AIJR Preprints
Section: Working Paper
Article Id: 336, Version: 1, 2021
DOI: https://doi.org/10.21467/preprints.336
URL: https://preprints.aijr.org/index.php/ap/preprint/view/336

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NOT PEER-REVIEWED

Version 1: Received: 20 August 2021 / Approved: 21 August 2021 / Online: 22 August 2021

Value of Newborn Screening Programs for Severe Combined Immunodeficiency

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ABSTRACT

Severe Combined Immunodeficiency Disease (SCID) is life-threatening disease of infancy and childhood characterized by recurrent infections and failure to thrive. Given the modern medical progress made available for treating SCID, early identification of these children is paramount to their wellbeing and overall survival into adulthood. Newborn screening (NBS) programs provide the opportunity to identify SCID patients before life-threatening infections can manifest. The T-cell receptor excision circles (TRECs) assay currently used for SCID screening has been shown to satisfy all parameters of an effective screening test. Its widespread use is indicated by the time-sensitive nature of the disease, its efficacy in reducing morbidity and mortality in these patients, and the cost-effectiveness of prompt recognition versus long-term management. While immensely beneficial, screening tests still hold limitations that require analyzing. Follow-up measures for SCID identification programs have identified ambiguity and inconsistency among testing algorithms across facilities and technical errors that have causes inaccurate results. Considering fewer than 20% of SCID patients report a positive family history and the lethal consequences of disease if left untreated, a screening program is a highly valuable tool for early diagnosis and prompt intervention.

Keywords: SCID, Newborn Screening, Excision Circles

1 Introduction

Infants born with Severe Combined Immunodeficiency Disease (SCID) are born seemingly healthy; yet face imminent danger from the moment they enter this world. These children possess genetic mutations that impede the development and function of immune cells, primarily T-cell, rendering them defenseless against a world of infinite pathogens [1]. Considered a pediatric emergency, SCID is life-threatening disease characterized by recurrent infections, cutaneous manifestations, and failure to thrive [1]. Initial presentation occurs within the first three months of life and, without medical treatment, often die before age two [2].

How to Cite:

Elisa J. Pirozzi, "Value of Newborn Screening Programs for Severe Combined Immunodeficiency". *AIJR Preprints*, 336, Version 1, 2021.

Therefore, prompt diagnosis is critical for survival in these patients. Newborn screening (NBS) programs provide an opportunity for that early identification and treatment of SCID patients.

Characterization of this disease includes patent disruption of T-cell differentiation and proliferation [3]. B-cells and Natural Killer (NK) cells may also be affected depending on the pathway affected by mutation [1]. The ensuing deficiency in antigen recognition and antibody production renders the patient compromised against invading pathogens. Incidence of SCID worldwide is estimated around 1 in 100,000; however, this number is suspected to be underreported [4]. The mutations implicated in SCID can be x-linked, autosomal recessive, or sporadic in nature and considerable heterogeneous pathogenicity exist amongst affected individuals. Regardless, lymphocytic dysfunction remains the universal consequence. Disruptions within 15 known genes and immune pathways have been identified to date [1, 7]. These include *d*efective lymphokine signaling, thymic dysgenesis, defective TCR and Ig gene rearrangement, toxic metabolites mediated apoptosis, or cell signaling before TCR selection [1]. The most common genetic mutation, accounting for 50% of SCID cases, is loss of the interleukin (IL) receptor common y-chain encoded for on the X-chromosome [5]. This deficit eliminates lymphocyte proliferation and apoptotic signals, class switching ability, and T-cell receptor rearrangement; critical processes to the immunogenic response [5]. Similarly, autosomal recessive mutations include deficiencies in JAK3, ADA, ζ chain–associated protein, RAG1/2 and MHC II for example [1]. Disruptions in these pathways cause varying degrees of malfunction among T-cell, B-cell, and NK cells.

