

Neonatal screening for severe combined immune deficiency

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Purpose of review

Severe combined immunodeficiency has been identified as a high-priority disease for inclusion in population-based newborn screening programs. In this review, the justification, advances to date and remaining challenges for universal severe combined immunodeficiency screening are outlined.

Recent findings

Severe combined immunodeficiency is treatable by hematopoietic stem cell transplantation, with best outcome if recognized and treated early in life. Universal screening of newborns could make possible prompt diagnosis and lifesaving treatment for all affected infants. One screening test using the dried blood spots already collected from all newborns involves quantitation of T cell receptor excision circles, and other test methods have been proposed and are being evaluated. Development of screening programs will require integration of screening, contacting infants with abnormal screen results for definitive testing, prompt treatment of affected infants, and outcome tracking.

Summary

Newborn screening for severe combined immunodeficiency is advancing toward pilot trials.

Keywords

bone marrow transplantation, early diagnosis, hematopoietic stem cell transplantation, live vaccine, newborn screening, primary immunodeficiency

Curr Opin Allergy Clin Immunol 7:522–527.

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Current Opinion in Allergy and Clinical Immunology 2007, 7:522–527

Abbreviations

HSCT hematopoietic stem cell transplantation
SCID severe combined immunodeficiency
TREC T cell receptor gene excision circle

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Introduction

Infants affected with severe combined immunodeficiency (SCID) die of the infectious complications of this disease unless a functional immune system can be provided, usually by hematopoietic stem cell transplantation (HSCT). Presymptomatic diagnosis, before onset of infections, affords the best opportunity for successful treatment. Most cases have no family history, however, and therefore diagnosis in the first weeks of life would require the institution of universal newborn screening. Efforts by immunologists, public health officials and patient advocates to develop appropriate screening tests and to plan pilot trials of SCID newborn screening are underway.

Scope, earlier work, and context

Patients with primary immunodeficiencies are often not diagnosed promptly because the infectious manifestations of these rare disorders are not initially distinguishable from routine infections in otherwise healthy individuals. For other classes of diseases not readily diagnosed clinically, population-wide screening has been instituted. Such screening could be advantageous for primary immunodeficiencies, with SCID as the initial disease category under consideration.

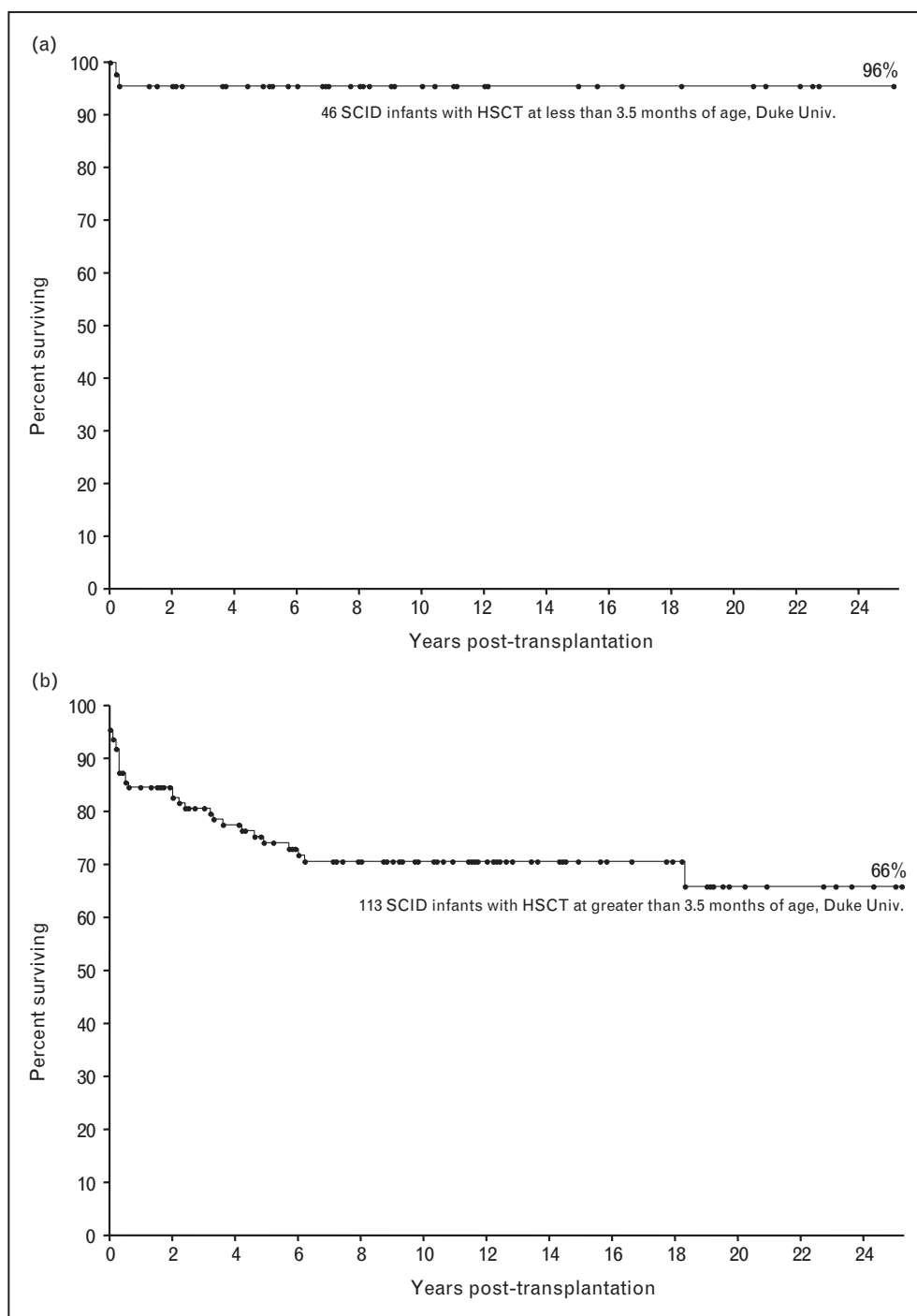
Natural course and incidence of severe combined immunodeficiency

