Newborn screening for severe combined immunodeficiency: breaking the bubble

Blom, M.

CHAPTER 1

General introduction

1

GENERAL INTRODUCTION

The story of David Vetter – 'The boy in the bubble' moved a world he couldn't touch
In the early 1970s, an unusual boy captured the world. On September 21st, 1971, David Phillip Vetter was born at the Texas Children's Hospital in Houston. After 20 seconds of exposure to the world, he was placed in an isolating, sterile, plastic bubble. David was diagnosed with severe combined immunodeficiency (SCID), a hereditary immune condition preventing him from fighting off infections caused by everyday pathogens. Without a working immune system, any germ he picked up could have been lethal. At the time of his birth in 1971, a bone marrow transplant from a matched donor (HLA matched family donor) was the only possible cure for SCID, but there was no matched donor available in David's family. As David grew older and doctors continued searching for a cure, David's life in the bubble became permanent. His mother Carol Ann explained, "*There was never any plan to keep David in there – in the bubble – indefinitely. To keep a child isolated, unable to touch, or feel, or smell, or enjoy, sounds cruel, perhaps. What did they expect us to do – take David out of the bubble, which would have been certain death?*". David had grown into an adolescent without a clear road forward, but medical advances provided new hope. By 1983, a new technical approach of bone marrow transplantation had been developed with unmatched donors. David's sister Katherine donated her marrow and David received the stem cell graft. At first, the procedure seemed to work, but a dormant and undetected Epstein-Barr virus (EBV) in Katherine's marrow, triggered the growth of Burkitt's lymphoma that overwhelmed David's body. Eventually, it became necessary to remove David from the bubble for what would be the last two weeks of his life. For the first time in his life, his parents were able to hold him without a sheet of plastic between them. David died two weeks after entering a world his body could not tolerate.

David Vetter inside his sterile bubble

David (age 12) with his mother Carol-Ann

|Courtesy Baylor College of Medicine Archives|

SEVERE COMBINED IMMUNODEFICIENCY

Severe combined immunodeficiency (SCID) is the most severe form of inborn errors of immunity (IEI) characterized by the absence or dysfunction of T-lymphocytes, often accompanied by the lack of B-lymphocytes and NK-cells affecting both cellular and humoral immunity [1]. SCID is a term used to describe a disease entity caused by various genetic defects. The incidence of SCID is estimated to be in 1 in 50,000 to 100,000 births, but varies depending on geographical and ethnic background [2-4]. Infants with SCID typically appear normal at birth, but develop severe infections in the first months of life. Without curative treatment, in the form of allogeneic hematopoietic stem cell transplantation (HSCT) or in some specific forms of SCID, gene therapy (GT), affected infants die within the first year of life [5]. Early definitive treatment, before the onset of infections, results in the best outcomes [6].

Disease mechanisms and molecular causes

SCID is primarily characterized by the absence or dysfunction of T-lymphocytes affecting both cellular and humoral immunity. Even if B-lymphocytes are present, they are barely functional due to the lack of T-cell help or due to intrinsic defects in B-cell function. Several molecular defects have been identified resulting in the aberrant development or absence of naïve T-lymphocytes. The IUIS expert committee has published and updated biannually a genotypic and phenotypic classification of all IEIs [7, 8]. For SCID, more than 20 different genetic defects been described. SCID gene lists have grown and become more complex as the discovery of novel IEI disorders has been occurring at an impressive rate [9]. Types of SCID can be classified by shared pathogenesis and immunological features (Figure 1).

Defects in Cytokine Receptors and Cytokine Signaling (T-B+ SCID). The most common form of SCID is X-linked SCID, caused by mutations in the *IL2RG* gene encoding for the common γ chain (γc). This common subunit is shared by cell surface receptors for various interleukin molecules (IL-2, IL-4, IL-7, IL-9, IL-15 and IL-21). IL-7 is involved in expansion of early thymocyte progenitors, whereas IL-15 plays a role in NK-cell development. Patients with X-linked SCID therefore lack both T-lymphocytes and NK-cells. The number of circulating B-lymphocytes is usually normal, but as B-cells do not undergo class switching due to lack of T-cell help, their function is impaired. X-linked SCID patients often have poor B-cell function post-HSCT, suggesting an intrinsic defect in B-cell function as well [10, 11]. The γc is bound to the intracellular tyrosine kinase Janus kinase-3 (JAK3) which is activated upon cytokine binding to the receptor and delivers γc-mediated intracellular signaling. Defects in the *JAK3*

gene result in an autosomal recessive form of SCID with a T-B+NK- phenotype similar to X-linked SCID. Mutations of the *IL7R* gene (encoding for the α chain of the IL-7 receptor) abrogate T-lymphocyte development, but do not interfere with B-cell and NK-cell development [1, 12].

