NEWBORN SCREENING FOR SCID IN BRAZIL

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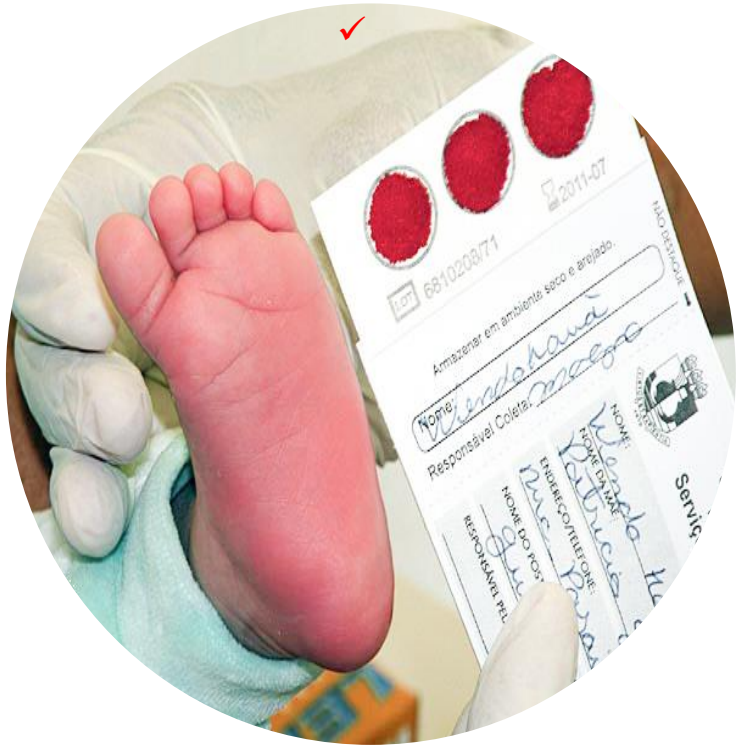

ICB USP

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J Clin Immunol (2014) 34:10–22
DOI 10.1007/s10875-013-9954-6

KEY REVIEW ARTICLE

Attending to Warning Signs of Primary Immunodeficiency Diseases Across the Range of Clinical Practice

Beatriz Tavares Costa-Carvalho • Anete Sevciovic Grumach • José Luis Franco • Francisco Javier Espinosa-Rosales • Lily E. Leiva • Alejandra King • Oscar Porras • Liliana Bezrodnik • Mathias Oleastro • Ricardo U. Sorensen • Antonio Condino-Neto

A group of > 420 monogenic diseases presenting with: recurrent infections, autoimmunity, inflammation, allergy, and cancer

Clinical & Experimental Immunology
The Journal of Translational Immunology

Clinical and Experimental Immunology | IMMUNODEFICIENCIES | doi:10.1111/cei.12495

The relevance of collaborative work: the Latin American Society for Immunodeficiencies (LASID) registry model

 Clinical and Experimental Immunology, **178:** 16–17

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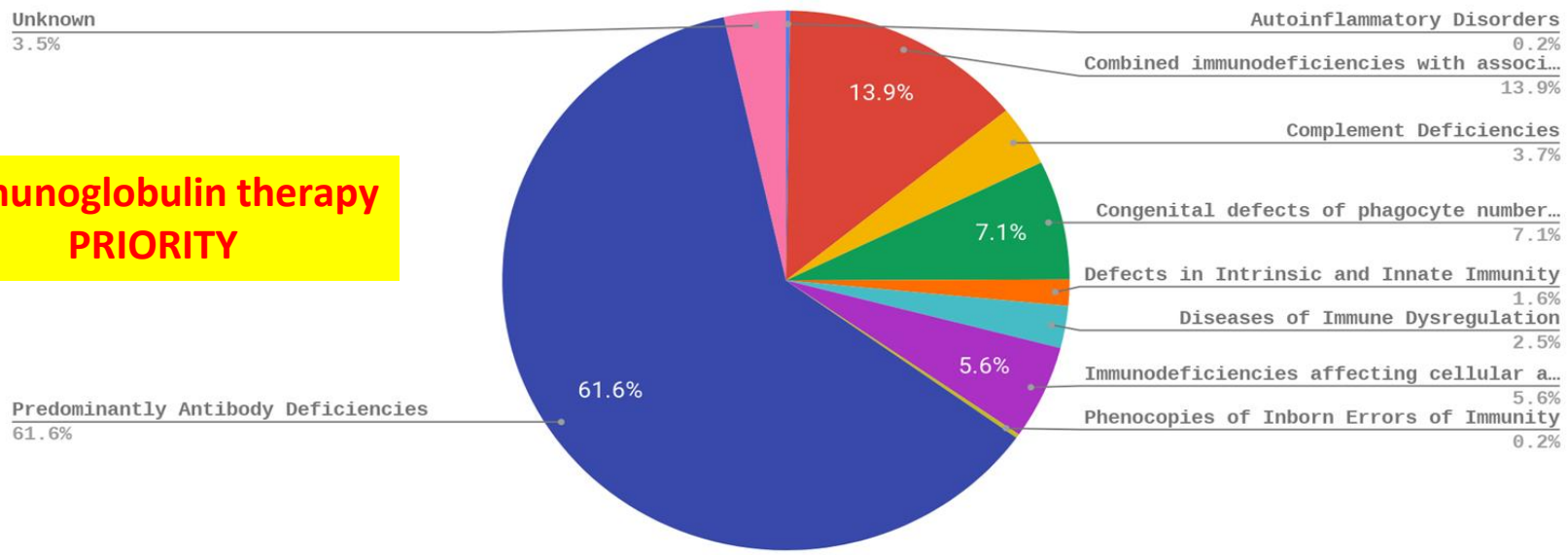
EDUCATION
10 YEARS !
~ 8000 CASES !

LASID
Latin American Society for Immunodeficiencies
Sociedad Latinoamericana de Inmunodeficiencias

PIDD LATAM

Immunoglobulin therapy PRIORITY



Autoinflammatory Disorders	18
Combined immunodeficiencies with associated or syndromic features	1071
Complement Deficiencies	282
Congenital defects of phagocyte number or function	548
Defects in Intrinsic and Innate Immunity	121
Diseases of Immune Dysregulation	194
Immunodeficiencies affecting cellular and humoral immunity	433
Phenocopies of Inborn Errors of Immunity	19
Predominantly Antibody Deficiencies	4738
Unknown	271
Total	**7695**

90% PIDs without diagnosis

Diagnosis delay 7 - 10 years

Severe Combined Immunodeficiency - SCID

- **Most severe form of PID**
- Newborns usually appear healthy at birth
- **Absence or low number of T cells that can be accompanied by** B or NK cell deficiency in number and function
- Recurrent infections
- Frequent diarrhea and failure to thrive
- Severe, sometimes fatal reactions to vaccination

(**BCG vaccination** in mandatory in the 1st mo of life in Br)

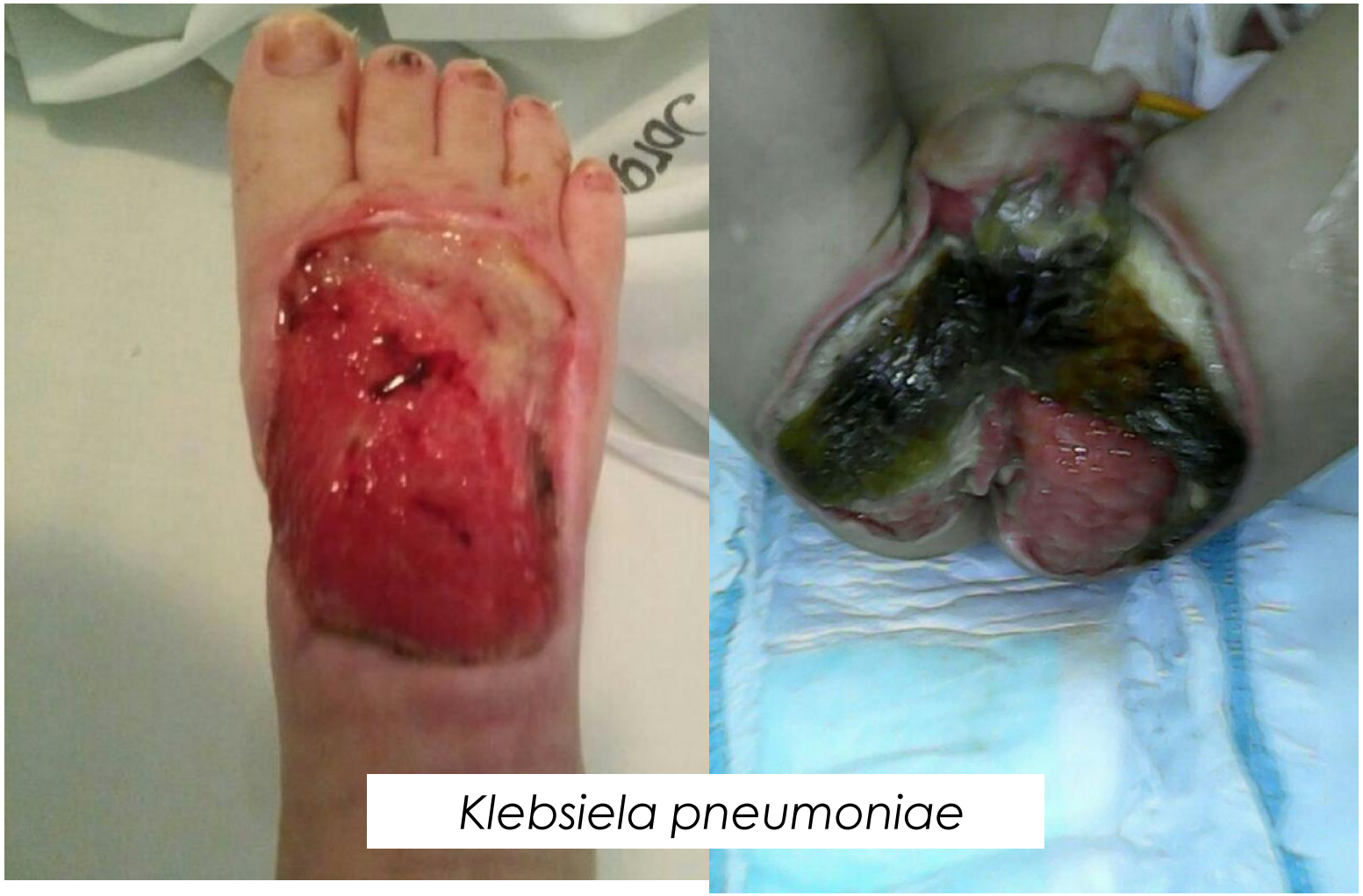
- **Fatal without immune reconstitution**

PEDIATRIC EMERGENCY

SCID – treatment

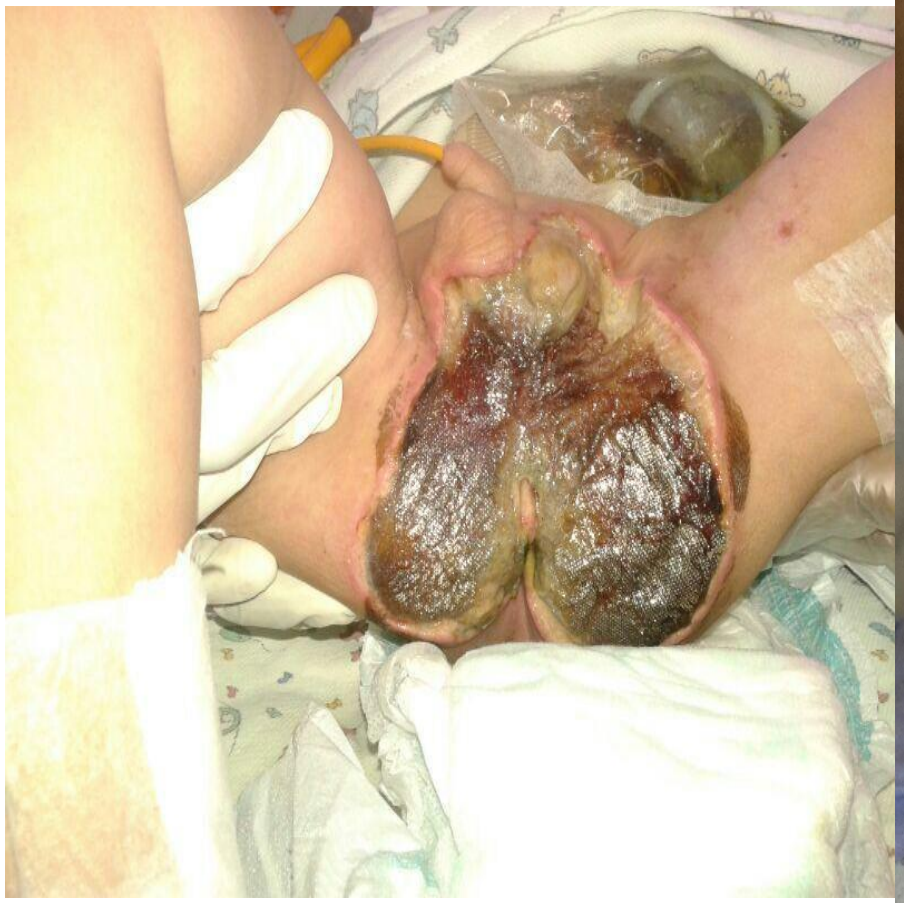
- Antibiotics, Antifungals, Antiviral
- Anti-BCG
- Immunoglobulin replacement
- Enzyme replacement (ADA) as indicated
- **Hematopoietic stem-cell transplantation (majority is haploidentical)**
- Gene therapy – sent abroad (US)