Symptoms of immune system malfunction generally arise before three months of age in these patients. The most commonly present with failure to thrive and repeat prolonged, progressive bronchiolitic type illness [1, 2]. Given their lack of defenses, common and opportunistic pathogens (i.e. Respiratory syncytial virus (RSV), cytomegalovirus (CMV), Pneumococcal jiroveci or invasive fungal infections) often trigger the swift decline and death within if left untreated [1, 2]. Physical exam may reveal atrophic lymphoid tissue; however, considering lymphoid structures in infants are generally very small, it can be difficult to appreciate their absence [2]. Chest x-rays will often exhibit lack of thymic shadow and hyperinflation with interstitial pneumonia [2]. Recognizing these manifestations is important for early diagnosis and subsequent referral for bone marrow transplant (BMT). By However, waiting for clinical symptoms to arise before diagnosis is possible has deleterious consequences.

2 Newborn SCID Screening Methodology

Recognizing SCID clinically is critical; yet, staving off symptomatic presentation is ideal. As such, NBS provides an opportunity to identify SCID infants prior to life-threatening infection development. Early screening would also expedite treatment and mitigate possible sequelae of severe infections. Considering the vast majority of infants tested will not be affected by SCID, testing sensitivity and specificity must be extremely high in order to be a useful tool. False negatives due to low sensitivity would result in missing affected individuals who would then be forgoing critical treatment, rendering NBS moot [14]. Similarly, low

specificity would result in false positives contributing to unnecessary family distress, higher costs, and further investigations [4]. Therefore, simply assessing total lymphocyte count would be inappropriate since some variations of SCID allow for normal quantities of B- and NK cells that may mask the primary deficiency of T-cells. Utilizing the same dried blood spot (DBS) drawn for other NBS genetic panels, the test exploits the phenotypic T-cell lymphopenia seen regardless of the exact mutated genotype and other lymphocytes' level of involvement. The T-cell receptor excision circles (TRECs) assay currently used for SCID screening has been shown to satisfy these parameters [6].

During development, T-cells undergo T-cell receptor (TCR) recombination to provide the repertoire diversity required for recognition of innumerable pathogens. This process involves DNA excision, rearrangement, and ligation to create TCR combinations unique to each T-cell. Superfluous excised DNA elements are joined at its ends, forming TRECs [4]. These byproducts, therefore, serve as a biomarker for naïve T-cell formation rate and indicate the status of the developing immune system. Polymerase chain reaction (PCR) primers that target the point of union identify the TRECs [4]. A real time quantitative polymerase chain reaction applied to the DBS sample quantifies the PCR product, TRECs in this instance [6]. The results are then qualified as normal, abnormal, or borderline based on an algorithmic cut-off value set by the testing location. Low concentrations of TREC copies designate an abnormal value and are indication for further investigations to query SCID. Under current US Department of Health guidelines, children with borderline results must be resampled; all confirmed abnormal results must see an immunologist within three days of result receipt [7]. This algorithm ensures affected infants receive appropriate care in a timely manner, since effective intervention is time sensitive.

3 SCID Screening Justifications

Making NBS for SCID routine certainly has clinical advantage. Since 2008 when the initial SCID NBS was implemented in Wisconsin, the US Department of Health and Human Service Secretary's Heritable Disorders of Newborns and Children Advisory Committee has successfully added SCID NBS to the existing genetic screening panel in all states [8]. The timeliness was in part due to how well SCID met the Wilson and Jungner criteria set for determining merited additions to the NBS panel. These conditions state the disorder must have indistinct physical exam features, an early asymptomatic period, high burden of untreated disease, and significantly improved mortality and morbidity upon intervention of accessible treatment [4]. Unquestionably, SCID meets each one and, therefore, has resounding justification for universal NBS.

