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Short Communication

Postnatal corticosteroid treatment as a risk factor for false positivity in severe combined immunodeficiency newborn screening

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Abstract From 2011, 37 children were referred to a hospital due to low levels of T cell receptor excision circles (TRECs) from newborn screening. Among them, three children were immunologically characterized and followed up to show that postnatal corticosteroid usage may be among the causes of false positivity in TRECs screening.

Introduction

Severe combined immunodeficiency (SCID) is one of the most severe forms of primary immunodeficiency which require urgent evaluation and treatment.[1] With the availability of newborn screening for this disease, early diagnosis and treatment with hematopoietic cell transplantation in specialized centers may be lifesaving and have greatly improved the prognosis of SCID patients.[1]

In 2010, newborn screening using dried blood spot (DBS) sampling for T cell receptor excision circles (TRECs) was first introduced in the US to identify infants with low T cell counts.[2] Since 2011, we have successfully identified and treated infants with severe immunodeficiency from newborns with low TRECs in the Taiwanese newborn screening program.[3] We found that most of the false-positive patients

Abbreviations: TREC, T cell receptor excision circle; SCID, Severe combined immunodeficiency; DBS, Dried blood spot; LGA, Large for gestational age.

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were premature newborns. Interestingly, we also discovered that another false-positive patient group with low TRECs in the screening test was term infants treated with corticosteroids. They had normal CD3 cell counts in the initial evaluation, and their TRECs increased to normal levels faster than other screen-positive groups.

Method

Study population

This study was conducted retrospectively and reviewed children from 2010 to 01-01 to 2021-11-30. We enrolled children referred to National Cheng-Kung University Hospital (NCKUH) for primary immunodeficiency diseases. Children were referred after failing DBS tests. We then arranged examinations including complete blood count, white blood cell (WBC) differential counts and lymphocyte subgroup analysis. This study was approved by the Institutional Research Board (IRB) of National Cheng Kung University Hospital (B-ER-109-210).

Study method

Medical records, including the complete admission history, outpatient department records, laboratory data, prescriptions, and antenatal prescriptions, were reviewed and compared in newborn patients with positive screening test results.

In screening for SCID through DBS testing, the TREC count threshold was set at 50 copies/microliter (cp/μL). A 3.2 mm disk of DBS from NBS card was punched into a 96-well plate and washed with 100 μL DNA purification solution one, followed by washing with 100 μL DNA elution solution two (Qiagen Science). DNA was eluted by adding 60 μL sterile water and shaking at 99 °C for 30 min. Real-time quantitative polymerase chain reaction (RT-PCR) for detecting TRECs was performed using LightMix Modular TREC and LightCycler® 480 Probes Master. The reactions were carried out on a LightCycler® 480 system. The copy numbers of TRECs were determined based on standard curves. DBS testing was performed within the first week of life for all newborns. If the TREC count was below the normal range, DBS test was repeated two weeks later. Additional DBS test for TRECs was performed two weeks after the second DBS test. Due to their extremely low TRECs (lower than one copy/μL), none of the confirmed SCID patients underwent third DBS test before receiving flow cytometry examination.

Clinical data, including the WBC count and cell markers (CD3, CD19, CD4, CD8, CD3-/CD (16 + 56)+) were acquired from blood samples and by flow cytometry. Diagnosis of SCID was confirmed when CD3+ lymphocytes were absence from flow cytometry. Chest plain films were applied on every referred child and serum calcium levels were also tested. Multiplex ligation dependent probe amplification was performed to diagnose DiGeorge disease.

Statistical analyses

Clinical data and TRECs were compared by using Mann-Whitney U test. A P value < 0.05 was considered statistically significant. TRECs between groups in sequence were compared by using Kruskal-Wallis Test. All analyses were carried out using IBM SPSS Statistics version 26.

Result

In total, 37 patients were referred due to low TRECs in SCID screening. Among them, four infants were diagnosed with SCID. These patients' families were not genetically related. All SCID patient were asymptomatic before being diagnosed with SCID. Among the non-SCID patients, eighteen were premature infants and three patients had DiGeorge syndrome. Two mothers took disease-modifying antirheumatic drug due to their systemic lupus erythematosus. One of them took azathioprine and the other took glucocorticosteroid with hydroxychloroquine (Table 1).

The three term infants had very small thymus shadow in their chest plain films. Gene surveys were hence performed to exclude the diagnosis of DiGeorge syndrome. Moreover, they were diagnostically worked-up through multiplex ligation-dependent probe amplification analysis or DNA microarray (Table 1).