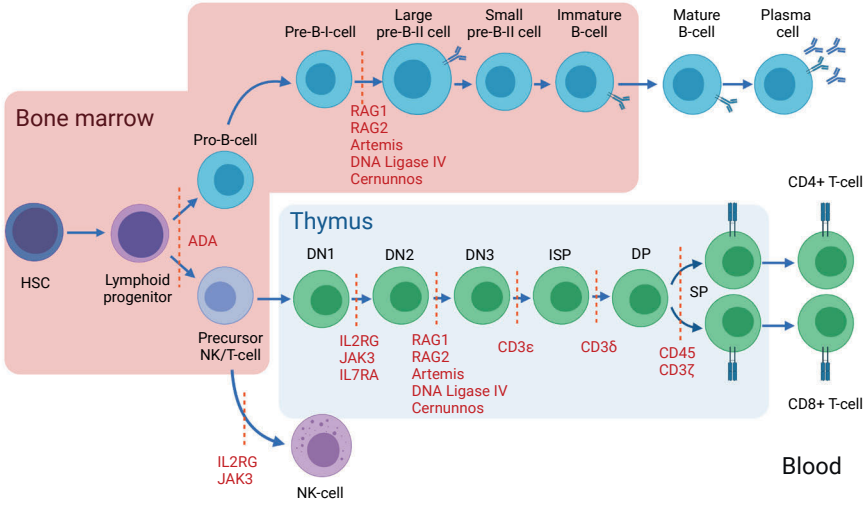

Figure 1. Schematic overview of development of human T-, B, and natural killer (NK) cells. Defects in the SCID genes with the highest incidence causing blocks in lymphoid development are indicated. Created with Biorender.com

Defective (pre-)T-cell receptor (TCR) signaling (T-B+ SCID). Defects in the key proteins involved in pre-TCR/TCR signaling can also lead to a SCID T-B+ phenotype. Mutations of the *CD3δ*, *CD3ε* and *CD3ζ* chains prevent formation of a functional CD3 complex leading to disrupted expression and signaling via the (pre)-TCR. Patients with CD3 deficiency have very low levels of mature circulating CD3+ T-cells, no CD4+ or CD8+ T-cells, and a total absence of γ/δ T-cells. T-B+ SCID can also be caused by variants in the tyrosine phosphatase *CD45* gene coding for a transmembrane protein required for T-and B-cell antigen receptor signal transduction [13].

Defects in Recombination of the Antigen Receptor Genes (T-B- SCID). A critical process during the T- and B-cell development is the somatic rearrangement of the antigen receptor genes on T- and B-cells, generating clonal diversity. *RAG1* and *RAG2* genes encode proteins that introduce DNA double-strand breaks at recombination signal sequences (RSSs), permitting V, D, and J gene rearrangements. *RAG1* or *RAG2* mutations result in a functional inability to form antigen receptors, disrupting

development of both T- and B-lymphocytes, whereas NK-cell development is not affected. Impaired V(D)J recombination may also be due to genetic defects in components of the non-homologous end joining pathway (NHEJ) such as defective DNA end-binding (DNA PKcs), DNA end-processing (Artemis/*DCLRE1C*) and DNA ligation (LIG4, XLF/Cernunnos). Genetic defects in one of the NHEJ factors are also characterized by radiosensitivity and accompanied with other manifestations such as microcephaly and facial dysmorphisms. Similar to RAG1 and RAG2 SCID, development of T- and B-lymphocytes is severely impaired in these genetic conditions while NK-cells proceed normally [14, 15].