The most significant benefit to screening for SCID is saving a life. Curative treatment is available for these patients, but recognizing them before life-threatening infection occurred was difficult prior to NBS. Hematopoietic stem cell transplantation (HSCT) functionally reboots the child's immune system giving them the ability to fight infection as a normal child can. The survival rate among SCID patients post HSCT is between 95-100% [9,10]. This extraordinary success rate hinges on an early diagnosis and prompt intervention. Ideally, HSCT

should be done before the child is three months old; survival rate is upwards of 96% [10]. Unfortunately, delayed diagnosis and intervention sees that number drop steeply to 66% [7, 10]. The older a child is, the greater likelihood of prior pathogen exposure and a severe infection developing. This complicates BMT as it requires immunosuppression; a life-threatening situation for patients with standing infection. Additionally, patients older than three months or with a history of infection generally have less successful BMT and often require booster transplants later in life [9]. This discrepancy highlights the crucial impact early screening makes. Screening with the TREC assay facilitates the earliest postnatal identification of SCID, expediting HSCT and improving patient outcomes.

In addition to lowered mortality, morbidity in these patients also declines with prompt SCID identification and treatment. Decreased instance of infection results in fewer sequelae that may negatively impact the child's health later in life, such as respiratory track damage or permanent organ damage [6,11]. For example, CMV infection carries risk of long-term brain, liver, and spleen damage, as well as growth impairment. Overall, patient prognosis and well-being significantly benefit by implementing TREC assay screening.

Identifying SCID patients is the primary target of the TRECs assay, but the test is also able to detect other immunologic abnormalities that expedite T-cell loss from peripheral circulation [4]. For example, DiGeorge syndrome, ataxia telangiectasia, CHARGE syndrome, and vascular leakage syndromes have associated T-cell lymphopenia and are commonly detected through the TREC assay [7]. If one test has the ability to flag multiple disorders without detriment to its intended target, increases its clinical advantage, applicability, and value.

Not only is TREC assay methodology efficient diagnostically, but is also in its exploitation of the established heel prick test. This streamlines the process for making the screening program routine and is also cost effective. Including equipment, labor, and materials, testing one infant via the TREC assay costs about $5 USD [12]. Conversely, delaying SCID diagnosis accrues roughly $2 million USD within the infant's first year due to the repeated, prolonged and intensive hospitalizations necessitated by severe infection [9]. The cost of care for one late SCID diagnosis would surpass the cost of screening entire cohorts in some locations [9]. Therefore, this highly sensitive, inexpensive test could notably decrease expenditures by decreasing frequency of hospitalizations and supportive treatment, reducing healthcare consumption, and minimizing risk of long-term costly sequelae [6,13].

4 SCID screening Limitations

The clinical benefits of SCID NBS are striking, but that does not preclude the current method from having its limitations. All screening programs require continuous monitoring so appropriate adjustments can be made to improve efficacy, outcomes, and cost benefit [4]. Follow-up measures for SCID screening programs have identified ambiguity and inconsistency among testing algorithms across facilities. Diagnostic performance is impacted by TREC cut-off value inconsistency [6]. Having differing values characterizing "normal", "abnormal" or "borderline" stratification permits the possibility of missing infants whose TREC level would

have raised concern under other parameters. Here exists potential for diagnostic inconsistency and skewed program efficacy measurements. Conversely, higher thresholds tend to increase frequency of false positives and "borderline" results, expanding the need for retesting and contributing to raising testing cost [14]. Since classification then determines the algorithm's follow up measures for a specific result, there is risk for inappropriate decisions to be made regarding need for reassessment or even treatment. Furthermore, if these follow-up protocols are also location dependent, the screening method acquires additional confounding variables. While the HHS set the timeframe for immunologist referral, the provision of follow-up information to all result groups lies at a local level. An adjustment for this limitation is to create clear follow up guidelines for each category applicable location-wide; however, without set TREC cut-off levels a certain amount of inconsistency will remain and a greater quantity of values will be deemed abnormal. Ultimately, more accurate cut-off values are needed in SCID screening.

