Leiden University Medical Center

M. Blom[1], I. Pico-Knijnenburg [1], S. Imholz[2], J. J. Schulze[3], J. Werner [3] and M. van der Burg [1]

[1]Department of Pediatrics, Laboratory for Pediatric Immunology, Leiden University Medical Center, Leiden, the Netherlands, [2]Centre for Health Protection, National Institute for Public Health and the Environment, Bilthoven, the Netherlands, [3]Department of Research and Development, Epimune GmbH, Belin, Germany

m.blom@lumc.nl

Second tier test options in newborn screening for inborn errors of immunity

Introduction

Newborn screening (NBS) for severe combined immunodeficiency (SCID) and X-linked agammaglobulinemia (XLA) is based on the detection of indirect markers for newly formed T-lymphocytes i.e. T -cell receptor excision circles (TRECs) and B-lymphocytes i.e. kappa-deleting recombination excision circles (KRECs). There is a range of neonatal disorders and conditions that lead to low T- and/or B-lymphocytes around birth, causing NBS for SCID and XLA to be accompanied by secondary findings and false positive referrals.

Objectives

- To explore different second tier options after TREC/KREC analysis
- To reduce the number of secondary findings, increase the positive predictive value of NBS for SCID/XLA and reduce the emotional impact for parents that is associated with a referral procedure (Blom et al. *J Clin Immunol.* 2021)

Methods

1. Second PCR with different primers

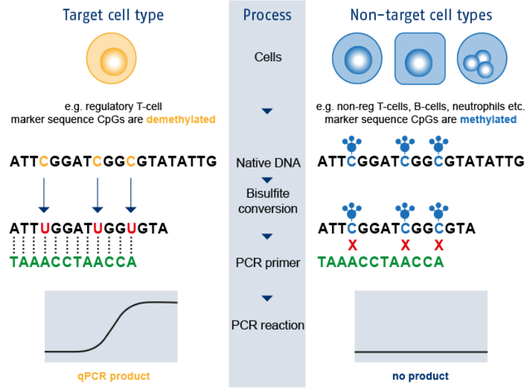
NBS cards of healthy newborns (N=80), newborns with low TREC levels (N=56) and low KREC levels (N=110) measured with the SPOT-it kit (ImmunoIVD) were analyzed with a second TREC/KREC assay (NeoMDx assay PerkinElmer).

2. TREC region sequencing

DNA was isolated for TREC region sequencing from NBS cards of healthy controls (N=12), idiopathic lymphocytopenia cases (N=4) and false positive referrals (N=8).

3. Epigenetic immune cell counting (Epimune GmbH)

Relative quantification of CD3+ T-cell and B-cell counts (BLC) was performed in NBS cards of healthy controls (N=311), newborns with low TRECs (N=58) and newborns with low KRECs (N=103).



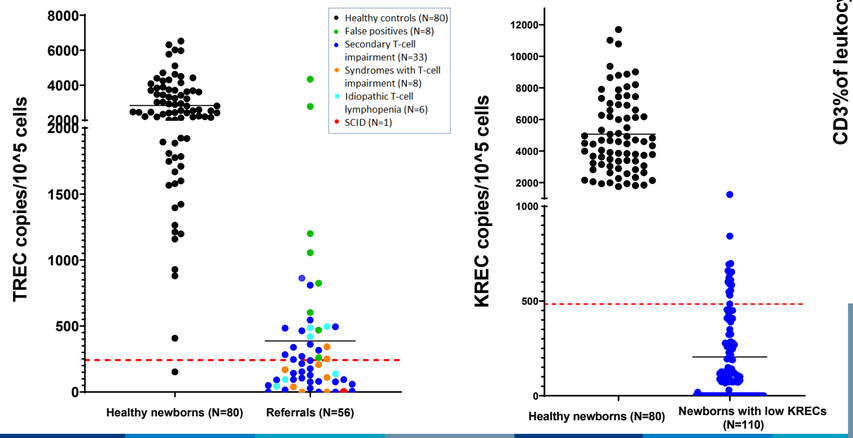
Method 3

Epigenetic immune cell counting was performed by amplification of lymphocyte-specific demethylated genomic regions (Baron et al. *Sci Transl Med*. 2018)

Results

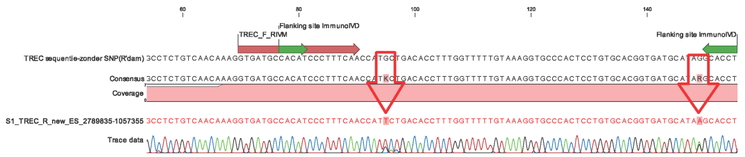
1. Second PCR with different primers

With the second TREC/KREC assay, 45% (25/56) of the newborns with low TRECs (left plot) and 15% (16 /110) of the newborns with low KRECs (right plot) had TREC/KREC levels above the cut-off of the manufacturer (red line).



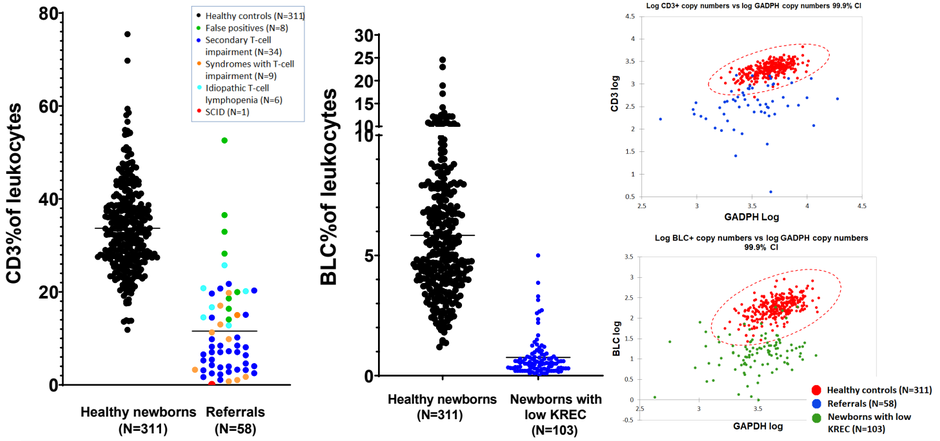
2. TREC region sequencing

Two SNPs were found in the TREC region of four false positive cases **(NC_000014.9:g.22475276G>T, NC_000014.9:g.22386840G>A** red arrows in the figure below**)** leading to a primer mismatch and no TREC amplification with the SPOT-it kit.



3. Epigenetic immune cell counting

26% (15/58) of the newborns with low TREC levels and 17% (18/103) of the newborns with low KREC levels had relative T- and B-cell counts within the 99.9% confidence ellipse of the healthy newborn controls.



Conclusions

- All second tier options have the potential to reduce the number of referrals and secondary findings in NBS for SCID and XLA.
- Second tier testing with NGS with a targeted SCID gene panel or BTK gene analysis are currently being explored as well.
- Sensitivity, specificity, costs, feasibility for the screening laboratories and throughput time should be further evaluated before second tier options can be implemented.

ImmunoIVD · PerkinElmer For the Better · EPIMUNE DIAGNOSTICS