SCID encompasses over 12 known and additional as-yet unknown single gene disorders that share the clinical phenotype of profound impairment of both cellular and humoral immune function [1,2]. Infants with SCID are healthy at birth and have no signs of their underlying disorder that can be picked up on routine newborn physical examination. They are, however, destined to die of infections in their first years of life unless they can be provided with a functional immune system [1,2]. HSCT and enzyme replacement (PEG-ADA to treat adenosine deaminase deficient SCID) have made these previously fatal diseases treatable [2–6,7–9,10]. Gene therapy is also a promising treatment despite occurrences of leukemia related to retroviral insertional mutagenesis in one early trial of X-linked SCID gene therapy [11,12]. Molecular diagnosis of mutations in SCID disease genes has made possible carrier and prenatal diagnosis in some families, after diagnosis in at least one proband who had developed recurrent, serious infections. Although most SCID is sporadic, in families known to be at risk for SCID affected infants have been identified by prenatal mutation diagnosis or postnatal evaluation.

Such infants have in many instances received HSCT before they are 3.5-months old. In this presymptomatic setting, HSCT treatment produces strikingly higher survival rates, lower morbidity and lower treatment costs than HSCT after the onset of chronic diarrhea, infections and failure to thrive [13]. Figure 1 shows the difference in

survival, in the largest single-institution series of HSCT for SCID at Duke University, between infants diagnosed and treated prior to 3.5 months of age (a) versus those treated later (b). Unfortunately most infants with SCID are not identified in the preinfectious period, and some die of infections without the correct diagnosis having

Figure 1 Kaplan–Meier plot of severe combined immunodeficiency patients transplanted at Duke University Medical Center



(a) Forty-six infants diagnosed and treated in the first 3.5 months of life. (b) One hundred and thirteen infants treated after age 3.5 months. Kindly provided by Rebecca Buckley. Reproduced with permission [15].

been made. For this reason, the true incidence of SCID is unknown. A minimal estimate of 1 in 100 000 births affected with SCID is based on cases referred to Dr Puck's laboratory for mutation determination [14], but most experts believe that there are at least two-fold more, resulting in about 100 new cases of SCID per year in the US [15].