Defects in Purine Pathway Enzymes (T-B- SCID). Autosomal recessive SCID is most commonly caused by mutations in the adenosine deaminase (*ADA*) gene leading to ADA deficiency (10% to 15% of all forms of SCID). ADA deficiency results in buildup of toxic metabolites, leading to premature lymphocyte precursor cell death. In the case of complete absence of enzymatic activity, accumulation of adenosine and deoxyadenosine will induce apoptosis, resulting in a T-B-NK- phenotype. Milder forms with residual ADA activity have been reported, leading to delayed diagnosis of immunodeficiency after several months (delayed onset) or even later occurring after two to three years (late onset). Purine nucleoside phosphorylase (PNP) is another enzyme of the salvage pathway of purine metabolism. PNP deficiency is unique among IEI as T-lymphocytes progressively decrease, while auto-immune hemolytic anemia and neurological impairment can occur as well [16, 17].

Impaired survival of lymphocyte precursors (T-B-SCID). A rare autosomal recessive form of SCID is reticular dysgenesis (RD). This rare condition is caused by mutations of the adenylate kinase 2 gene (AK2). AK2 deficiency is not only associated with blocked lymphoid differentiation, but also results in apoptosis of the myeloid precursors. Patients with RD may present with neutropenia, deafness and in some cases with anemia and thrombocytopenia [18].

Hypomorphic mutations in SCID genes. Hypomorphic mutations in several genes that cause SCID can give rise to an incomplete defect leading to a leaky SCID phenotype, a less profound combined immunodeficiency (CID) phenotype or in Omenn syndrome. Both leaky SCID and Omenn syndrome can be associated with presence of variable numbers of T-lymphocytes with poor immune function. Auto-immune manifestations are common in these patients due to inadequate control of autoreactivity and the infiltration of target tissues by activated and oligoclonal T-cells. Omenn syndrome was originally described in patients with mutations in *RAG 1* and *RAG2,* but has now been identified in a growing list of other leaky SCIDs with mutations in *Artemis*, *IL7RA*, *LIG4*, *ADA* and *IL2RG [19]*.

Clinical manifestations

Without adaptive immunity, patients with SCID are prone to severe, recurrent infections caused by both non-opportunistic and opportunistic pathogens. Patients are usually born asymptomatic, but develop life-threatening infections, failure to thrive and in some cases chronic diarrhea in the first months of life. Opportunistic infections such as *Pneumocystis jiroveci* pneumonia (PCP) and viral infections can have fatal outcomes in SCID patients. Bacterial infections are less common in part because of the presence of maternal Ig-antibodies in early infancy. With the exception of mucocutaneous candidiasis, severe invasive fungal infections are rare in SCID patients. In countries with neonatal BCG vaccination programs, Bacillus Calmette-Guerin (BCG)-vaccine-related complications may occasionally be the presenting feature in immunized SCID patients. Non-infectious clinical manifestations consist mainly of graft versus host disease (GvHD) caused by the patient's inability to reject allogenic lymphocytes acquired either from mother *in utero* or from unirradiated blood transfusion [1, 15].

Leaky SCID patients usually survive beyond 12 months of age and can present with recurrent infections and immune dysregulation including auto-immune manifestations such as auto-immune cytopenia and EBV-driven lymphoproliferative disease. It is important to consider and recognize atypical SCID presentation in children presenting beyond the first year of life [20]. Patients with Omenn syndrome can present with a progressive erythematous rash (erythroderma) which may often cause alopecia and loss of eyebrows and eyelashes. These symptoms can be present at birth but can also evolve over the first weeks of life. Lymphadenopathy, hepatosplenomegaly, high IgE levels and eosinophilia are frequent findings. Patients with Omenn syndrome often suffer from diarrhea, failure to thrive and persisting infections as seen in other forms of SCID [21].

Diagnostics

Awareness of clinical manifestations and laboratory features that indicate an underlying cellular immunodeficiency amongst primary caregivers and pediatricians is critical in the diagnostic process of SCID. Flow cytometric immunophenotyping of (naïve) T-, B-, and NK-cells is the classically recommended method in the diagnostic work-up in case of a suspicion of SCID. SCID is primarily characterized by very low or absent naïve T-cells (< 200 naïve $CD4^+$ T-cells/μL). Interpretation of flow cytometric results is more complicated in patients with Omenn syndrome or leaky SCID, as the patients can present with high numbers of oligoclonal T-cells or maternal engraftment. A detailed analysis of T-cell subsets is therefore of utmost importance. Diagnostic criteria that describe the most important features of SCID might facilitate diagnosis of SCID, helping physicians regardless of their familiarity with IEIs (Table 1) [22].