SCID – pediatric emergency

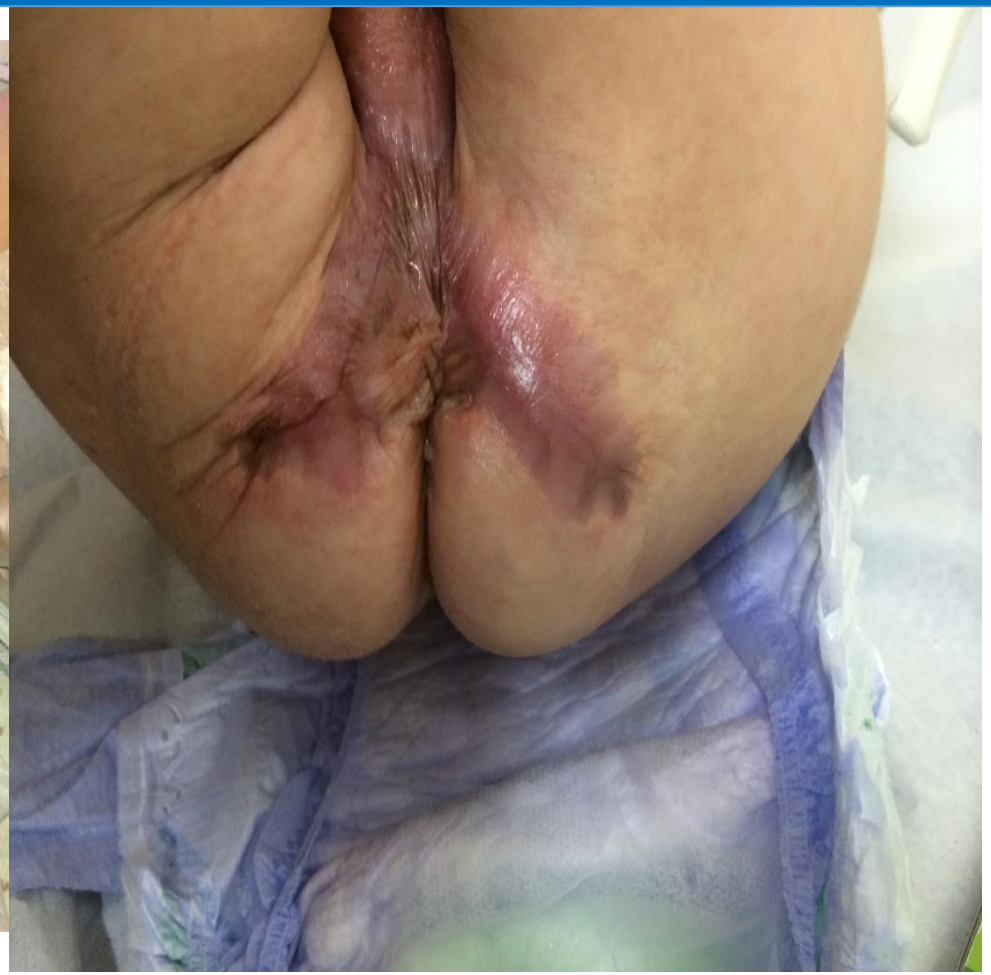


Klebsiela pneumoniae

SCID – pediatric emergency

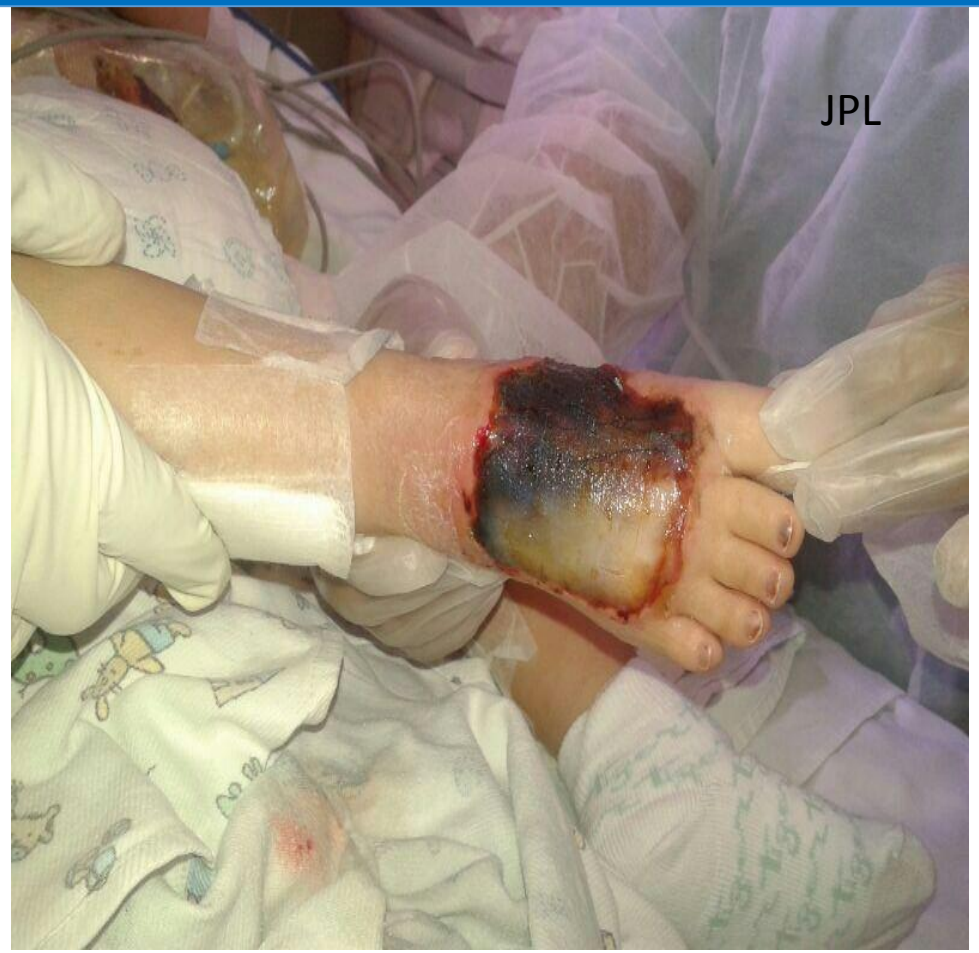


Klebsiela pneumoniae

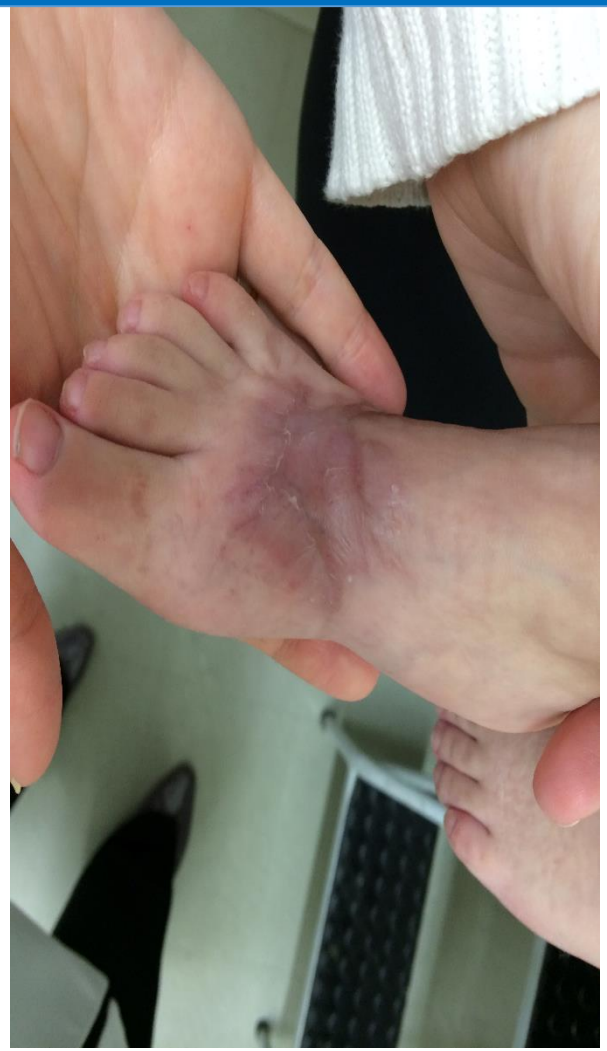


JPL: received BCG **twice**!