Depending on the patient cohort under investigation and disease incidence in the area, adapting the cut-off value is reasonable, but structure is again necessary for viability [6]. TREC level variability at birth, especially in premature infants, is such as instance [9]. Called leaky SCID, this cohort exhibit transient T lymphocyte reduction that can fall within the normal TREC value in newborn screening [14]. This too contributes to greater false negatives results and the need for further investigation to ensure affected infants are not overlooked. Therefore, cutoff values for varying gestational ages should be population based. While it is possible to modify screening algorithms to account for pre-term patients through retesting a second DBS, the high burden of further evaluation is again highlighted as a clinical disadvantage [9].

Catchment of non-SCID causes of T-cell lymphopenia also contributes to additional "abnormal" designations that then require further differentiation. This ability to flag other causes one test is cost-effective, efficient, and medically beneficial. Unfortunately, it comes at the expense of prolonging time before definitive treatment, which ultimately is the motivation behind early screening. Most of these other causes require interventions alternative to BMT as appropriate treatment and therefore, must be precisely distinguished by immunologists [7]. This additional step necessitated by the TREC assay's limited specificity results in increased immunologist case load, healthcare resource consumption, and risk of infection with BMT delay.

Correlation between delayed diagnosis and increased infection rates motivated SCID NBS proposals initially. As discussed previously, prompt diagnosis begets faster transplantation, resulting in better outcomes. However, despite being the earliest instance for postnatal SCID identification, the TREC assay screening method does not eliminate the possibility of nosocomial or congenital infection [4]. Circulating maternal IgG antibodies still present in infants at the time of the heel-prick test were thought to provide enough protection against infection until BMT could be completed. However, T-cell deficiencies leave infants particularly unprotected against certain viruses whose destruction is primarily T-cell mediated [2, 4]. For instance, CMV, adenovirus, and herpesviruses remain acutely pathogenic even in

the screened SCID patient population [1, 4]. NBS identifies their need for heightened protective measures (i.e. isolation, supplemental IgG, prophylactic antimicrobials), but does not alleviate this clinical disadvantage, especially if the transmission source is maternal in origin [4].

Depending on the specific genetic mutation causing the immunodeficiency, the TREC assay may not be sensitive enough. For instance, contributory B cell deficiencies would go undetected and increase the possibility delayed diagnosis and treatment [9, 14]. To bolster this limitation, some NBS locations in the US are augmenting the TREC assay with a kappa-deleting element recombination circle (KREC) assay. Screening diagnostic value is improved by expanding the test's reach to include mutations such as late-onset ADA, Nijmegen breakage syndrome, and X-linked agammaglobulinemias [9]. As genetic testing improves identifying additional SCID contributary mutations, further screening adaptations may be required to ensure it remains the best method of identifying all SCID patients.

Technical errors during sample collection impact NBS program value just as intrinsic disadvantages do. During DBS collection, using a heparinized central lines or capillary tubes for sample draw can impact TREC assay levels [7]. Additionally, false negative results have been associated with small blood spots and sample compression during testing [15]. False negatives confer the greatest concern as it means a child with SCID is missed and intervention not initiated. However, inflated abnormal or borderline results also have consequences, including higher referral rates for further evaluation. While it is arguably better to provide a safety net by over-screening, it must balance the resultant necessary retests and higher costs.

5 Conclusion

Considering that fewer than 20% of SCID patients have a family history of the disease and the lethal consequences if left untreated, a screening program is a highly valuable tool for early diagnosis. The TREC assay has shown high sensitivity and good efficacy in identifying T-cell lymphopenia, irrespective of the exact genetic mutation. This broad catchment range beneficially influences prophylactic anti-infection measures and expedition of curative treatment. However, implementation inconsistency across programs acts as a nidus for clinical disadvantages that impact results and follow up measures, undermining of screening's original timely goals. However, further research is needed into streamlining and standardizing the process to facilitate better programs globally, improve analysis, and consistently identify all infants affected by SCID. Ultimately, the possible decreases in mortality and morbidity with NBS in SCID patients certainly bolster the value of making it a routine process.

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