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LETTER TO EDITOR

Abnormal Newborn Screen in a WHIM Syndrome Infant

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To the Editor,

T cell receptor excision circle (TREC) quantification was added to newborn screening in 2008 to identify newborns with severe combined immunodeficiency (SCID). However, the vast majority of infants identified by TREC screening do not have SCID on confirmatory testing [1]. Because of the novelty of the test, there is a paucity of information regarding long-term prognosis and differential diagnosis of non-SCID infants with low TREC levels. Here we present a case of warts, hypogammaglobulinemia, infections, and myelokathexis (WHIM) syndrome that presented on newborn screen with low TREC levels.

A full-term white male was born to a mother with WHIM syndrome, a rare autosomal dominant immunodeficiency disorder caused by gain-of-function CXCR4 mutations that are characterized by panleukopenia due to abnormal leukocyte distribution. The infant developed fever, tachypnea, and hypoxia 30 h after birth and was admitted to the neonatal intensive care unit for empiric intravenous antibiotics pending culture results. Within another 30 h, vital signs had returned to normal and hypoxia had resolved. Blood and cerebrospinal fluid cultures were negative. Physical examination did not reveal any syndromic abnormalities. The initial absolute lymphocyte count (ALC) and absolute neutrophil count (ANC) were 600 cells/μL and 9000 cells/μL, respectively. However, the newborn screen (NBS) detected abnormally low levels of TRECs (Table 1). On day 6 after birth, flow cytometry detected T and B cell lymphopenia. Genetic testing revealed that the patient was a heterozygote for the most common WHIM mutation, *CXCR4* (c.1000C > T (p.Arg334Ter)), which was also present in the mother. An echocardiogram detected patent ductus arteriosus, pulmonary artery stenosis, and an atrial septal defect or patent foramen ovale. Five months after birth, the ANC was 32 cells/μL and prophylactic antibiotics were started. This case illustrates that neutropenia, a common laboratory finding in WHIM syndrome, may be absent at birth, possibly due to stress-induced neutrophil mobilization from the bone marrow. Patients, however, might still be identified by low TREC levels on the newborn screen.

Evaluation of TRECs has been added to NBS programs in all states to be a screening tool for severe combined immunodeficiency (SCID). In the setting of an abnormal TREC level, flow cytometry is performed to evaluate T, B, and natural killer cells, as well as naive and memory phenotype T cells. Among three million consecutive infants tested in California prior to 2017, 562 viable infants were referred for flow cytometry resulting in 213 (37.9%) of those infants with T cell lymphopenia and 50 (8.9%) diagnosed with SCID [1]. Therefore, the majority of infants with low TRECs are not diagnosed with SCID or lymphopenia.

If SCID is ruled out, as in our case, the clinical relevance of an abnormal TREC level is not always clear. Infants identified on NBS are outliers with results well below three standard deviations below the mean and therefore merit careful consideration. In a small case series with limited follow-up, idiopathic T cell lymphopenia represented a generally benign condition [2]. In other populations, however, low TREC levels have been associated with disease progression, increased infection rates, complication rates, and mortality [3]. Additionally, a

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Table 1 Patient laboratory values

Category Days after birth	1	3	6	10	Reference range
WBC cells/μL	10,800	2900 (L)		3200 (L)	5000–21,000 cells/μL
ANC cells/μL	9000	1020 (L)		220 (L)	1500–10,000 cells/μL
ALC cells/μL	600 (L)	1250 (L)		2460	2000–17,000 cells/μL
TRECs Ct value	34.47* (H)	32.35		32.80	< 34
RNAse P Ct value	22.03	23.53		24.65	< 25
TRECs/μL	4.7 (L)	21.9		15.9	> 6.7 copies/μL
TRECs/μL percentile**	0.095	2.97		1.41	
CD3+ cells/μL			1817 (L)		2500–5500 cells/μL
CD3+CD4+ cells/μL			1432 (L)		1600–4000 cells/μL
CD3+CD8+ cells/μL			335 (L)		560–1700 cells/μL
CD19+ cells/μL			240 (L)		300–2000 cells/μL
CD45+ cells/μL			2582		2000–17,000 cells/μL
CD16+CD56+ cells/μL			482		100–1900 cells/μL

ALC absolute lymphocyte count, *ANC* absolute neutrophil count, *Ct* cycle threshold number, *RNAse P* ribonuclease P, *TRECs*, T cell receptor excision circles, *WBC* white blood count

*Day 1 TRECs Ct, RNAse P Ct, and TRECs/μl are the triplicate medians. **Percentile based on TRECs Ct values from all samples received in 2018 with valid RNAse *P* values processed by the Maryland State Laboratory

wide variety of non-SCID primary immunodeficiencies has presented with low TREC levels [1]. This has led some to consider whole-genome sequencing (WGS) for infants with abnormal NBS results. Thoughtful consensus statements, though, advocate that routine WGS has not been shown to be in the best interest of the child, their parents, or society at large; WGS should only be used as part of a targeted approach. Ultimately, the detection of low TREC levels in NBS leaves immunologists to provide individualized assessments and tailored counseling for each patient. With that purpose, this case further expands the differential diagnosis of an abnormal TREC screen.