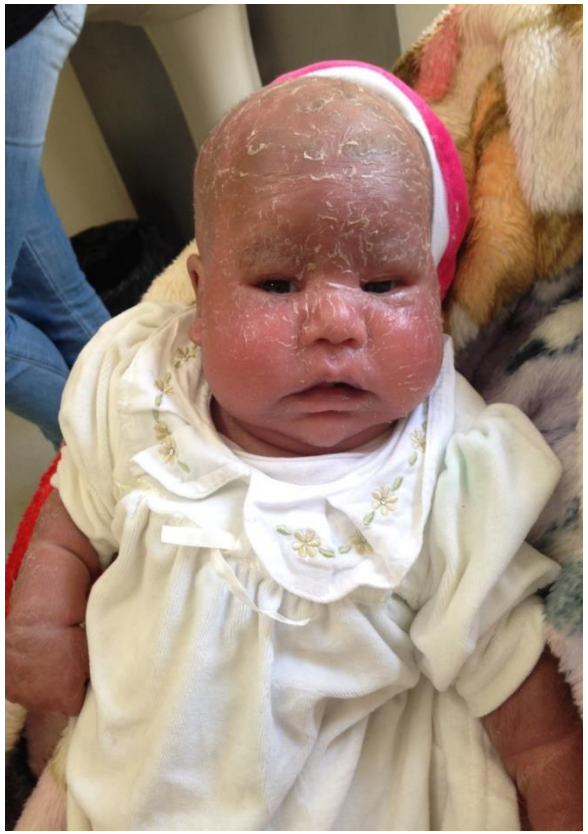
SCID – pediatric emergency

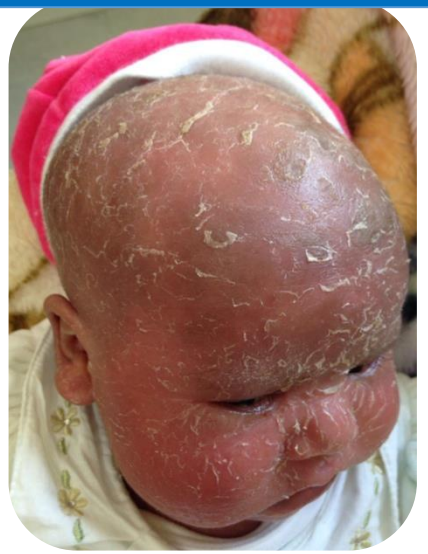


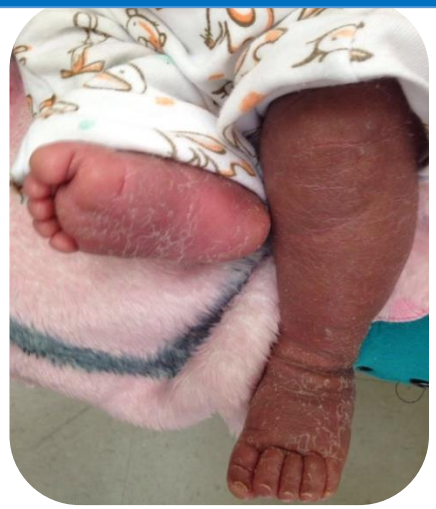
Klebsiela pneumoniae

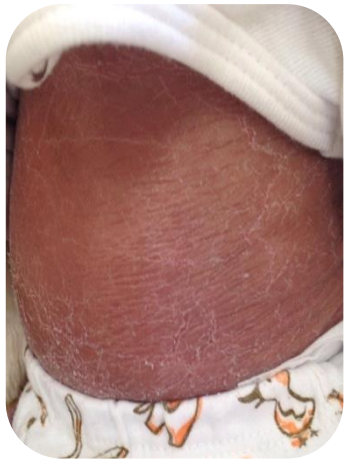


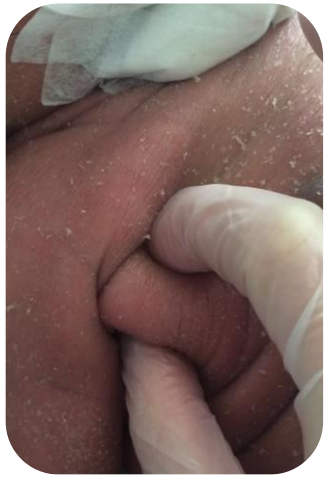
SCID – pediatric emergency





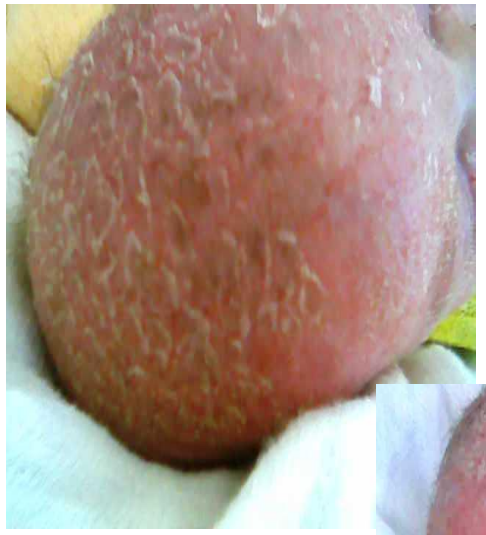




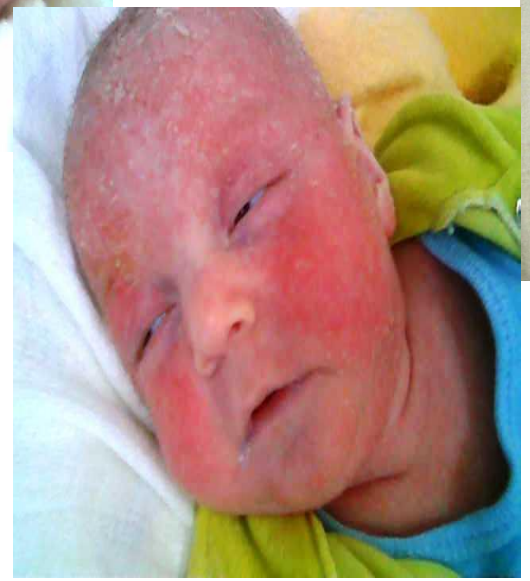


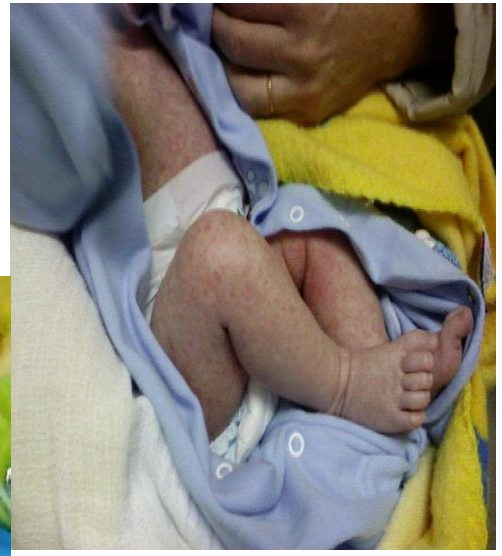
Omenn Syndrome / SCID
Died before BMT

SCID – pediatric emergency



Omenn / SCID – Pre-Transplant

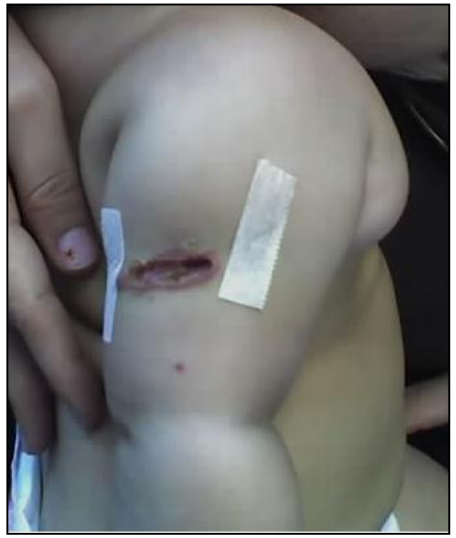


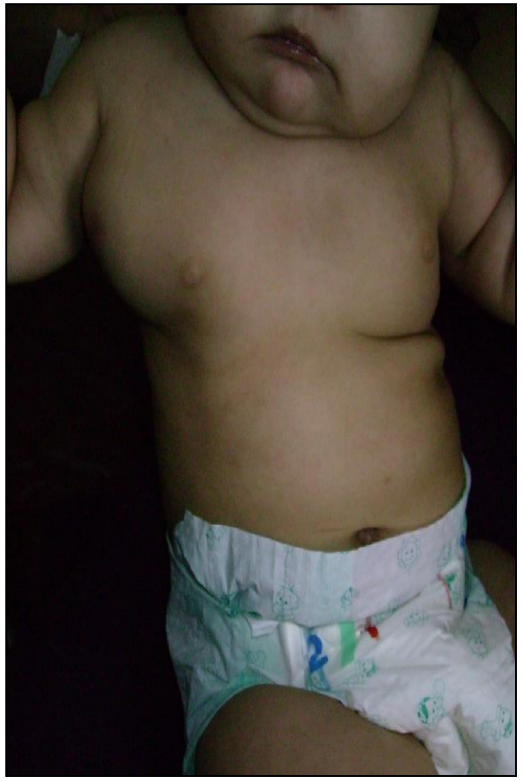


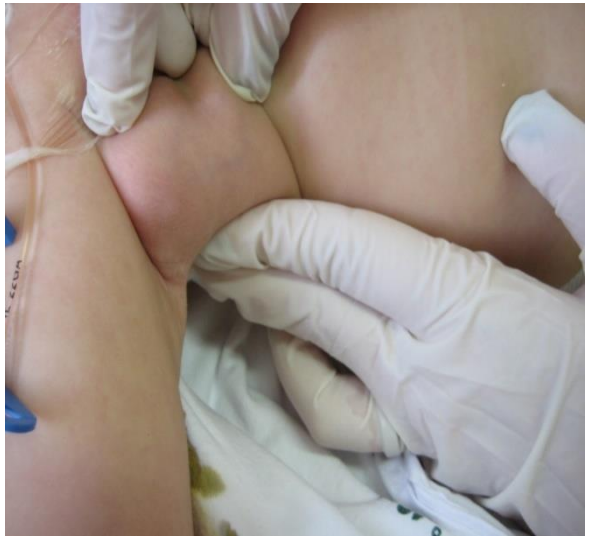
Post-transplant

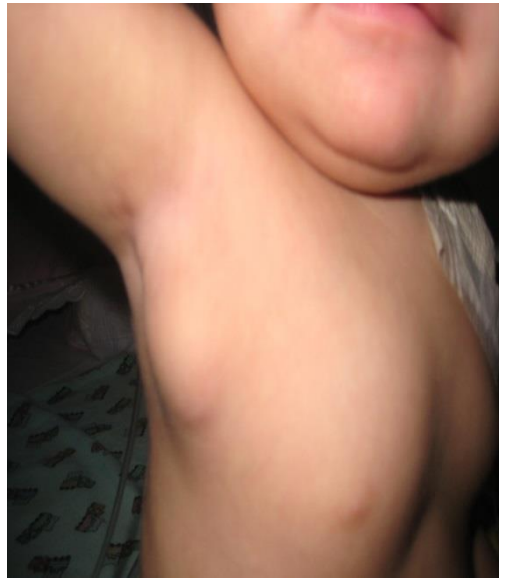
SCID – pediatric emergency

BCG complications