Newborn screening

Routine screening of all newborns for genetic disease began with testing for phenylketonuria using the Guthrie procedure on infant blood spotted onto filter paper and air-dried. Currently all states in the US collect dried blood spots for mandatory screening of all newborns for a variety of conditions. Criteria for including diseases in screening panels first suggested by Wilson and Jungner in 1968 [16] were as follows:

- (1) The condition being screened for should be an important health problem.
- (2) The natural history of the condition should be well understood.
- (3) There should be a detectable early stage.
- (4) Treatment at an early stage should be of more benefit than at a later stage.
- (5) A suitable test should be devised for the early stage.
- (6) The test should be acceptable.
- (7) Intervals for repeating the test should be determined.
- (8) Adequate health service provision should be made for the extra clinical workload resulting from screening.
- (9) The risks, both physical and psychological, should be less than the benefits.
- (10) The costs should be balanced against the benefits.

Clearly, from the early days of screening there were value judgments required in the selection of diseases for screening, but priority has always been given to conditions for which there is effective treatment; for which the best outcomes are achieved with treatment instituted soon after birth; and which are not readily recognized in the nursery except by means of a special test. The number of conditions currently screened for varies between countries and between states within the US, but is increasing to encompass multiple assays for metabolic disorders, hypothyroidism, hemoglobinopathies, and other genetic diseases such as cystic fibrosis [17]. In 2005, an extensive report, assembled by Watson *et al.* of the American College of Medical Genetics, established the goal of working toward evidence-based, uniform standards for newborn screening tests. This report was endorsed by the Advisory Committee to the Secretary for Health and Human Services, and several states have since moved to increase the number of conditions screened for. It is anticipated that tests for further con-

ditions to be added to newborn screening panels in the future will be subjected to review using evidence-based criteria. In this report, SCID was noted to meet many criteria for inclusion in newborn screening panels, but at the time no SCID screening assay had been sufficiently validated for a complete rating to be possible [18].

Severe combined immunodeficiency screening conceptualization

A universal newborn screening program for SCID could in theory result in early diagnosis of all cases, allowing for optimal treatment to achieve the best possible outcomes. At a conference in 2001 entitled 'Applying Public Health Strategies to Primary Immunodeficiency Diseases' Buckley reported that because the majority of SCID infants have low numbers of circulating lymphocytes, a complete blood count and manual differential count could alert healthcare providers to suspect the diagnosis [2,13,19]. Because some infants have maternal lymphocytes, and some, especially those with *IL2RG*, *JAK3* and *IL7R* gene defects have substantial numbers of B lymphocytes, however, overlap between healthy infants with the lowest lymphocyte counts and SCID infants with the highest lymphocyte counts makes absolute lymphocyte counts alone problematic as a test.

A further workshop seeking public health partnerships to advance SCID newborn screening was held at the CDC in November 2006. By this time Chan and Puck [14] had introduced T cell receptor excision circle (TREC) copy number as an analyte that could distinguish T cell lymphopenic SCID infants from healthy controls and could be performed using the dried blood spots already collected routinely from newborns. In early 2007 [20], the Wisconsin newborn screening program announced its intention to pilot SCID newborn screening on a large scale.

In May 2007, a SCID Newborn Screening Working Group was convened in San Francisco with the goal of fostering teamwork among experts in newborn screening, trial design, immunologic diagnosis and SCID transplantation so that integrated approaches to SCID screening could be efficiently pursued [15]. Emphasizing that developing a successful newborn screening program requires integrated efforts in many disciplines, the participants reviewed recent additions to newborn screening panels and how they have been brought on line with pilot trials in one or more states [21^{••}]. Successful genetic screening programs, including any program planned for SCID, must include 12 fundamental elements [15]:

- (1) universality; all babies should be included (some states require parental permission);

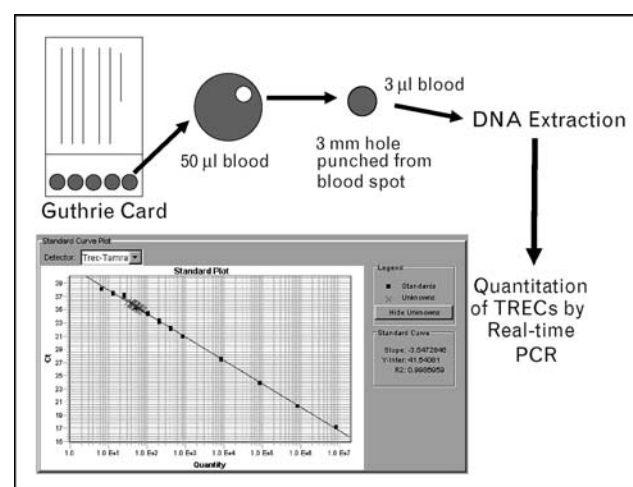
- (2) prescreening education of parents, primary care providers;
- (3) specimen quality control; every nursery, every day (obtain, transport to central lab);
- (4) laboratory screening tests, cutoffs, standards, analysis, quality control;
- (5) data integrity (HIPAA compliance);
- (6) timely notification and follow-up;
- (7) appropriate definitive diagnostic work-up;
- (8) appropriate referral for treatment; immediate and long-term care of affected infants;
- (9) education, reassurance for families with false positive tests;
- (10) continuous monitoring of all phases of program, including outcomes;
- (11) cost-effectiveness assessments to justify continuation, suggest improvements;
- (12) acquisition of new knowledge about the conditions identified by screening.