A term baby had low TREC count at the first and the second DBS test. He was a large for gestational age (LGA) newborn and infant of a diabetic mother. Severe hypoglycemia (<10 mg/dL) was recorded and intravenous glucose infusion was given. Corticosteroid was prescribed due to refractory hypoglycemia and was discontinued before he was referred. His cytometry results revealed that lymphocyte subsets of CD3, CD19, CD4, CD8, and CD3-/CD (16 + 56)+ were within normal ranges. We arranged another DBS about two weeks after previous DBS test and the result was 166.5 cp/μL. The second infant was admitted because she was small for gestational age and had respiratory distress and hypoglycemia. Her serum glucose level was around 50 mg/dL despite optimal amount of formula milk with calory fortifier. Her insulin, free thyroxine and thyroid-stimulating hormone levels were normal. However, morning cortisol level was low, and corticosteroid was prescribed later. Her results of flow cytometric analysis were normal, which made the diagnosis of SCID unlikely. We also observed a rebound in TRECs after cessation of corticosteroid, which increased from 4.7 cp/μL to 369.6 cp/μL. The third infant was an LGA newborn found to have spontaneous desaturation episodes shortly after birth. Hypoglycemia was found after admission. Glucose infusion, and intravenous corticosteroid were given but in vain. His glucose level was stabilized after we prescribed glucagon. His cytometry results were also normal for lymphocyte subpopulations. TREC count after cessation of corticosteroid was 64.4 cp/μL. All three infants shared a history of corticosteroid treatment and a rebound in TRECs after discontinuing corticosteroids.

As corticosteroid treatment reduced TRECs from newborn while prematurity was the most common cause of low TREC results, we compared TRECs in sequence between premature infants with and without corticosteroid prescription, term infants with corticosteroid prescription and SCID patients (Fig. 1). The first and the second TRECs among three groups were similar except SCID patients' TRECs, which were very low in comparison with TRECs of other groups. The third TRECs of term infants with

Table 1 Clinical and laboratory data between different groups.

	SCID	Prematurity without steroid use	Prematurity with steroid use	Term with steroid use	Immunomodulator used	DiGeorge syndrome	Others
Patient number	4	15	3	3	2	3	7
Sex, male (%)	75%	80%	33%	66%	50%	100%	29%
BW (gm)	3040.00 ± 532.54	* 1120.40 ± 497.92	683.33 ± 85.33	3273.67 ± 2090.82	2781.00 ± 281.43	3025.00 ± 521.00	2714.29 ± 363.32
Age (weeks)	38.00 ± 1.00	* 28.21 ± 4.64	24.00 ± 1.00	34.67 ± 4.16	38.50 ± 0.71	37.00 ± 1.73	37.86 ± 1.35
WBC (10^3/μL)	9366.67 ± 3203.64	6660 ± 1777.16	10333.33 ± 5519.36	8166.67 ± 2672.70	5250.00 ± 1343.50	10566.67 ± 4215.84	6442.86 ± 2021.43
CD3 (%)	2.90 ± 2.43	* 54.00 ± 14.47	48.23 ± 9.44	71.87 ± 6.03	59.1 ± 29.42	37.70 ± 12.88	* 44.41 ± 19.77
CD19 (%)	66.98 ± 19.79	* 36.66 ± 13.88	42.27 ± 6.02	17.23 ± 11.49	32.65 ± 33.59	43.30 ± 7.88	* 32.40 ± 13.43
CD4 (%)	2.33 ± 2.08	* 35.44 ± 11.90	32.03 ± 8.26	49.47 ± 7.50	37.30 ± 31.82	24.30 ± 10.97	* 33.96 ± 16.44
CD8 (%)	0.00 ± 0.00	* 17.84 ± 4.99	23.73 ± 6.93	21.80 ± 5.31	18.20 ± 0.85	12.57 ± 5.87	14.04 ± 10.01
TREC 1 (cp/μL)	0.03 ± 0.06	* 27.91 ± 8.76	32.13 ± 3.95	26.27 ± 17.58	24.50 ± 34.51	33.57 ± 15.75	* 19.2 ± 13.43
TREC 2 (cp/μL)	0.10[a]	22.01 ± 10.25	18.90 ± 9.48	7.33 ± 2.40	15.20 ± 21.35	29.00 ± 14.99	17.33 ± 10.05
TREC 3 (cp/μL)	[b]	21.68 ± 6.98	28.3[a]	200.17 ± 155.36	38.90[a]	20.83 ± 13.80	23.85 ± 8.04

BW: body weight at birth, WBC: white blood cell, TREC: T cell receptor excision circle, cp: copies, Immunomodulator use: infants whose mothers took immunomodulators during pregnancy, *: p value < 0.05 compared with the SCID group, a: only one infant received 2nd DBS, b: no infant received 3rd DBS.