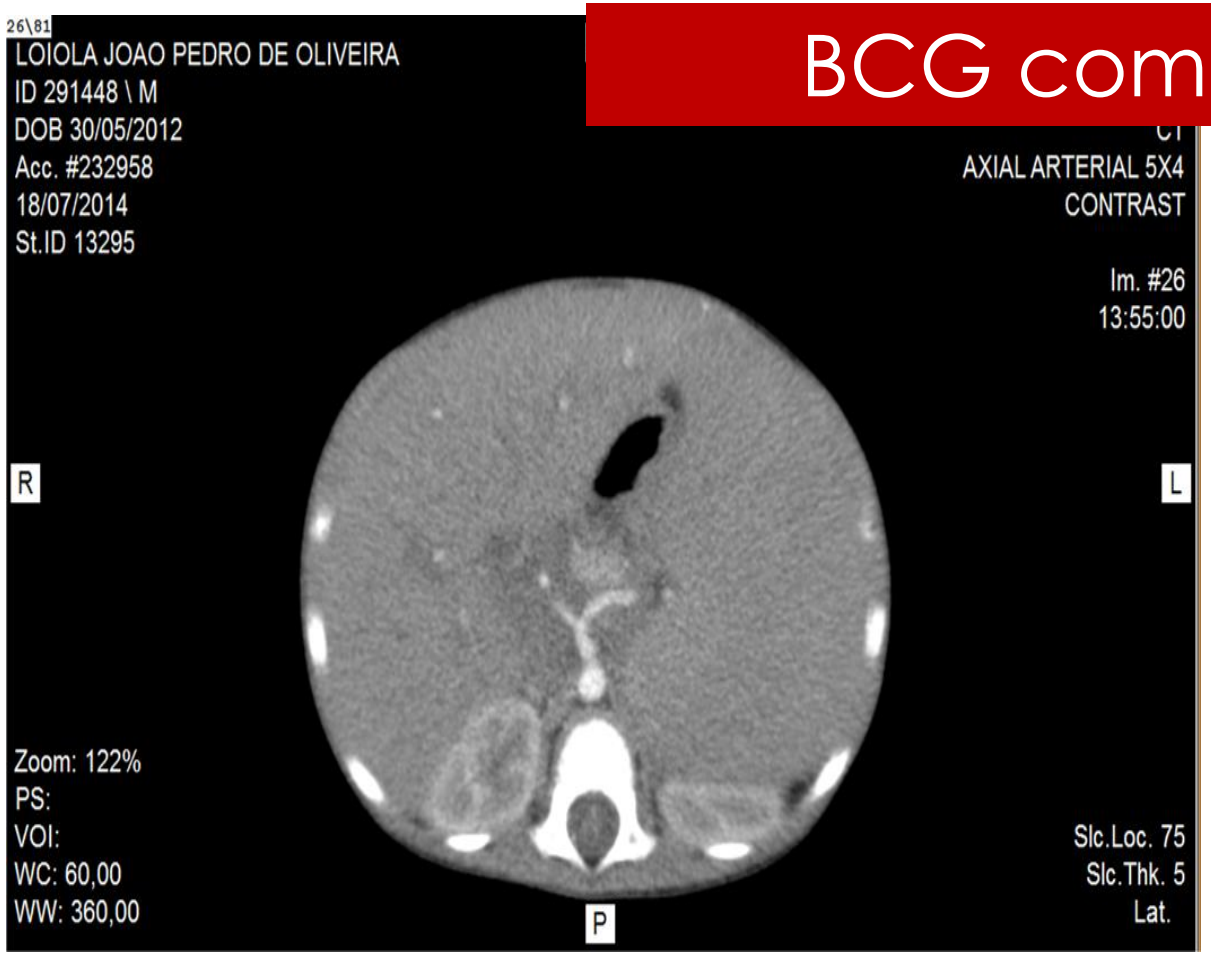


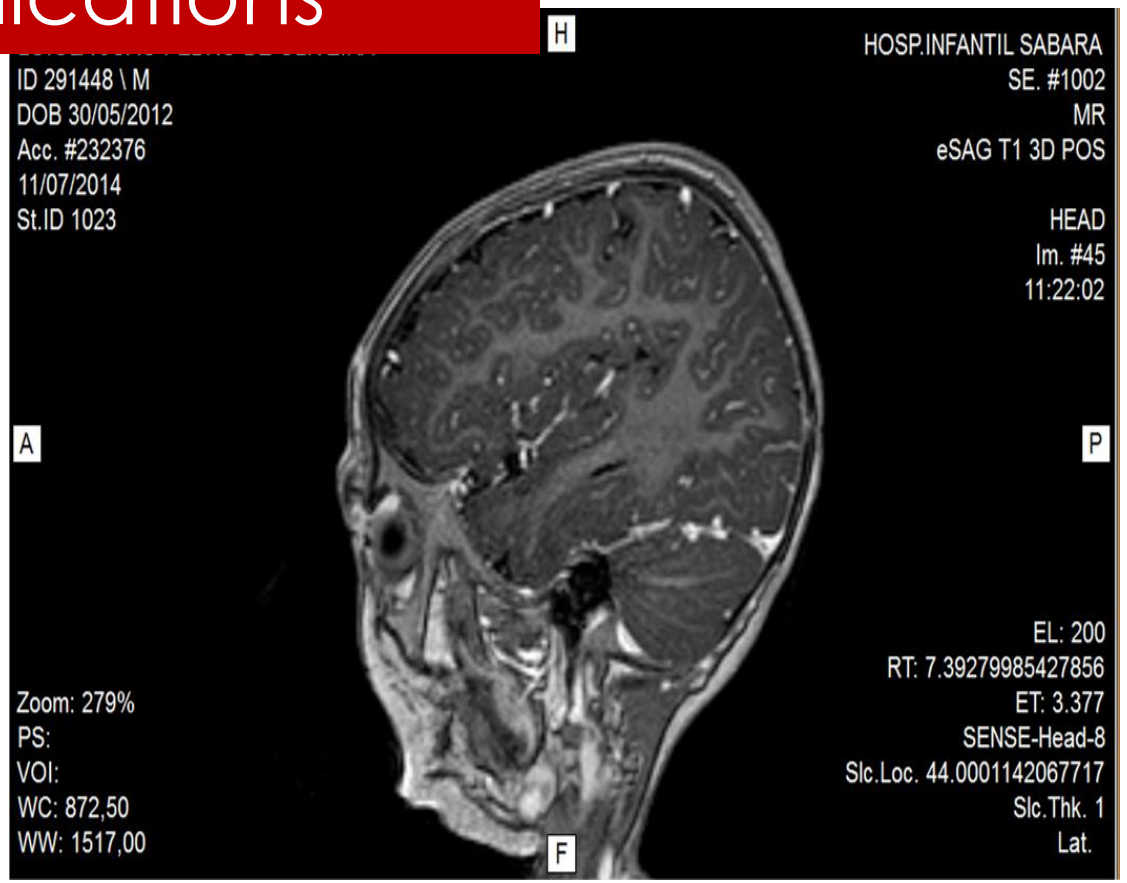




SCID – pediatric emergency

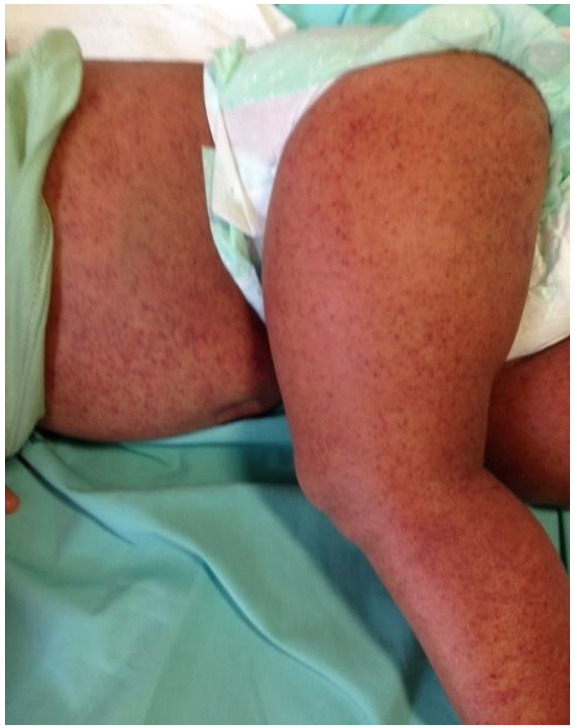
BCG complications

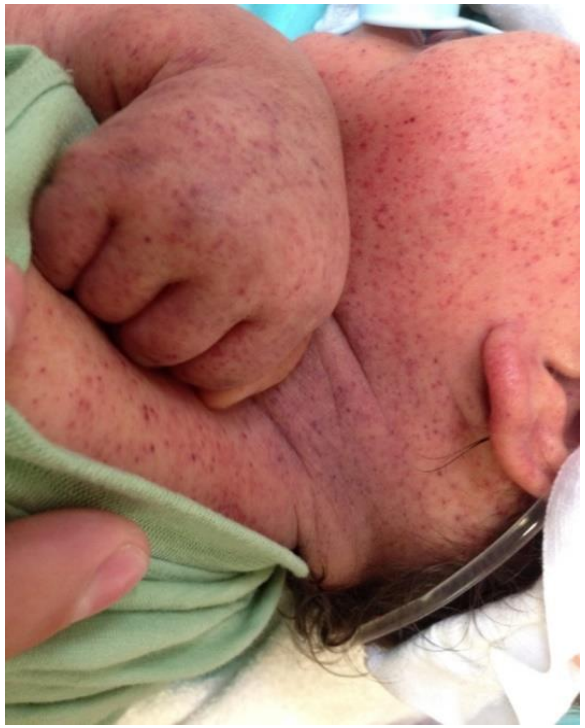




JPL: BCG isolated in the lungs, brain and bone marrow

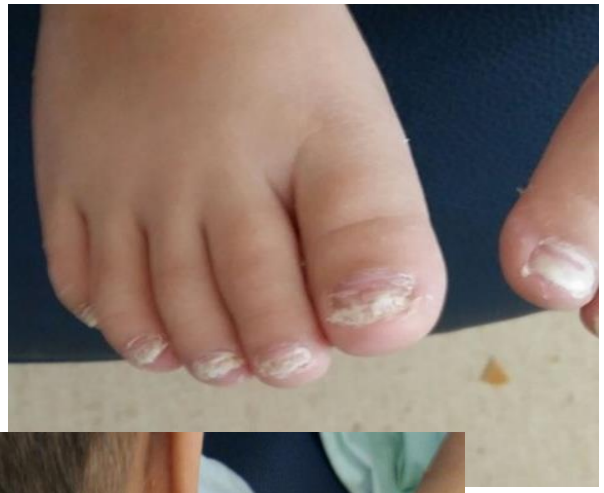
SCID – pediatric emergency

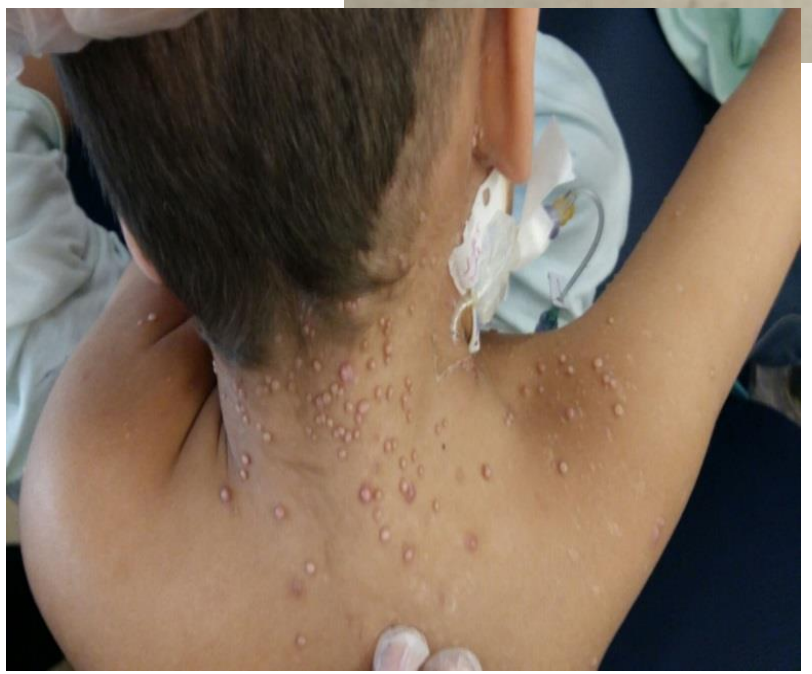




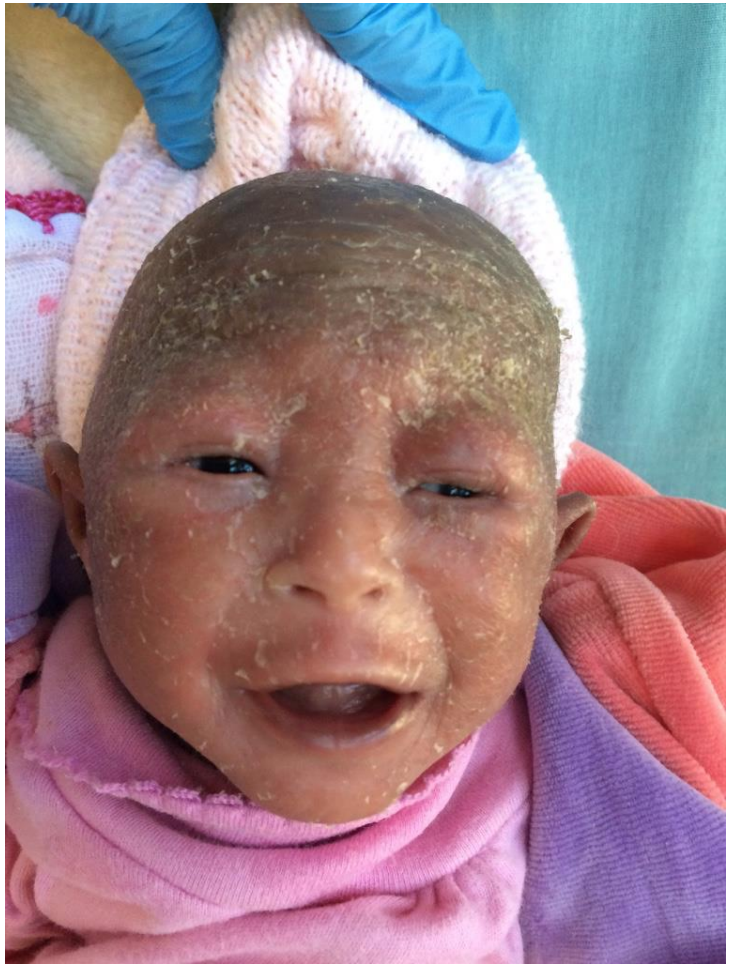
SCID / death post-transplantation

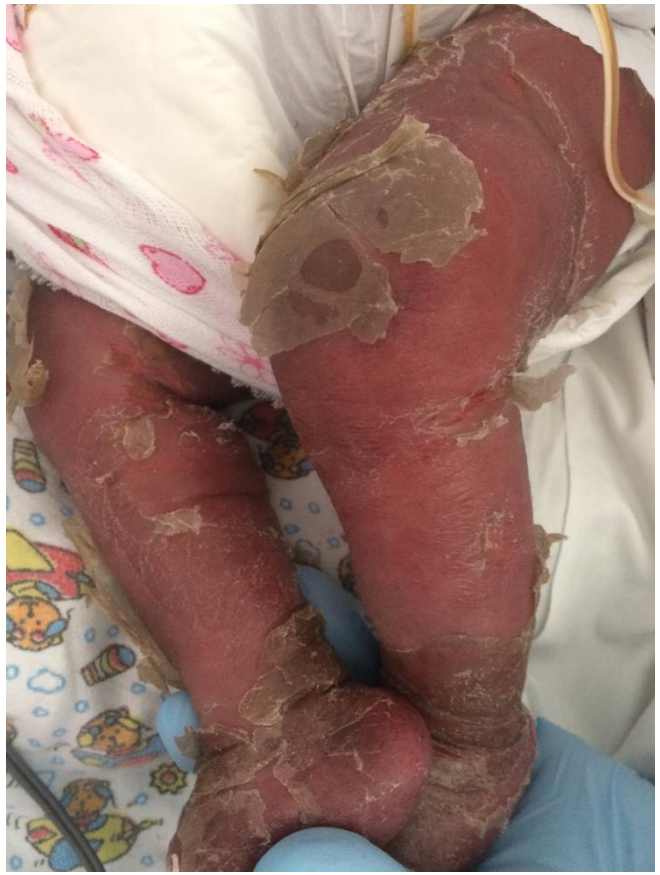
Leaky SCID
Moluscum





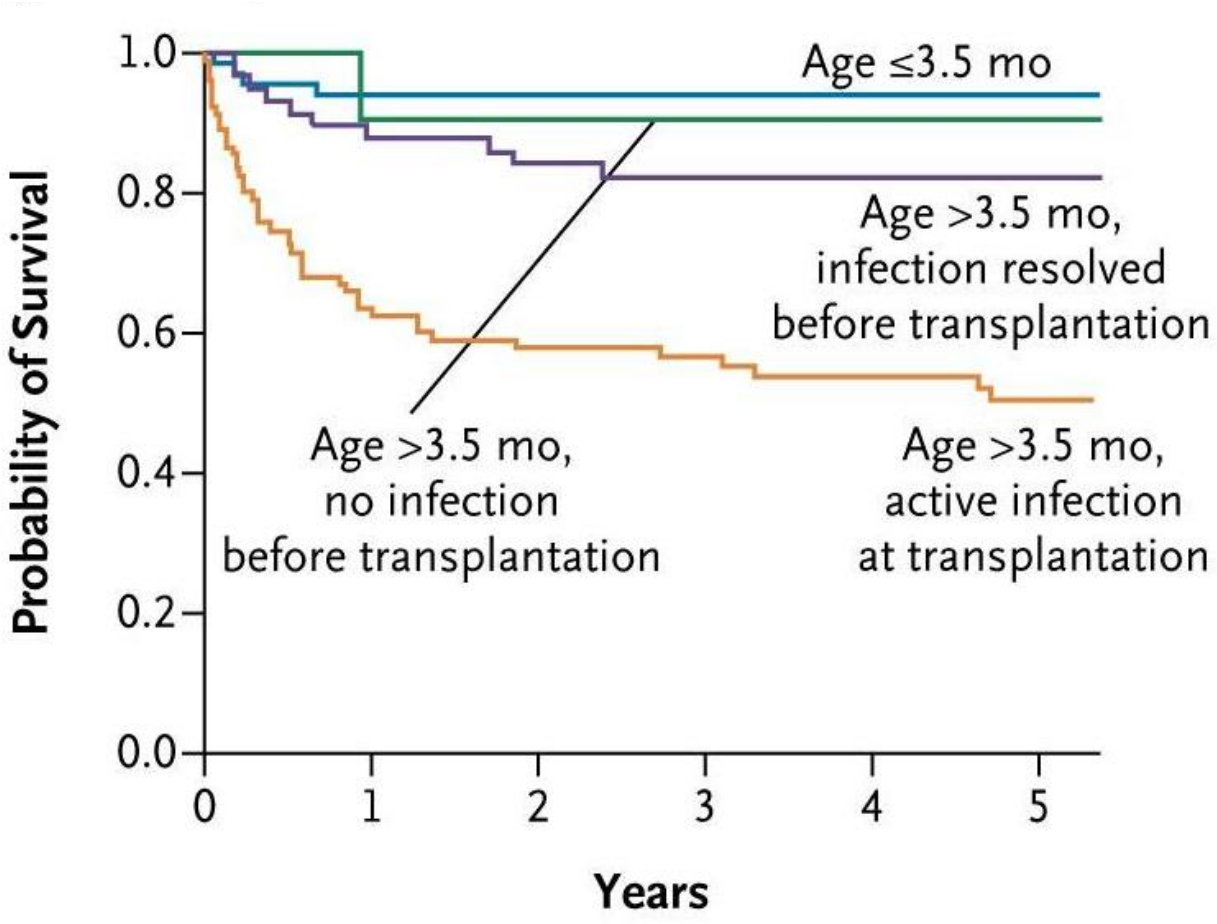
SCID – pediatric emergency





Omenn Syndrome
PRE x POST-TRANSPLANT

SCID – pediatric emergency

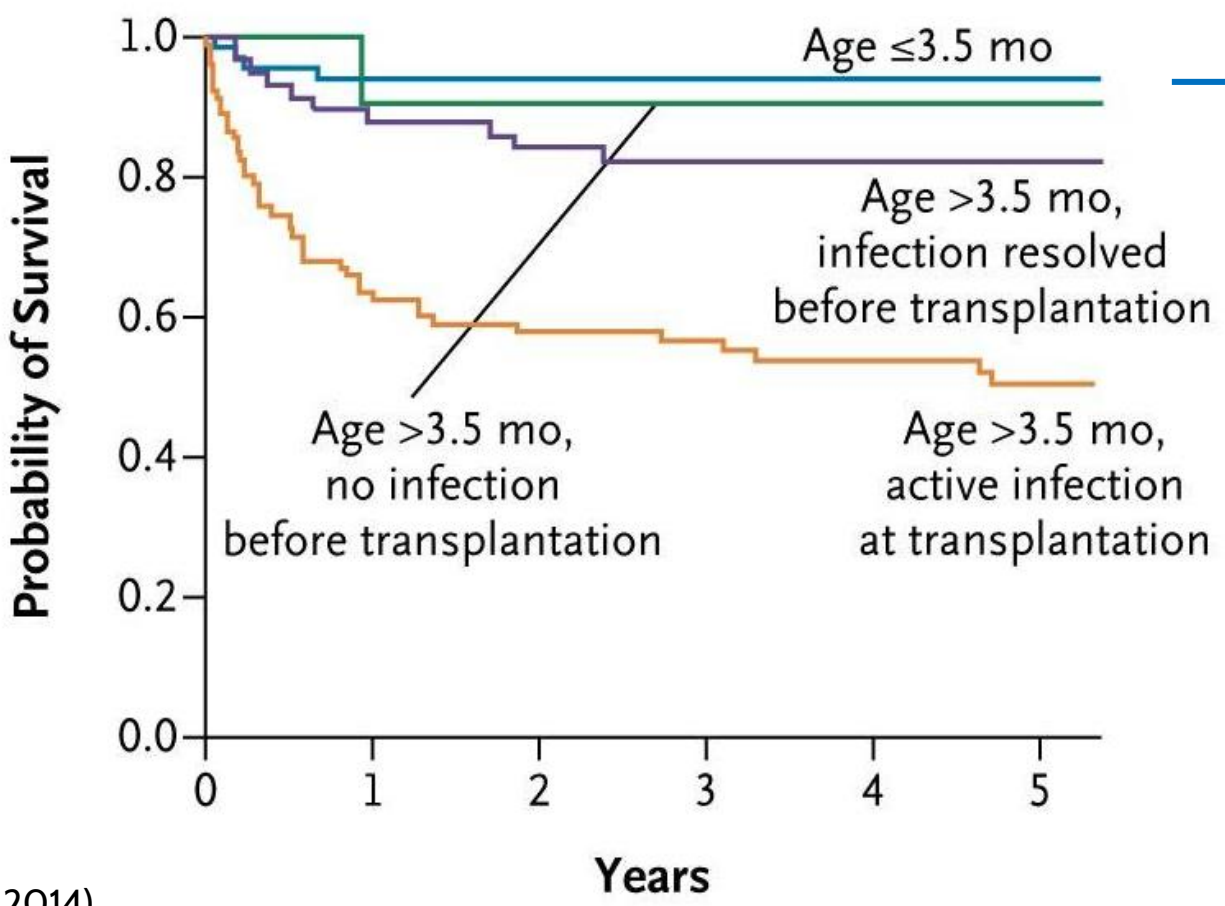


240 SCID transplanted
2000 - 2009
25 centers

(Pai *et al.*, 2014)