Severe combined immunodeficiency screening test methodologies

There is not yet an assay that has been proven appropriate as a single-step screening test for SCID. Desirable features of an assay include using the dried blood spots already collected by state laboratories in addition to high sensitivity and specificity, low unit cost and scalability for high throughput. These features have been modeled in a hypothetical cost-benefit analysis by McGhee *et al.* [22]. The main barrier to implementation of currently proposed tests is the rate of false positive or indeterminate test results. Because of the rarity of SCID, the positive predictive value of a screening test needs to be very high to be accepted by the newborn screening community and pediatricians. Screening analytes are based on the inability of all SCID patients to make normal numbers of T cells, regardless of genotype, but must take into account the possibility of maternally engrafted T cells and the fact that T-B⁺ SCID patients may have high numbers of B cells.

Quantitation of TRECs has been the most extensively tested method to date. These DNA circles are byproducts of T cell receptor gene rearrangement in developing thymocytes joined together by the same recombinases that produce the mature T cell receptor gene from V, D and J gene segments. A particularly frequent intermediate TREC in humans can be assayed from peripheral blood DNA by quantitative PCR [23,24]. Puck and Chan showed that SCID patients, whose gene defects result in low output of mature T cells, have low or undetectable TRECs, as opposed to healthy newborns, who have high numbers of TRECs. Both blood of newly diagnosed SCID infants spotted onto standard filters and, more importantly, actual SCID infant Guthrie cards recovered from state

Figure 2 The T cell excision circle (TREC) assay on dried blood spots



The Guthrie card, or standard filter card used to collect heel-stick blood, is obtained routinely from every newborn. At the screening laboratory a 3 mm punch, corresponding to about 3 µl of whole blood, is submitted for DNA extraction. Quantitative PCR is performed, and the number of TRECs is determined by comparison with standard serial dilutions of a plasmid containing the TREC sequence. Genomic DNA copy number can also be measured, such as with actin-specific primers to indicate DNA quality and approximate number of nucleated blood cells.

laboratories have shown extremely low to undetectable TRECs (Fig. 2) [14,15]. Around 1.4% of anonymous, outdated Guthrie cards, however, have had indeterminate results due to poor DNA recovery, measured by low copy number of genomic actin in the same DNA sample used to enumerate TRECs. Repeat DNA isolation from the same sample may decrease the rate of indeterminate samples, and fresh samples appear to yield superior DNA with lower rates of indeterminate results. In Wisconsin, a commitment has been made to pilot SCID screening on a large scale within the next year using the TREC test as a first-line test [20].

In Maryland and 11 other states in the US, a second blood spot filter card is requested from all infants during the first month of life. In these states, an indeterminate TREC test could be followed up automatically with a TREC assay on the second specimen, with a substantial reduction, to 0.1% or less, of persistently abnormal or indeterminate readings. In locales that do not get a routine second dried blood spot, the screening laboratory could ask for a repeat sample if the first one has proven inadequate.

Other assays for SCID newborn screening include immunoassays for IL-7 and T-cell specific proteins, as being evaluated by McGhee [25] and Ken Pass, respectively [15]. These protein-based assays might become first-line or second-line screening tests; or they could be used in

combination with the TREC assay in a two-tier system. High IL-7 levels are associated with T-lymphocytopenic states, and 11 of 13 SCID patients in one study had greater than 15 pg/ml of IL-7 in serum or extracts from dried blood spots, while samples from controls generally had low or undetectable IL-7 [25]. It is not yet known, however, whether an association of high peripheral blood IL-7 levels with SCID can be developed into a robust newborn screening tool for SCID using dried blood spots. CD3 either alone or in combination with CD45 or other T-cell proteins could be assayed by sensitive, high-throughput Luminex bead capture technology or equivalent methods [15]. It is important to note that these assays are currently in the preliminary stages of exploration. Also, it is not clear whether the samples that are indeterminate with a TREC assay would have clear results with protein-based assays; it is possible that samples of poor quality for one assay would fail for all.