The TREC assay may be especially important for identifying WHIM syndrome patients since patients often present without a family history of the disease, neutropenia, or lymphopenia. In our case, the mother was known to have WHIM syndrome; however, approximately 45% of WHIM cases are caused by de novo mutations [4]. In addition, patients with WHIM syndrome often experience severe congenital neutropenia that can be repeatedly missed since their ANC rises during infection. Additionally, this case indicates that the most penetrant WHIM phenotype, neutropenia, may be absent at birth possibly due to stress-induced neutrophil mobilization from the bone marrow. Lymphopenia affects approximately 80–88% of WHIM patients [4]. In the present case, the $CD3^+$ T cell count on day 6 was 1817 cells/μL, which may or may not have been defined as T cell lymphopenia because published normal values vary [1]. Therefore, assessing a patient's immune system with TREC levels provides additional screening for WHIM patients who may have unaffected parents, or a normal ANC and ALC. These WHIM patients may otherwise go undetected.

The knowledge that low TREC levels may be present in infants with WHIM syndrome may help with our understanding of the pathophysiology of the disease. The specific TRECs evaluated by NBS are the δRec-ψJα signal joint TRECs; these are formed in over 70% of αβ T cells during rearrangement of the *TCRA* gene. Consequently, these are used as a surrogate marker of thymic output. Thus, low TREC levels can be indicative of lymphopenia or impaired αβ T cell lymphopoiesis. In WHIM syndrome, low TREC levels might be anticipated based on frequent concomitant lymphopenia. Indeed, CXCR4 signaling has been shown to affect lymphocyte proliferation and trafficking. While this would certainly result in lymphopenia, our case suggests lymphopenia may not be the only cause of low TRECs. In the present case, our patient's lymphocyte count doubled between days 1 and 3 after birth, while his TRECs increased by more than four-fold. By day 10, the lymphocyte count had continued to increase, yet the TRECs decreased. These disproportional and discordant changes suggest that lymphopenia alone does not account for the TREC levels observed in our patient. In a recent case series of WHIM patients with lymphopenia, all patients evaluated demonstrated a disproportionately low subset of recent thymic emigrants [5]. This indeed would suggest a hyperfunctional CXCR4 receptor impairs lymphopoiesis. Data from human transplant data suggests the natural ligand of CXCR4 (CXCL12) is downregulated in WHIM patients and corresponds with the αβ to γδ T cell ratio. Taken together, CXCR4 signaling may be a factor in the αβ to γδ T cell ratio

and increased signal lymphopoiesis, simultaneously contributing to other causes of CXCR4-mediated lymphopenia.

In conclusion, a low TREC level detected in NBS programs is a useful screening tool for SCID, but it is also a valuable tool for other primary immunodeficiency disorders with associated lymphopenia or impaired T cell development. WHIM syndrome should be included in the differential diagnosis of patients presenting with low TRECs levels. The TREC assay may be particularly useful in WHIM patients who can present without neutropenia, lymphopenia, or a family history of immunodeficiency.

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Author's Contributions ME, MP, and DM provided the medical care for the patient; DM and PM performed the gene sequencing, and ME, MP, PM, and DM wrote the manuscript. All authors read and approved the final manuscript.

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Compliance with Ethical Standards Informed consent was obtained from the parents.

Conflict of Interest The authors declare that they have no conflicts of interest.

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Abbreviations *ALC,* Absolute lymphocyte count; *ANC,* Absolute neutrophil count; *Ct,* Cycle threshold number; *CXCR4,* C-X-C chemokine receptor type 4; *CXCL12,* C-X-C motif chemokine 12; *DBS,* Dried blood spots; *NBS,* Newborn screen; *RNAse P,* Ribonuclease P; *SCID,* Severe combined immunodeficiency; *TRECs,* T cell receptor excision circles; *WBC,* White blood count; *WGS,* Whole-genome sequencing; *WHIM,* Warts, hypogammaglobulinemia, infections, myelokathexis

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