SCID – pediatric emergency

240 SCID transplanted (2000 – 2009) - 25 centers



Higher success (> 95%)

- before 3.5 months
- without active infections

> 3.5 m and with active infections

Survival drops to ~ 50%

(Pai *et al.*, 2014)

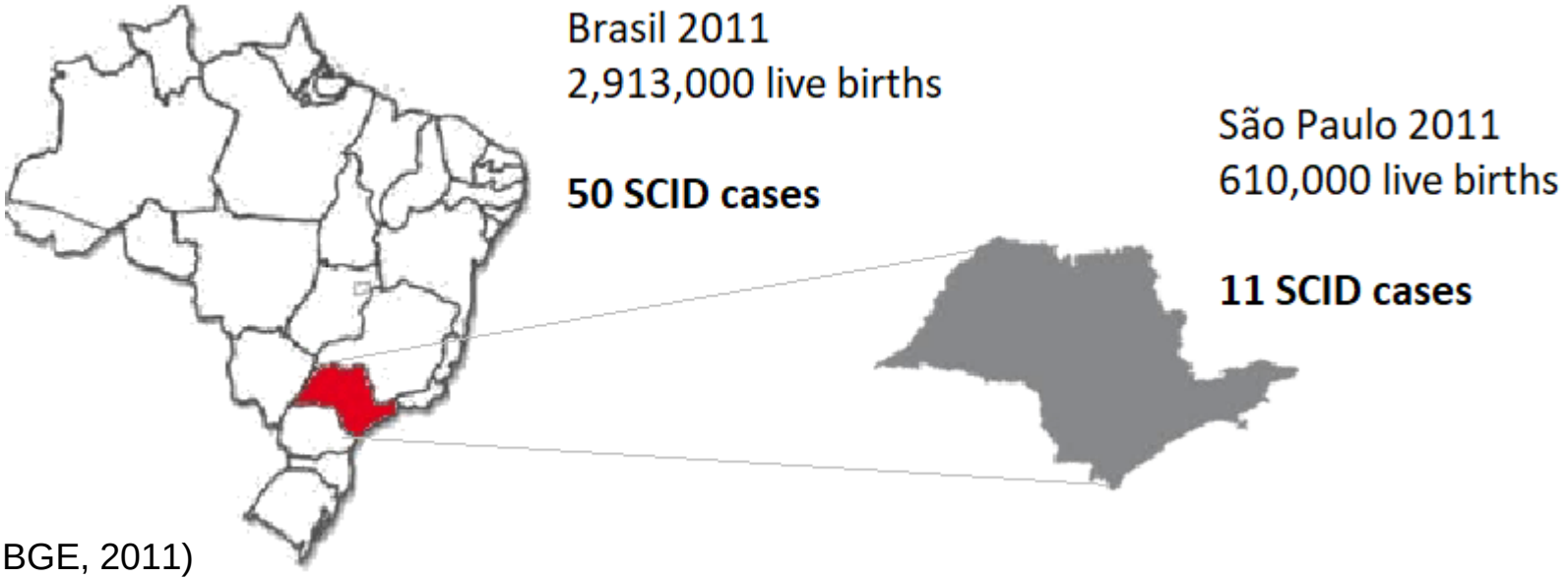
Newborn screening for SCID

- **2005:** Chan and Puck described TREC quantification as a technique for NBS for SCID
- **2007:** SCID was nominated for addition to the screening panel in the USA
- **2008:** first NBS pilot for SCID in Wisconsin
- **2010:** start of NBS pilot in São Paulo
- **2014**: start of NBS pilot in Curitiba (Parana, Rondonia, RJ and SP)
- **2016:** beginning of the PRONAS SABARA / PENSI / USP pilot program

SCID: current scenario in Brazil

- Retrospective study with **patients suspected of SCID 1996-2010 (= 14 years)**
- **70** possible cases in 65 families
  - Only 7 confirmed cases
  - 50% of patients died and 23 of those did not undergo BMT

(Mazzucchelli et al., 2014)



(IBGE, 2011)

For 3 million births / year: 750 cases in 15 y (90% of pt. were missed!)

Using incidence for the US: **1: 58,000 live births**

HSCT for PIDs: current scenario in Brazil

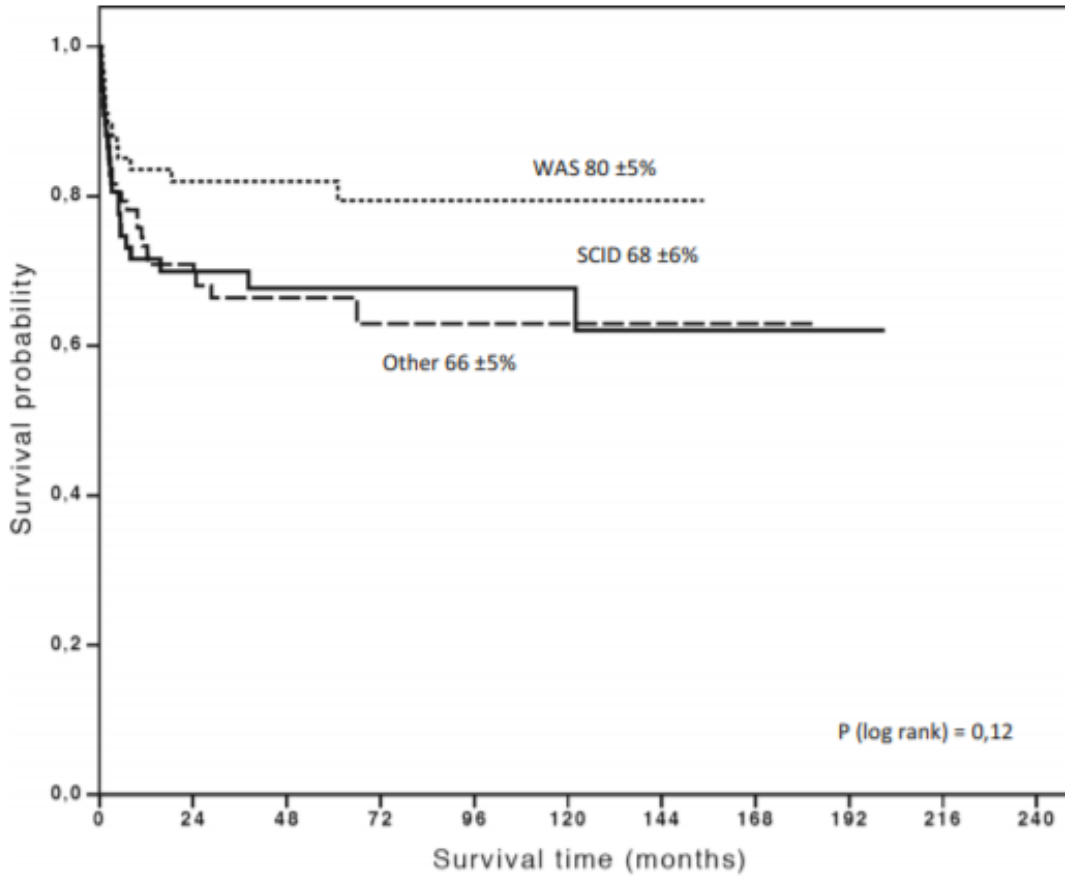
Journal of Clinical Immunology (2018) 38:917–926
https://doi.org/10.1007/s10875-018-0564-1

ORIGINAL ARTICLE

Transplantation of Hematopoietic Stem Cells for Primary Immunodeficiencies in Brazil: Challenges in Treating Rare Diseases in Developing Countries

Juliana Folloni Fernandes[1,2] · Samantha Nichele[3] · Liane E. Daudt[4] · Rita B. Tavares[5] · Adriana Seber[6] · Fábio R. Kerbauy[2] · Adriana Koliski[3] · Gisele Loth[3] · Ana K. Vieira[7] · Luiz G. Darrigo-Junior[8] · Vanderson Rocha[9,10] · Alessandra A. Gomes[9] · Vergílio Colturato[11] · Luiz F. Mantovani[1,2] · Andreza F. Ribeiro[2] · Lisandro L. Ribeiro[3,12] · Cilmara Kuwahara[13] · Ana L. M. Rodrigues[13] · Victor G. Zecchin[6] · Beatriz T. Costa-Carvalho[14] · Magda Carneiro-Sampaio[1,15] · Antonio Condino-Neto[16] · Anders Fasth[17] · Andrew Gennery[18] · Ricardo Pasquini[3,12] · Nelson Hamerschlak[2] · Carmem Bonfim[3,12,13]

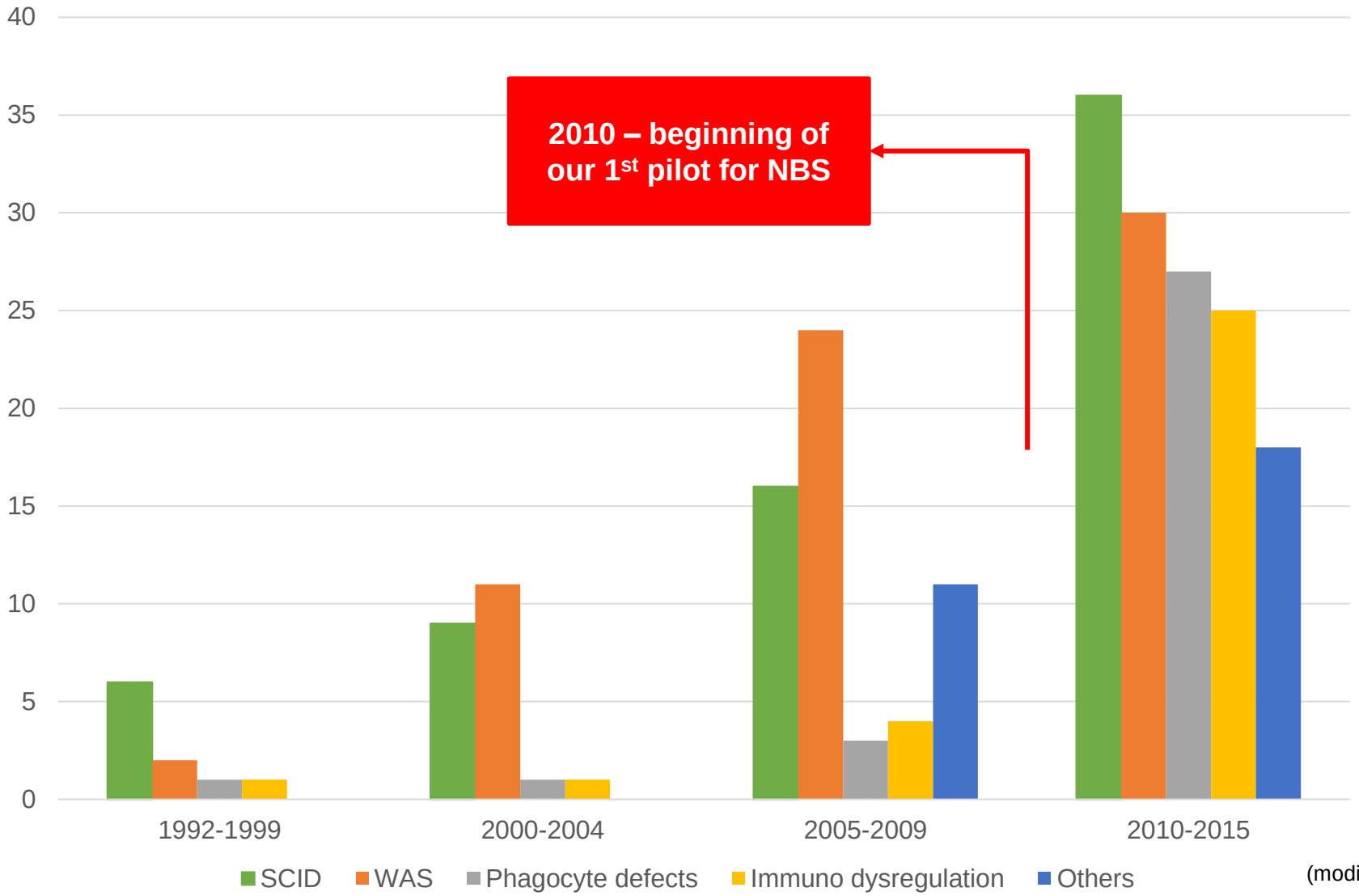
- 1990-2015: **221 patients** from 11 centers (**only 67 SCIDs – 30%**)
- Median age at transplantation: **22m**
- **HVGD – 33%**
- **5y OS: 71.6%**
- **Deaths were 24% due to infection (55%) or GVHS (13%)**



HSCT for PIDs: current scenario in Brazil

- SCIDs = 67
  - **Genetic diagnosis: 27%** (18/67)
  - Data on BCG vaccination: 53 were vaccinated out of 60 (88%)
  - **53% (28/53) – disseminated BCG infections**
  - **Majority was transplanted after 6 months of age**
    - Diagnostic delay
    - Late referral to a transplant unit
    - Long time to find a compatible donors
    - Lack of beds in the few public hospitals that perform this type of transplant

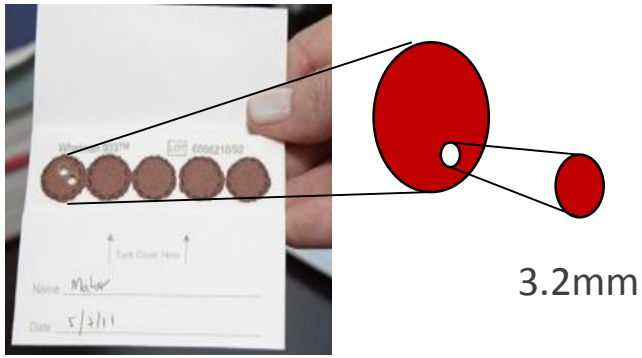
HSCT for PIDs: current scenario in Brazil



(modified from Fernandes et al., 2018)

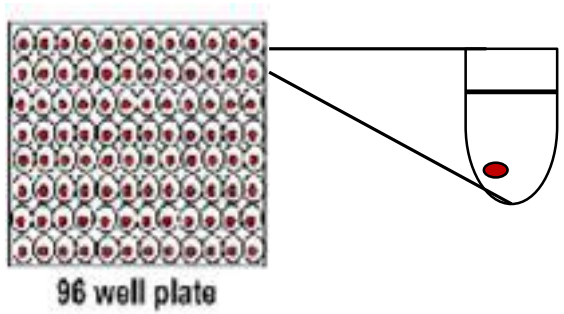
How was newborn screening for PIDD established in Brazil?