Table 1. Diagnostic criteria for typical SCID, leaky SCID and Omenn syndrome. Table adapted from the PIDTC classification, 2014 [22].

Typical SCID
Absence or very low number of T-cells (CD3 T-cells < 300/microliter), AND no or very low T-cell function (< 10% of lower limit of normal) as measured by response to phytohemagglutinin (PHA) **OR** T-cells of maternal origin present
Leaky SCID
Reduced number of CD3 T-cells • For age up to 2 years < 1000/microliter • For > 2 years up to 4 years < 800/microliter • For > 4 years < 600/microliter • Absence of maternal engraftment • 30% of lower limit of normal T-cell function (as measured by response to PHA)
Omenn syndrome
• Generalized Skin Rash • Absence of maternal engraftment. • Detectable CD3 T-cells, ≥ 300/microliter • Absent or low (up to 30% of normal) T-cell proliferation to antigens to which the patient has been expose If the proliferation to antigen was not performed, but at least 4 of the following 10 supportive criteria, at least one of which must be among those marked with an asterisk (*) below are present, the patient is eligible for the diagnosis Omenn syndrome • Hepatomegaly • Splenomegaly • Lymphadenopathy • Elevated IgE • Elevated absolute eosinophil count • Oligoclonal T-cells measured by CDR3 length or flow cytometry* • > 80% of CD3+ or CD4+ T-cells are CD45RO+ • Proliferation to PHA is reduced < 30% of lower limit of normal* • Proliferative response in mixed leukocyte reaction is reduced < 30% of lower limit of normal* • Mutation in SCID-causing gene*

In addition to flow cytometry, HIV-infections which could also cause severe recurrent infections and T-cell deficiency must be ruled out. Functional assays, assessing T-cell function can be done by *in vitro* measurement of responses to mitogens such as phytohemagglutinin (PHA). It is important to evaluate the humoral immunity by measurement of Ig levels while taking maternal transplacental antibodies into account. Every effort should be made to identify infections, and biopsy material including culture of appropriate tissue specimens and PCR may be needed to identify infecting pathogens. The definite diagnosis of SCID is ascertained by genetic analysis to identify the underlying disease-causing defect. Next generation sequencing (NGS) based on targeted panel

sequencing or whole exome sequencing (WES) with filter for SCID genes are increasingly used to identify variants in known SCID genes. WES or whole genome sequencing allow the identification of genetic defects in new IEI candidate genes [23, 24].

Treatment

Isolation and supportive care. Infants suspected of having a SCID should be placed in protective isolation with strict handwashing procedures to minimize exposure to (hospital-acquired) infections. Prophylaxis for bacterial infections and PCP should be started as soon as possible, while antifungal prophylaxis should also be considered [25]. Active infections should be treated vigorously. Discontinuation of breast feeding in CMV positive mothers is an ongoing topic of discussion, however, the risk of a neonatal CMV infection transmitted through breast milk in these severely immunocompromised newborns, may outweigh the benefit of breast-feeding [26]. Antiviral prophylaxis such as valganciclovir should be considered while awaiting maternal CMV results [27]. Blood products should be CMV-negative and irradiated to avoid the risk of transfusion GvHD. Live attenuated vaccines, such as rotavirus and varicella, should be avoided.