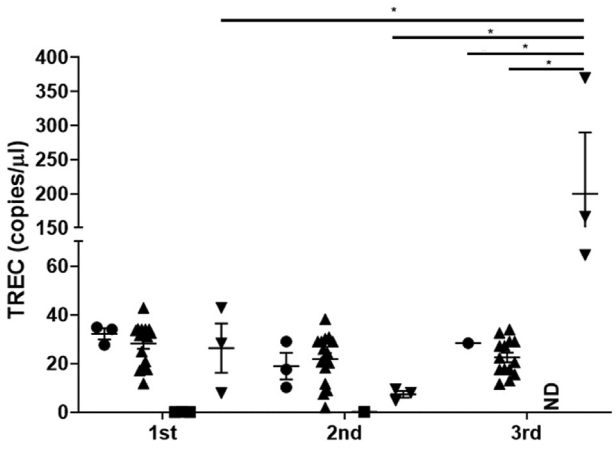

Figure 1. TREC trends among different diagnosis groups (■: SCID (severe combined immunodeficiency); ●: Prematurity, with steroids; ▲: Prematurity, without steroids; ▼: Term, with steroids; *: p value < 0.05).

corticosteroid treatment were significantly higher than those of the other two groups, and were also higher than previous two TRECs.

Discussion

Primary immunodeficiency disorders are often revealed through recurrent infection.[4,5] For SCID, early protective isolation followed by hematopoietic cell transplantation is still the best strategy for patient survival.[1] Newborn TREC assay enables early intervention.[1] In addition to SCID, several disorders can result in a low TREC condition. These include DiGeorge syndrome, trisomy 21, trisomy 18, CHARGE syndrome, VACTERL association, ataxia-telangiectasia, and Jacobsen syndrome. Premature newborns had higher rates of false positives than term newborns according to previous reports on the TREC assay.[2] Less T-lymphocyte precursors undergoing gene rearrangement might be the cause of low TRECs in premature infants. Moreover, corticosteroids, which are often used antenatally in premature infants, reduced TRECs by downregulating T-cell maturation. As we found no apparent rebound in three times of TREC follow up in premature newborns, we surmised that it is due to the counteracting effects of corticosteroid cessation, and prematurity of T lymphocytes. On the other hand, the impact of corticosteroid may be correlated with its dose. In our term infants with corticosteroid group, their indications (hypoglycemia or adrenal insufficiency) require higher dose of corticosteroid than prematurity infants (hypotension or respiratory distress syndrome). As human TRECs are highest in infancy and decrease rapidly with age,[6] TRECs are expected to return to normal ranges when prematurity newborns grow up with cessation of corticosteroid and decline when they grow older.

One patient's mother took azathioprine, of which the active metabolites consist of thioguanine nucleotide, which may affect the T cell proliferation 1 week after taking the drug.[7] Another mother took prednisone and hydroxychloroquine. Considering the immature liver and renal function and the long-term intrauterine exposure to these immunosuppressive drugs of these infants, TRECs could take longer times to rebound.

Corticosteroids affect multiple proinflammatory genes by binding to and blocking their promoter sites. Moreover, corticosteroids directly induce T lymphocyte apoptosis.[8] Together, these effects lead to a low TREC count in newborns antenatally treated with corticosteroid. This report, for the first time, highlighted the importance of postnatal corticosteroid treatment in causing the false positive results in term neonates.

Attempts to reduce false-positive results from TREC assays have been proposed[9] by using second-tier next-generation sequencing (NGS) in SCID screening. This report revealed that postnatal glucocorticoid usage in newborns can cause significantly low TRECs. A rebound in the TRECs is a signature finding after discontinuing corticosteroids. By investigating the medication history of corticosteroid use and repeating DBS test after corticosteroid cessation, we can recognize false positives induced by corticosteroids, spare children from unnecessary phlebotomy and avoid costs of flow cytometry or NGS. In this study we also found no trend of rebound between three times of DBS in prematurity newborns and confirmed SCID infants, both of which are distinct from the changes of TRECs in term infants treated with corticosteroids. The characteristic patterns revealed by this study hence are helpful in lessening false alarm and avoiding misdiagnosis in interpreting TREC screening results.

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