Plasmid w/ TREC/KREC/ACTB sequences (no kits)

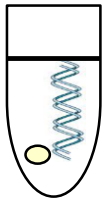


3.2mm

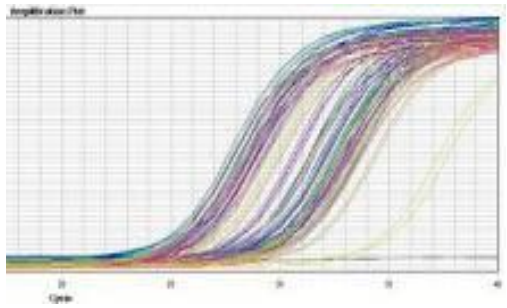
Punch



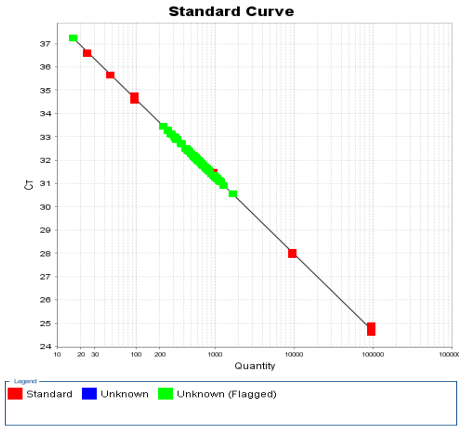
DNA elution



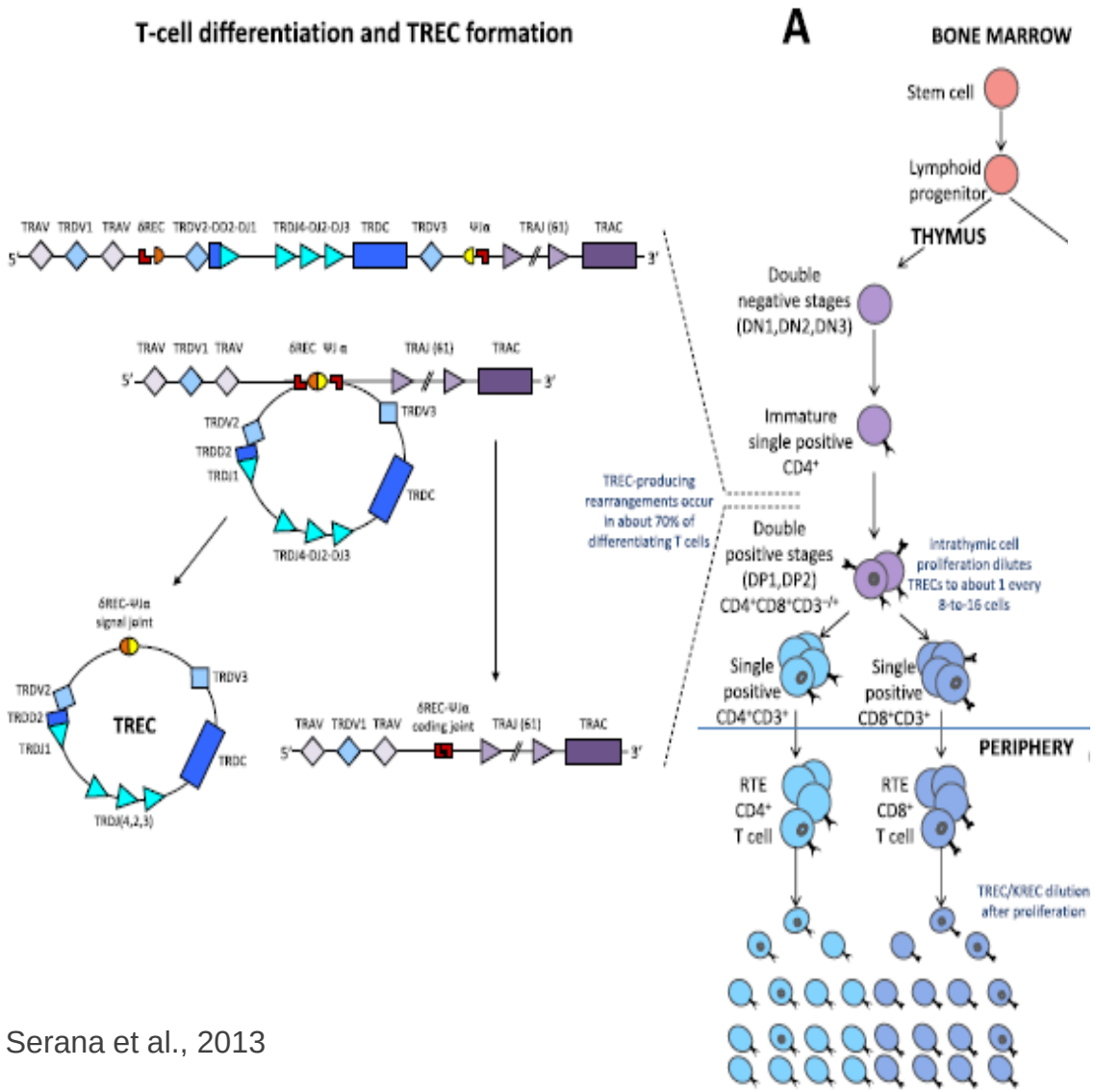
qPCR



Analysis



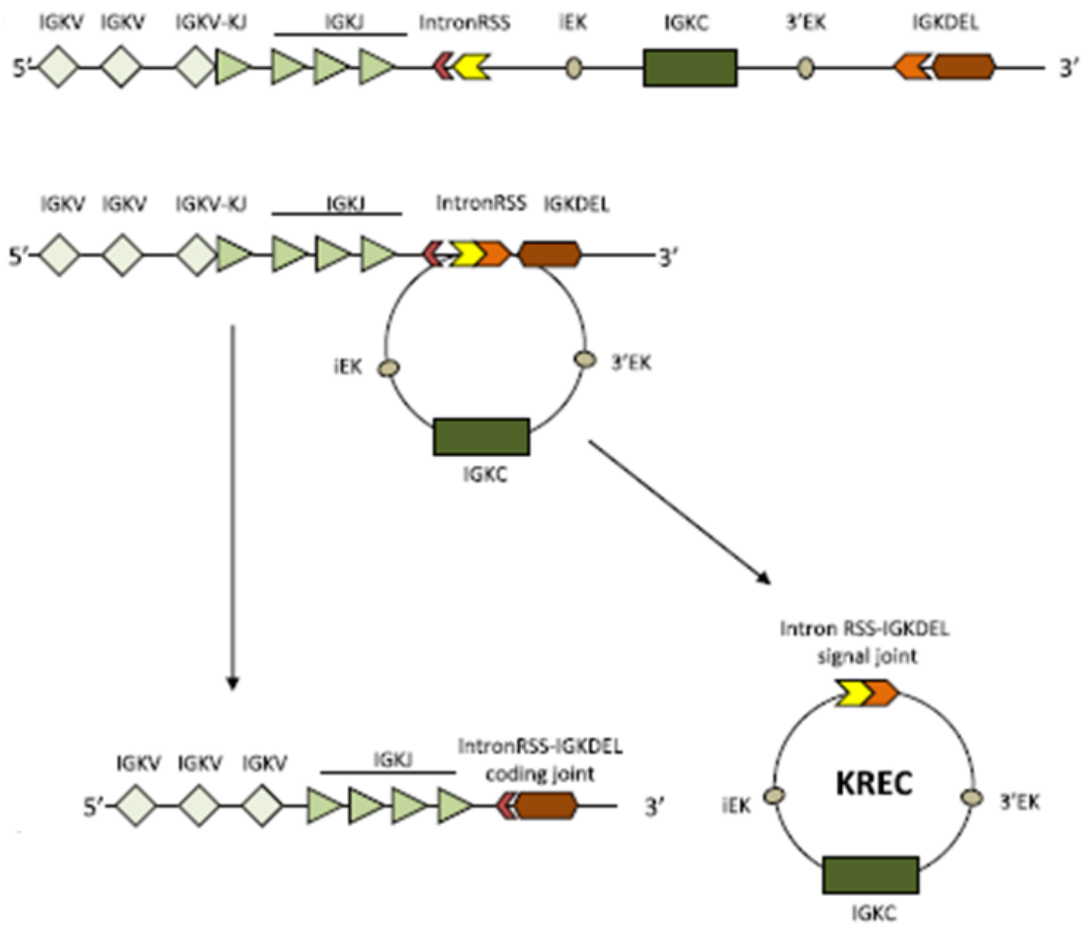
TRECs – T cell Receptor Excision Circles



Serana et al., 2013

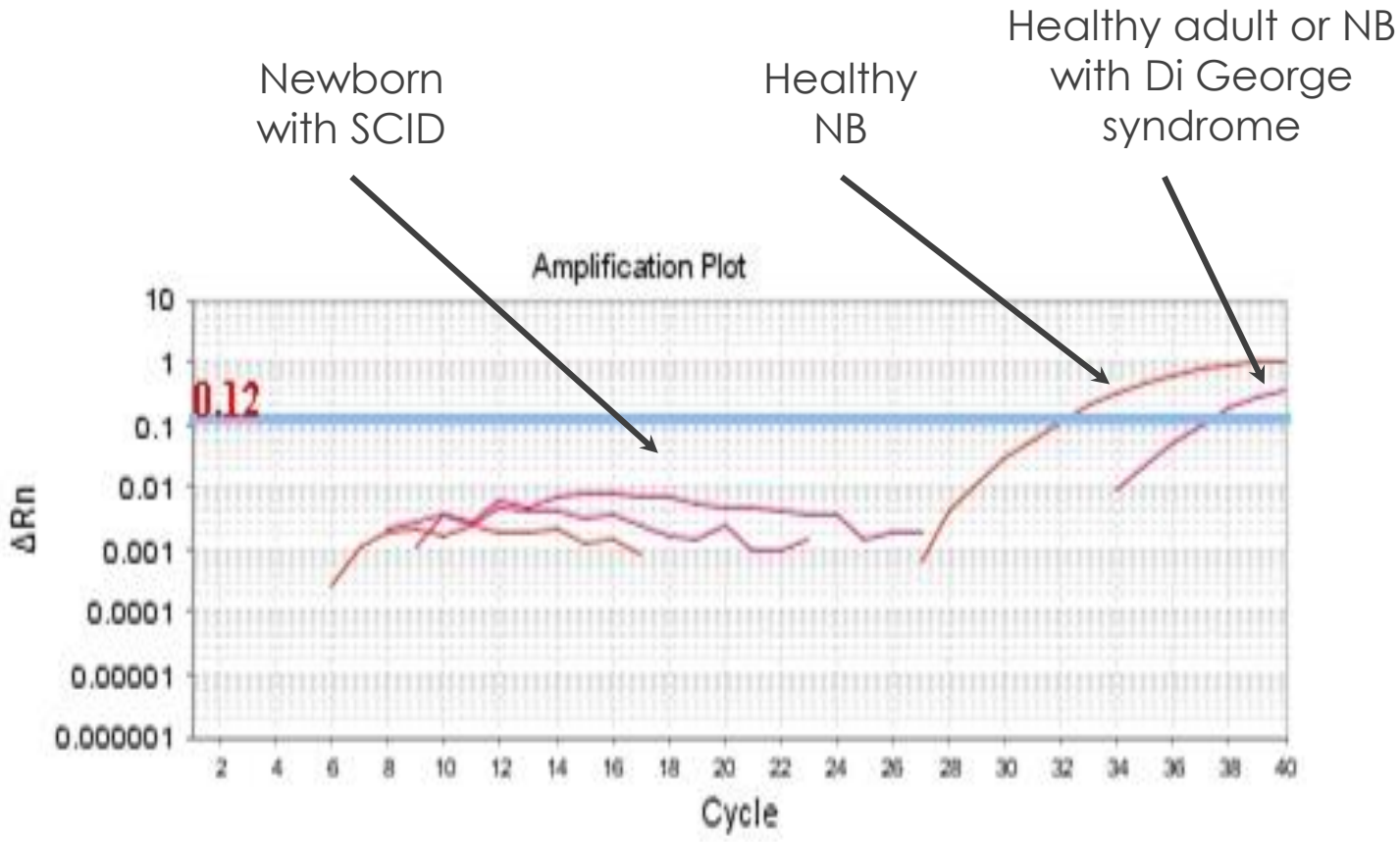
- Produced in the **thymus** during the process of genetic recombination needed for development of the **T cell receptor (TCR)**
- Does not undergo clonal expansion
- Approximately **1 TREC = 1 naive T cell**

KREC - K-deleting recombination excision circles

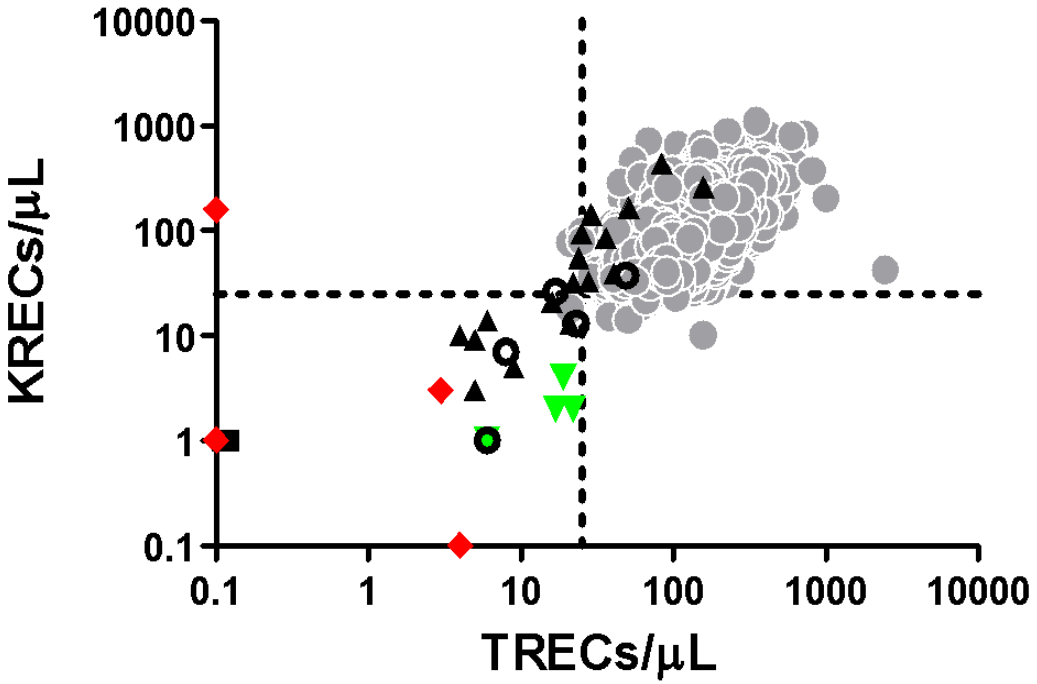


- Produced in the bone marrow during the genetic recombination needed for the development of the **B cell receptor** (BCR)
- Does not undergo clonal expansion
- approximately: **1 KREC = 1 naive B cell**
- Allows the detection of **agamaglobulinemia** and also guides SCID genetic investigation

