal screening for severe combined immunodeficiency in Brazil☆,☆☆

Marilia Pyles Patto Kanegae[a], Lucila Akune Barreiros[a], Juliana Themudo Lessa Mazzucchelli[b], Sonia Marchezi Hadachi[c], Laura Maria de Figueiredo Ferreira Guilhoto[c], Ana Lúcia Acquesta[d], Isabel Rugue Genov[b,e], Silvia Maia Holanda[f], Regina Sumiko Watanabe Di Gesu[g], Ana Lucia Goulart[b], Amélia Miyashiro Nunes dos Santos[b], Newton Bellesi[h], Beatriz Tavares Costa-Carvalho[b], Antonio Condino-Neto[a,*]

[a] *Department of Immunology, Instituto de Ciências Biomédicas, Universidade de São Paulo (USP), São Paulo, SP, Brazil*
[b] *Department of Pediatrics, Universidade Federal de São Paulo (UNIFESP), São Paulo, SP, Brazil*
[c] *APAE-SP, São Paulo, SP, Brazil*
[d] *Hospital Geral de Pirajussara, São Paulo, SP, Brazil*
[e] *Hospital Pimentas Bonsucesso, Guarulhos, SP, Brazil*
[f] *Amparo Maternal, São Paulo, SP, Brazil*
[g] *Hospital Nossa Senhora da Conceição, Porto Alegre, RS, Brazil*
[h] *Clínica de Medicina Preventiva do Pará (CLIMEP), Belém, PA, Brazil*

Received 1 July 2015; accepted 5 October 2015
Available online 18 May 2016

KEYWORDS
SCID;
Neonatal screening;
TRECs;
T lymphocytes;
Combined immunodeficiency;
Primary immunodeficiency

Abstract

Objective: To apply, in Brazil, the T-cell receptor excision circles (TRECs) quantification technique using real-time polymerase chain reaction in newborn screening for severe combined immunodeficiency and assess the feasibility of implementing it on a large scale in Brazil.
Methods: 8715 newborn blood samples were collected on filter paper and, after DNA elution, TRECs were quantified by real-time polymerase chain reaction. The cutoff value to determine whether a sample was abnormal was determined by ROC curve analysis, using SSPS.
Results: The concentration of TRECs in 8,682 samples ranged from 2 to 2,181 TRECs/μL of blood, with mean and median of 324 and 259 TRECs/μL, respectively. Forty-nine (0.56%) samples were below the cutoff (30 TRECs/μL) and were reanalyzed. Four (0.05%) samples had abnormal results (between 16 and 29 TRECs/μL). Samples from patients previously identified as having severe combined immunodeficiency or DiGeorge syndrome were used to validate the assay and

☆ Please cite this article as: Kanegae MP, Barreiros LA, Mazzucchelli JT, Hadachi SM, Guilhoto LM, Acquesta AL, et al. Neonatal screening for severe combined immunodeficiency in Brazil. J Pediatr (Rio J). 2016;92:374–80.
☆☆ This study was carried out at the Department of Immunology, Instituto de Ciências Biomédicas, Universidade de São Paulo (USP), São Paulo, SP, Brazil.

* Corresponding author.
E-mail: condino@icb.usp.br (A. Condino-Neto).

http://dx.doi.org/10.1016/j.jped.2015.10.006

all of them showed TRECs below the cutoff. Preterm infants had lower levels of TRECs than full-term neonates. The ROC curve showed a cutoff of 26 TRECs/μL, with 100% sensitivity for detecting severe combined immunodeficiency. Using this value, retest and referral rates were 0.43% (37 samples) and 0.03% (3 samples), respectively.
Conclusion: The technique is reliable and can be applied on a large scale after the training of technical teams throughout Brazil.

PALAVRAS-CHAVE
SCID;
Triagem neonatal;
TRECs;
Linfócitos T;
Imunodeficiência combinada;
Imunodeficiência primária

Triagem neonatal para imunodeficiência combinada grave no Brasil

Resumo
Objetivo: aplicar no Brasil a técnica de quantificação de *T-cell Receptor Excision Circles* (TRECs) por *polymerase chain reaction* em tempo real para triagem neonatal de imunodeficiência combinada grave (SCID) e avaliar se é possível realizá-la em larga escala em nosso país.
Métodos: foram coletadas 8.715 amostras de sangue de recém-nascidos em papel filtro e, após eluição do DNA, os TRECs foram quantificados por *polymerase chain reaction* em tempo real. O valor de corte para determinar se uma amostra é anormal foi determinado pela análise de curva ROC utilizando-se o programa SSPS.
Resultados: a concentração de TRECs em 8.682 amostras analisadas variou entre 2 e 2.181 TRECs/μL de sangue, com média e mediana de 324 e 259 TRECs/μL, respectivamente. 49 (0,56%) amostras ficaram abaixo do valor de corte (30 TRECs/μL) e foram requantificadas. Quatro (0,05%) mantiveram resultados anormais (entre 16 e 29 TRECs/μL). Amostras de pacientes com diagnóstico clínico prévio de imunodeficiência combinada grave e síndrome de DiGeorge foram utilizadas para validar o ensaio e todas apresentaram concentração de TRECs abaixo do valor de corte. Recém-nascidos prematuros apresentaram menores níveis de TRECs comparados aos nascidos a termo. Utilizando a curva ROC em nossos dados, chegamos ao valor de corte de 26 TRECs/μL, com sensibilidade de 100% para detecção de imunodeficiência combinada grave. Utilizando este valor, as taxas de repetição e encaminhamento, ficaram em 0,43% (37 amostras) e 0,03% (3 amostras), respectivamente.
Conclusão: A técnica é factível e pode ser implantada em larga escala, após treinamento técnico das equipes envolvidas.

Introduction

Severe combined immunodeficiencies (SCID) comprises a heterogeneous group of diseases characterized by severe defects in the development and function of T, B, and NK lymphocytes. They are recognized as pediatric emergencies, as they lead to severe and recurrent infections and are fatal in the first two years of life, if not diagnosed and treated adequately.[1,2]

Due to the disease severity and the urgency in diagnosis and treatment, in 2005, United States researchers developed the newborn screening (NBS) for SCID, which consists of quantifying T-cell receptor excision circles (TRECs) using real-time polymerase chain reaction (PCR) (qRT-PCR).[3,4] TRECs are small circular pieces of DNA formed during normal thymic processing, when T-cell receptor gene rearrangement occurs. As they do not replicate during cell division, they work as markers for the number of ''naïve'' T-cells recently emigrated from the thymus, and they are reduced in all forms of SCID.[5]

In most cases, NBS is the only way to detect SCID before infections occur, as more than 80% of cases have no family history of primary immunodeficiency.[6]

A significant altered finding observed after the development of neonatal screening was the disease incidence. As many children die before the diagnosis is attained, the incidence of SCID was estimated at 0.10/10,000; after the American experience, this figure is currently close to 0.17/10,000 (1:58,000), or almost two-fold higher.[6,7]

In Brazil, there is no neonatal screening test for primary immunodeficiencies applied during medical routine assessment and the diagnosis of these diseases falls short of what is required.[8–10] For this reason, the aim of this study was to apply the TRECs quantification technique by qRT-PCR in the NBS for severe combined immunodeficiencies and assess whether it is possible to do so on a large scale in Brazil.

Methods

All samples were collected following the current ethical standards of the Research Ethics Committee of ICB-USP (Opinion 967/CEP) and the parents/guardians of the newborns signed the informed consent. The concentration of TRECs was determined after blood samples were collected on 903 filter paper cards through heel puncture, which is