Another new approach is to detect mutations, either previously defined or as yet unreported, in known SCID genes by using resequencing microarray chips [26]. This technology is being investigated by Puck and Lebet in collaboration with Mansfield and Warrington at Affymetrix, Inc [15]. Still at a preliminary stage, this approach may have a substantial false negative rate; however, it would immediately yield a specific gene diagnosis if positive.

Severe combined immunodeficiency screening program development

Although SCID has unique features, the recent addition in public health programs of an expanded range of metabolic disorders and cystic fibrosis to newborn screening panels can provide a roadmap for the development of pilot trials and eventual widespread adoption of newborn screening for SCID. Addressing the elements listed in Table 2 will require new interactions between the pediatric immunology community and experts in newborn screening and public health. Major efforts to prepare health providers and prospective parents will be as important as test development. Additional aspects of planning for screening were addressed at the San Francisco meeting [15] as summarized below.

Recalling infants with abnormal or indeterminate screening test results

Available data with the TREC test indicate that dried blood spots without detectable TRECs will mostly represent poor quality or quantity of DNA extracted. These samples will have low genomic DNA and the result could be classified as indeterminate. There was consensus among the pediatric immunologists that while indeterminate test results will be problematic, any infant who truly lacks TRECs, has absent CD3, or has any test result that signifies a lack of T cells, should be brought to

medical attention. Profound T cell lymphocytopenia for any reason is believed to confer a risk of life-threatening infections. Infants who do not have SCID, but have indeterminate or 'false positive' tests may have serious primary immunodeficiencies other than SCID, such as DiGeorge syndrome.

From the public health perspective, however, recalling babies may have negative consequences. A large number of 'false alarms' is costly and causes parental anxiety that may lead to increased utilization of health services [27,28].

Confirmatory testing

State screening programs will need networks of immunology specialists available to be contacted in addition to the primary care provider for infants with screening results that need follow-up. The newborn screening labs and public health departments will need to work with immunologists to plan for the number of infants anticipated to require follow-up. Standardized evaluations will need to be designed. Ideally, a nationally coordinated workup would be used for all screen-positive infants with results deposited in a central databank to help monitor the performance of the screening process as well as provide population-based data on immune parameters.

The definitive diagnosis and treatment for SCID will likewise require recruitment of clinical specialists who have not traditionally been involved with newborn screening. For infants confirmed to have SCID, another immune deficiency or equivocal results on the confirmatory test panel, additional evaluation and treatment or follow-up would be indicated.

Treatment

Early treatment, generally by allogeneic HSCT, is recognized to be optimal for SCID. Given that most patients lack an HLA matched related donor, however, no single best approach to HSCT is currently agreed upon. The launching of newborn screening is eagerly anticipated by the pediatric immunology and transplant community in part as an impetus to establish multicenter collaborations to determine the best treatments for very young infants with SCID.

Long-term outcomes measurements

Newborn screening programs will require ongoing assessments not only of survival, but also long-term outcomes in order to justify continuation of screening or suggest improvements in the entire sequence from screening to treatment and beyond. Few studies of quality of life of HSCT survivors with SCID are available, but one new study from Italy found that the majority of such patients have a good clinical outcome [29].

Conclusion

Primary immunodeficiencies are frustrating to patients, families and clinicians alike because the diagnosis is rarely suspected before the occurrence of severe infections or even tragic loss of life. Early recognition by presymptomatic screening would afford the ideal opportunity for effective treatment. SCID will be a prototype for immunodeficiency screening in the near future, and carefully planned and conducted pilot trials will form the basis to push for universal newborn screening for SCID. If suitable tests can be developed, additional primary immunodeficiencies should also be candidates for screening.

Acknowledgements

This work was supported by USIDNET, the Jeffrey Modell Foundation, and Strategic Opportunities Support from the Clinical and Translational Sciences Institute, UCSF. Dr Puck is a consultant for USIDNET.

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- of special interest
- of outstanding interest

Additional references related to this topic can also be found in the Current World Literature section in this issue (p. 589).

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