Serana et al., 2013


Newborn with SCID
Healthy NB
Healthy adult or NB with Di George syndrome
Amplification Plot
0.12
ΔRn
10
1
0.1
0.01
0.001
0.0001
0.00001
0.000001
2 4 6 8 10 12 14 16 18 20 22 24 26 28 30 32 34 36 38 40
Cycle

TRECs x KRECs



- Ataxia-telangiectasia
- Amostras encaminhadas
- Agamaglobulinemia
- SCID
- Hipogamaglobulinemia

Cutoff values chosen to detect not only classic SCID, but also other diseases

Cutoff:
TREC < 25 molecules / uL of blood
KREC < 20 molecules / uL of blood

TREC and KREC values for 4,490 different samples

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[b], Beatriz Tavares Costa-Carvalho[b], Antonio Condino-Neto[a,*]

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[d] Hospital Geral de Pirajussara, São Paulo, SP, Brazil
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[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

ORIGINAL ARTICLE

http://dx.doi.org/10.1590/1984-0462/;2017;35;1;00013

NEWBORN SCREENING FOR SEVERE COMBINED IMMUNODEFICIENCIES USING TRECS AND KRECS: SECOND PILOT STUDY IN BRAZIL

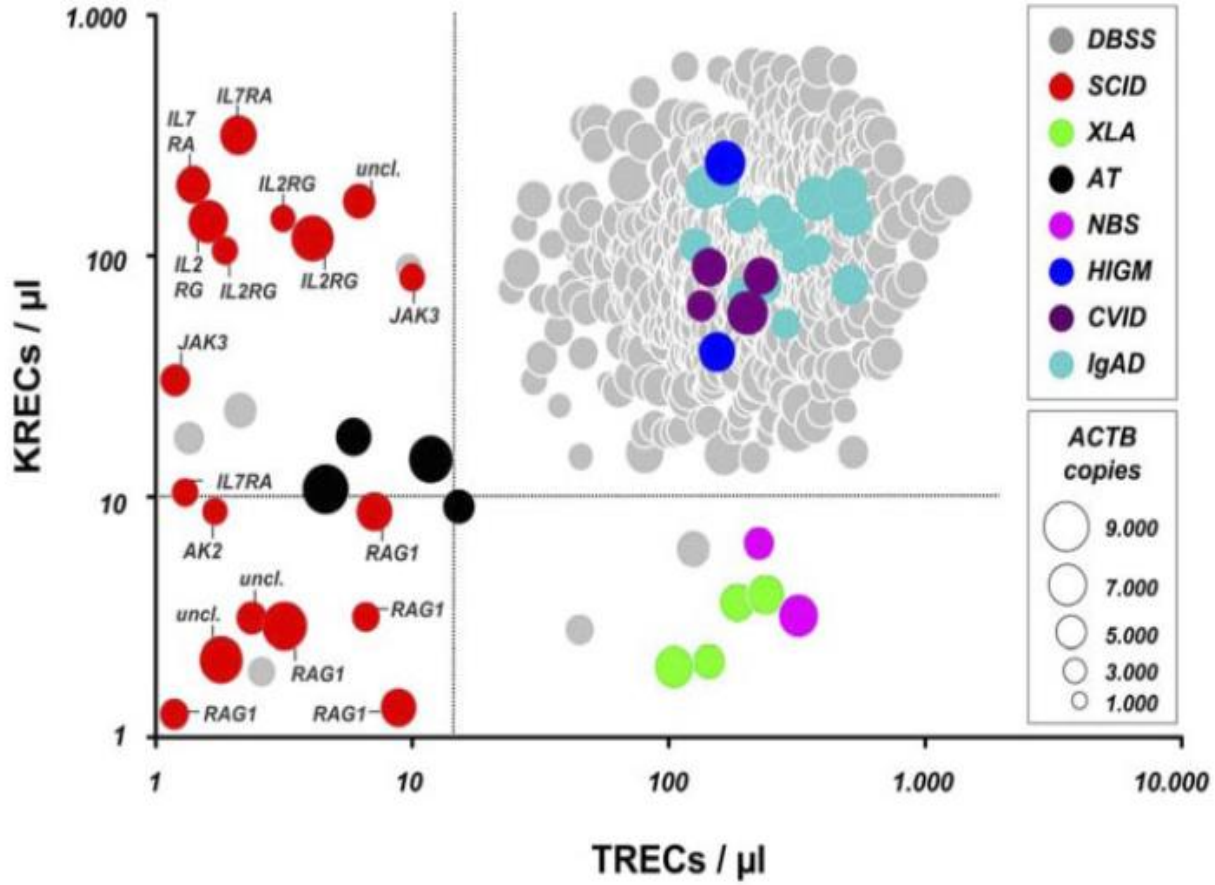
Triagem neonatal de imunodeficiências graves combinadas por meio de TRECs e KRECs: segundo estudo piloto no Brasil

Marilia Pyles P. Kanegae[a]*, Lucila Akune Barreiros[a]*, Jusley Lira Sousa[a], Marco Antônio S. Brito[a], Edgar Borges de Oliveira Junior[a], Lara Pereira Soares[b], Juliana Themudo L. Mazzucchelli[c], Débora Quiorato Fernandes[d], Sonia Marchezi Hadachi[e], Silvia Maia Holanda[f], Flavia Alice T. M. Guimarães[g], Maura Aparecida P. V. V. Boacnin[h], Marley Aparecida L. Pereira[i], Joaquina Maria C. Bueno[b], Anete Sevciovic Grumach[i], Regina Sumiko W. Di Gesu[k], Amélia Miyashiro N. dos Santos[c], Newton Bellesi[l], Beatriz T. Costa-Carvalho[c], Antonio Condino-Neto[a]

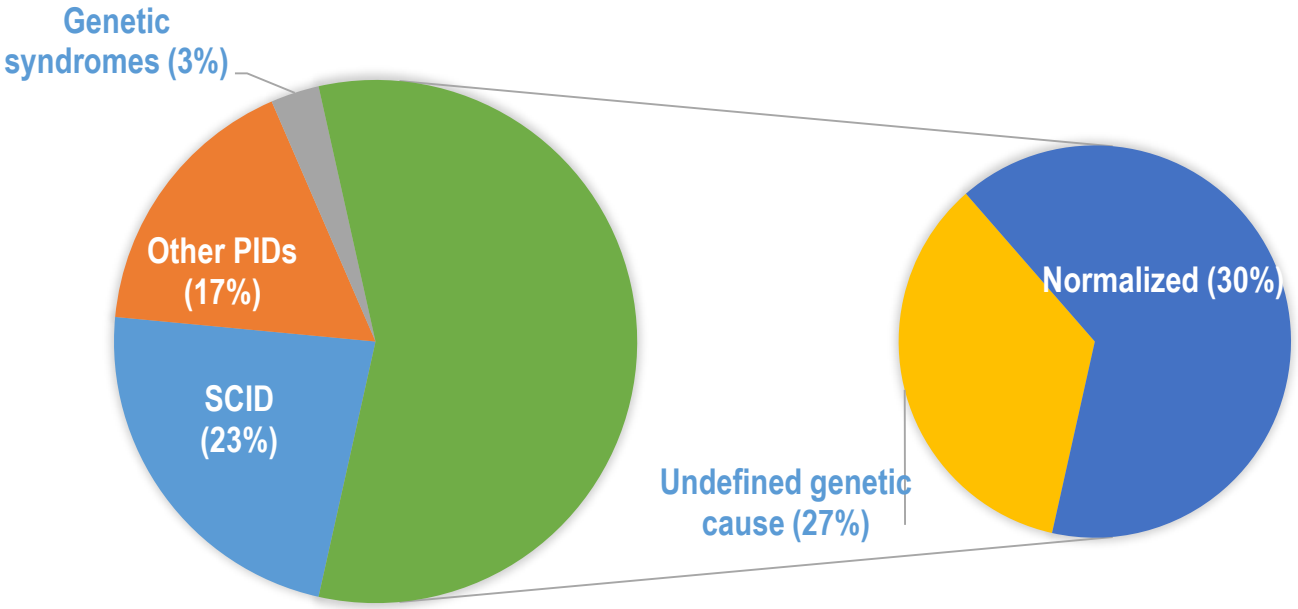
blood
2012 119: 2552-2555
Prepublished online November 30, 2011;
doi:10.1182/blood-2011-08-371021

Neonatal screening for severe primary immunodeficiency diseases using high-throughput triplex real-time PCR

Stephan Borte, Ulrika von Döbeln, Anders Fasth, Ning Wang, Magdalena Janzi, Jacek Winiarski, Ulrich Sack, Qiang Pan-Hammarström, Michael Borte and Lennart Hammarström



Possible causes for low TRECs worldwide



(Maraucher et al., 2017)

(Dorsey & Puck, 2017)

Syndromes with T cell deficiency	
DiGeorge / 22q11.2 deletion	57%
Trisomy 21	13%
Ataxia Telangectasia	3%
CHARGE syndrome	2%

Secondary T lymphopenia	
Congenital heart defect.	25%
Other congenital abnormalities	38%
Fetal hidropsy	13%
Neonatal Leukemia	3%
Maternal immunosuppresive drugs	3-5%
Extreme preterm birth	7%
Idiopatic T lymphopenia	3%

LIH – NBS in Brazil from 2011 to 2019

- **Curently: 4th Pilot Project (PRONAS – 2016-2019)**
  - N. samples: 20.457
  - TRECs and/or KRECs below 10 molecules/uL of blood = 51 (0.24%)

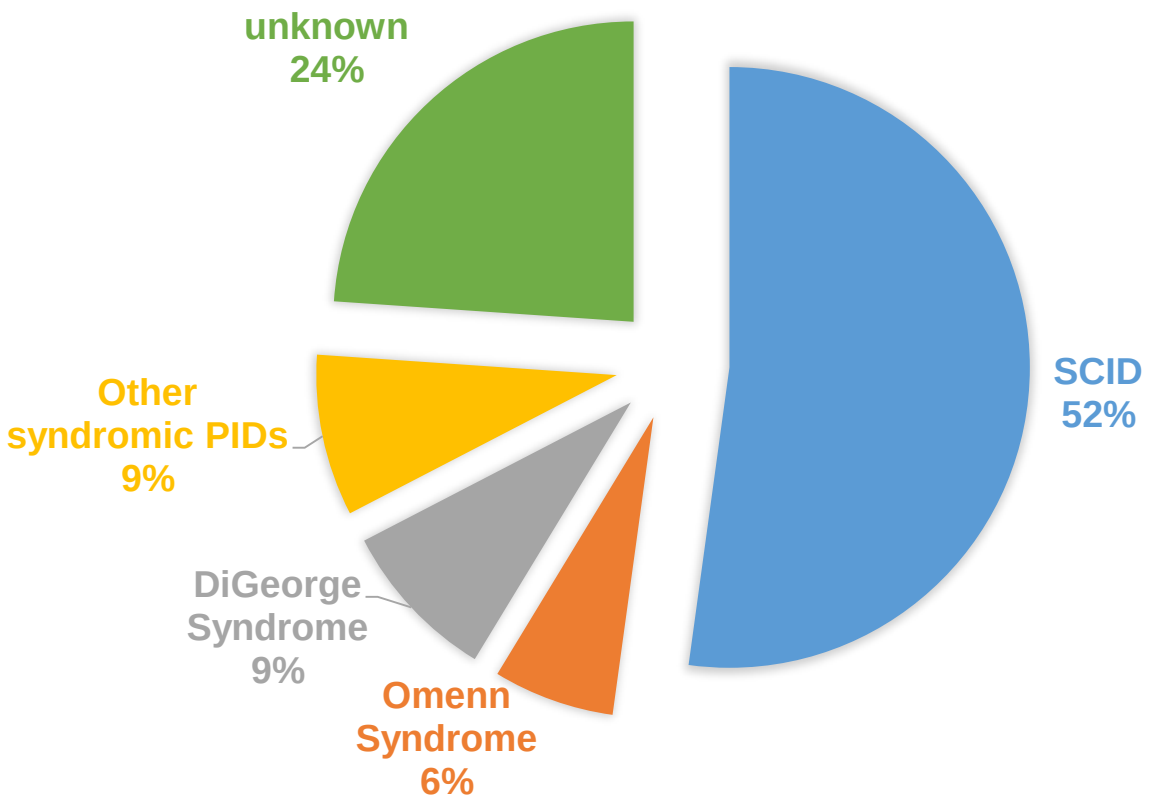
MINISTÉRIO DA SAÚDE

PRONAS
Programa Nacional de Apoio à Atenção da Saúde da Pessoa com Deficiência

- Total samples for NBS from 2011 to 2019: **~ 76,000**
  - **57 altered TRECs from NBS (0.16%)** – **<u>2 were SCID detected at birth</u>**

    (preterms whose values normalized later are not included in this number)
  - **30 pt. with genetic investigation finalized -> genetic variant found in 20 pt. (~67%)**

Identified causes of low TRECs in Brazil



(normalized TRECs are not included in this result)

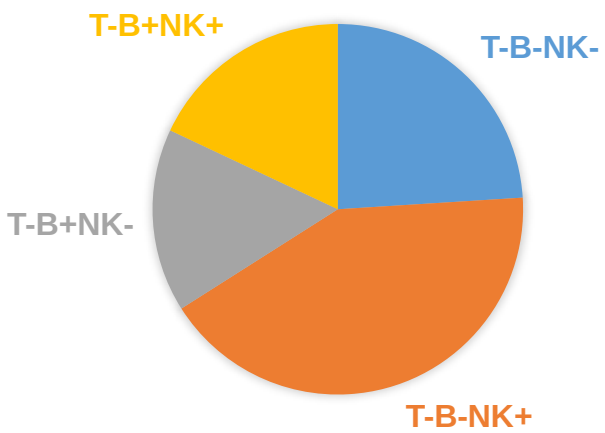
LIH – SCID cases (2011-2019)

- **49 suspected SCID patients (symptomatic or referred due to positive Family history of SCID/PID)**

  (investigated in 2 different institutions – USP and UNIFESP)

- 39% female and 61% males

- Immunophenotypes:



- 61% with known outcome

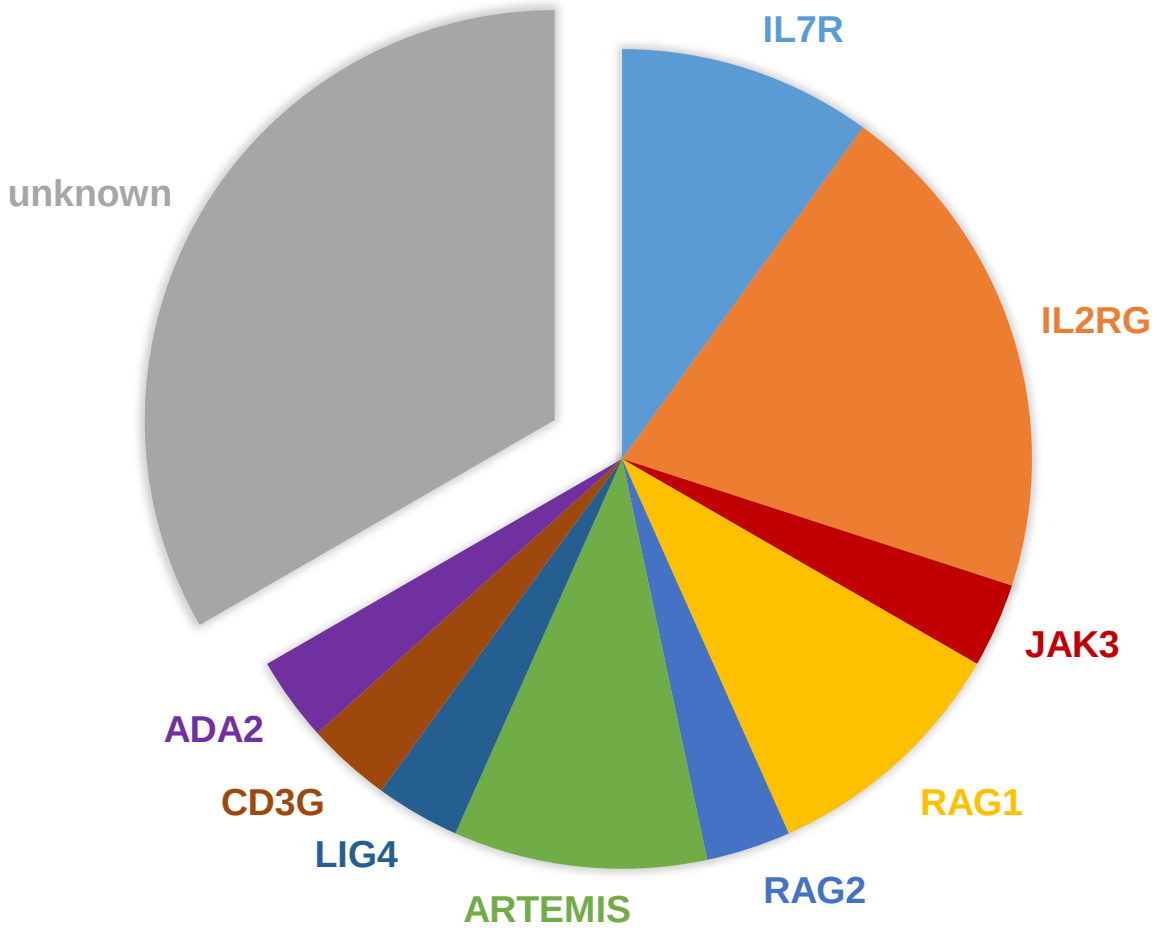
- **Survival post BMT: 47% (14/30)**

Need to **improve communication among investigators**, physicians and transplant team

LIH – SCID cases (2011-2019)

Genetic investigation

- 30/49 pt. = 65% (Sanger or WES)
- Genetic variant identified: 20/30 (67%)
- 33% are still unknown



From screening to genetic counseling

- Primary investigation- simple exams (blood cell count, Igs)
- Relevance of supporting the family throughout the process

TRIAGEM PARA

SCID e AGAMA

Imunodeficiência Combinada Grave
e Agamaglobulinemia

IMUNO GENIC

Laboratório APAE DE SÃO PAULO

VOCÊ TEM

50 MOTIVOS

PARA INDICAR O MELHOR
E MAIS COMPLETO

TESTE DO PEZINHO

O Teste do Pezinho SUPER + SCID e AGAMA
detectam juntos cinquenta doenças.

Este material é destinado a profissionais de saúde.

IMUNO GENIC

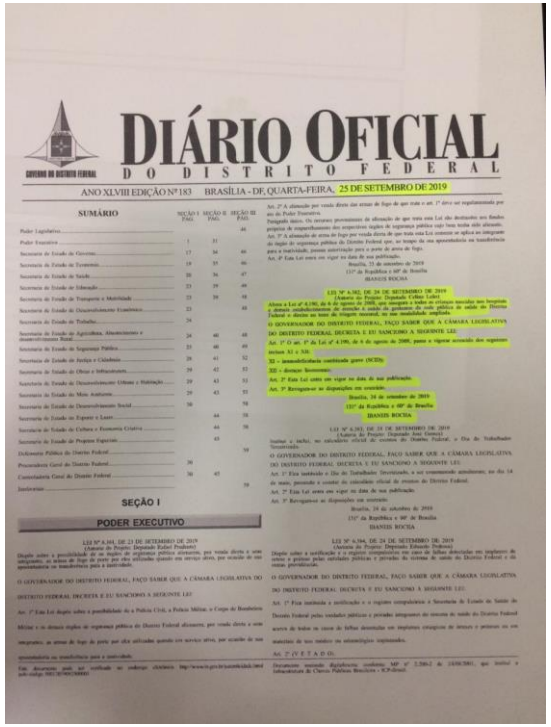
Laboratório APAE DE SÃO PAULO

Rua Loefgren, 2109 – Vila Clementino
04040-033 – São Paulo/SP
Tel.: (11) 5080 7023
e-mail: testedopezinho@apaesp.org.br
www.apaesp.org.br

PERSPECTIVES

AMB
Associação Médica Brasileira

ANS Agência Nacional de Saúde Suplementar



DIÁRIO OFICIAL
DO DISTRITO FEDERAL

ANO XLVIII EDIÇÃO Nº183 BRASÍLIA - DF, QUARTA-FEIRA, 25 DE SETEMBRO DE 2019

SUMÁRIO

SEÇÃO I

PODER EXECUTIVO

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ORIGINAL ARTICLE

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

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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).

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ARTIGO ORIGINAL

http://dx.doi.org/10.1590/1984-0462/;2017;35;1;00013

TRIAGEM NEONATAL DE IMUNODEFICIÊNCIAS GRAVES COMBINADAS POR MEIO DE TRECS E KRECS: SEGUNDO ESTUDO PILOTO NO BRASIL

Newborn screening for severe combined immunodeficiencies using TRECs and KRECs: second pilot study in Brazil

Marilia Pyles P. Kanegae[a,*], Lucila Akune Barreiros[a,*], Jusley Lira Sousa[a], Marco Antônio S. Brito[a], Edgar Borges de Oliveira Junior[a], Lara Pereira Soares[b], Juliana Themudo L. Mazzucchelli[c], Débora Quiorato Fernandes[d], Sonia Marchezi Hadachi[e], Silvia Maia Holanda[f], Flavia Alice T. M. Guimarães[g], Maura Aparecida P. V. V. Boacnin[h], Marley Aparecida L. Pereira[i], Joaquina Maria C. Bueno[b], Anete Sevciovic Grumach[j], Regina Sumiko W. Di Gesu[k], Amélia Miyashiro N. dos Santos[c], Newton Bellesi[l], Beatriz T. Costa-Carvalho[c], Antonio Condino-Neto[a]

RESUMO

Objetivo: Validar a quantificação de *T-cell receptor excision circles* (TRECs) e *kappa-deleting recombination circles* (KRECs) por reação em cadeia de polimerase (*polymerase chain reaction*, PCR) em tempo real (qRT-PCR), para triagem neonatal de imunodeficiências primárias que cursam com defeitos nas células T e/ou B no Brasil.
Métodos: Amostras de sangue de recém-nascidos (RN) e controles foram coletadas em papel-filtro. O DNA foi extraído e os TRECs e KRECs foram quantificados por reação duplex de qRT-PCR. O valor de corte foi determinado pela análise de *Receiver Operating Characteristics Curve*, utilizando-se o programa *Statistical Package for the Social Sciences* (SSPS) (IBM®, Armonk, NY, EUA).

ABSTRACT

Objective: To validate the quantification of T-cell receptor excision circles (TRECs) and kappa-deleting recombination excision circles (KRECs) by real-time polymerase chain reaction (qRT-PCR) for newborn screening of primary immunodeficiencies with defects in T and/or B cells in Brazil.
Methods: Blood samples from newborns and controls were collected on filter paper. DNA was extracted and TRECs, and KRECs were quantified by a duplex real-time PCR. The cutoff values were determined by receiver operating characteristic curve analysis using SPSS software (IBM®, Armonk, NY, USA).

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[l]Clínica de Medicina Preventiva do Pará (CLIMEP), Belém, PA, Brasil.
Primeira autoria partilhada entre Marilia P. P. Kanegae e Lucila A. Barreiros.
Recebido em 12 de maio de 2016; aceito em 02 de outubro de 2016; disponível on-line em 08 de março de 2017.

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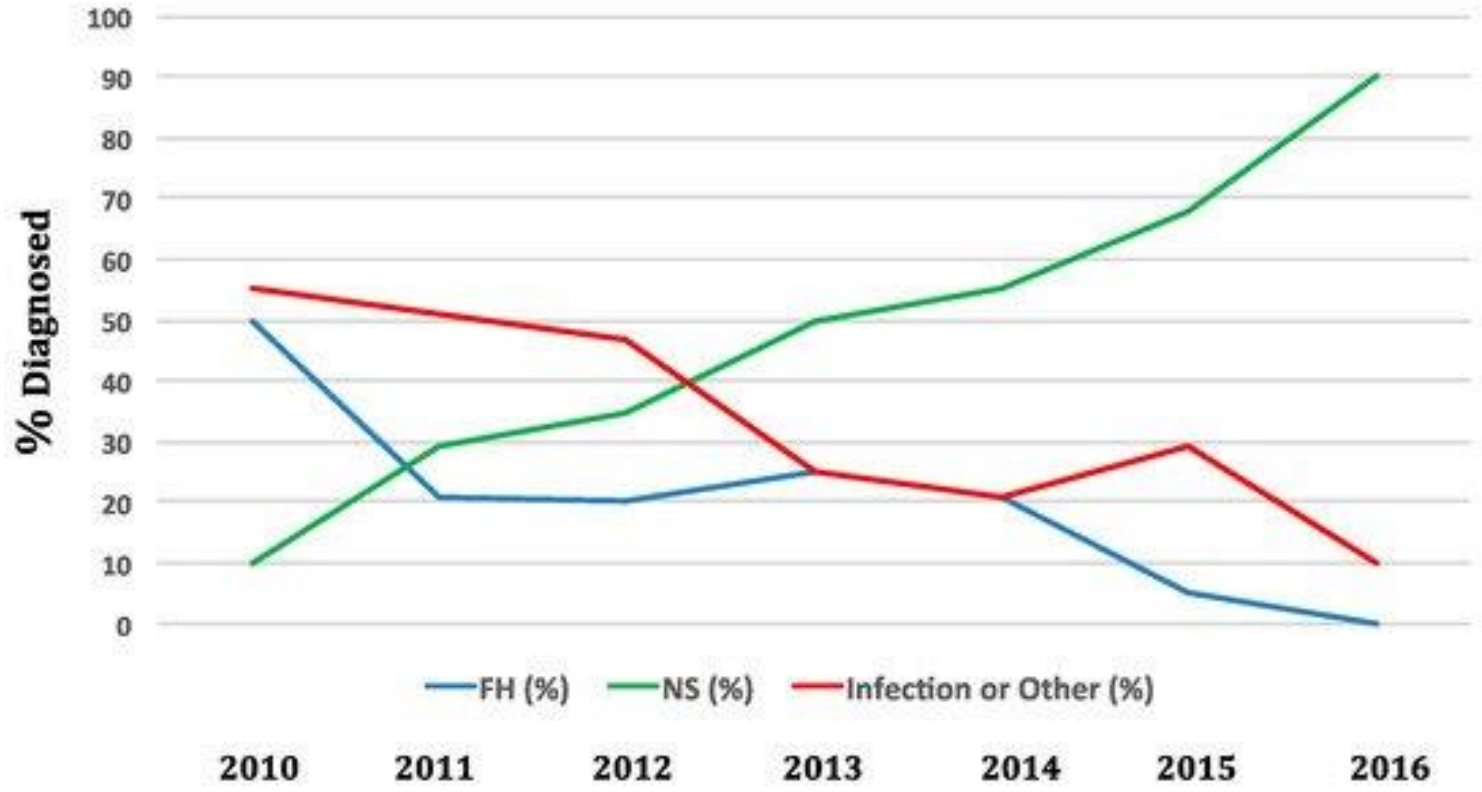
Partners:

Diagnosis, treatment and genetic couseling

- **Find** the patient for confirmatory exams
- **Lymphocyte immunophenotyping with memory subsets**
  - Challenge in Brazil - very few labs perform the full phenotyping (neither public or health insurance cover)
- Referral to BMT – there are still few beds reserved to PID pt. in public hospitals
- Genetic investigation and counseling of the family

Obs.: atypical SCIDs might need additional exams, such as lymphoproliferative response to mitogens and TCR repertoire analysis → very hard to find in Br

Newborn screening for SCID



More cases of low TRECs that are not classic SCID!

(Dorsey & Puck, 2017)