HSCT. HSCT has been the gold standard for treatment of SCID ever since the first stem cell transplantations in North America and Europe in 1968 [28, 29]. This lifesaving treatment reconstitutes a functional immune system by infusion of donor stem cells. Various stem cell sources can be used, including stem cells from bone marrow, mobilized peripheral blood stem cells or those harvested from umbilical cord blood. Donor types include HLA identical siblings, other matched family donors, (mis-)matched unrelated and mismatched related donors. Since the case of David Vetter in 1971, survival after HSCT has continued to improve due to refinement of HLA-tissue typing methods, improved methods of isolating $CD34^+$ hematopoietic stem cells (HSCs) and development of more effective (*ex vivo*) T-cell depletion methods. In addition, molecular detection of viral infections has enabled pre-emptive treatment of viremia and more effected treatment of transplant-related complications have led to an overall survival (OS) of 85 to 90% post HSCT [6, 30, 31]. There are a number of factors associated with better survival and outcomes after HSCT, but having an HLA-matched sibling donor and absence of active infections or organ damage prior to transplantation seem to be the most important ones [6, 32, 33]. A successful transplant procedure is lifesaving and in most cases curative with patients leading normal lives off medication, but some complications might occur. There is the risk of rejection or graft failure requiring a second transplant, in particular, when no conditioning is used. The role of chemotherapy conditioning regimens pre-HSCT is an ongoing topic of discussion. HSCT for SCID can be performed without any conditioning regimens which is associated with a lower incidence of GvHD without chemotherapy-induced toxicity [5]. However, condition regimes that contain (a certain level) of myeloablative agents are associated

with better donor myeloid engraftment and better T- and B-lymphocyte reconstitution [34]. Especially for patients with absent or non-functioning B-lymphocytes, conditioning is usually needed to acquire normal B-lymphocyte function post-HSCT [35, 36]. There are many patients after HSCT living with the effects of poor immunity or sequelae of both pre- and post-transplant complications. Approximately 25% of patients require life-long immunoglobulin replacement therapy because of the absence of donor B-lymphocyte engraftment [35]. One of the most significant adverse events of HSCT is the development of GvHD. GvHD occurs due to the recognition of host MHC antigens by donor T-cells leading to a range of symptoms and manifestations. GvHD can be categorized in acute GvHD, usually developing within three months post-HSCT and chronic GvHD. *Ex vivo* T-cell depletion of the graft, GvHD prophylactic mediation, serotherapy in the conditioning regimen and cyclophosphamide after graft infusion are strategies to prevent GvHD. Some sequelae relate to the specific genetic defect such as human papillomavirus-associated warts in patients with IL2RG/JAK3 SCID, neurodevelopmental disorders in ADA deficiency or late toxicity after HSCT with growth retardations and endocrinologic deficiencies in Artemis patients [37, 38].

Enzyme replacement therapy (ERT). For ADA deficiency, ERT with polyethylene-glycosylated ADA injections is an alternative treatment. In the short term, ERT may allow some immune reconstruction and clearance of infection. However, ERT is expensive and results in only partial immune reconstitution, therefore it is often used as a 'bridge' treatment before proceeding to definitive therapy [39].

Gene therapy (GT). While advances in HSCT have resulted in improved outcomes, the procedure is still associated with a risk of mortality and morbidity from GvHD. These severe complications mandated a search for new treatment options leading to the pursuit of genetically modified autologous hematopoietic cells transduced with a vector. GT has the potential to correct genetic defects across hematologic lineages without many of the complications of HSCT [40]. SCID is an ideal candidate due to the clear link between defined monogenetic defects and clinical phenotype and the ability to repair the defect in the immune cells by manipulating the readily accessible HSCs. In addition, as SCID patients lack T-lymphocytes, a selective growth advantage is conferred to the corrected progenitor cells if the transgene is expressed [40]. Autologous stem cells circumvent the need for a suitable matched donor and abrogate the need for immune suppression as GvHD prophylaxis [41]. GT has gone through several developmental stages with first clinical trials with retroviral vectors for X-linked SCID and ADA-SCID dating back to the late 90 [42-45]. However, the use of γ-retroviral vectors was associated with severe complications such as vector-related leukemia and myelodysplastic events caused by insertional oncogenesis [46, 47]. Since then, safer GT approaches have been developed

including self-inactivating (SIN) γ-retroviral and lentiviral vectors which have substantially less transactivation potential [48, 49]. These advances have even led to the marketing approval of a GT product in Europe for ADA-SCID patients who lack a suitable donor for HSCT [50]. GT has significantly improved over the last two decades. The infrastructure to manufacture and deliver cellular therapies advances and an increasing number of clinical trials report high efficacy and excellent safety. While alloHSCT still remains the first choice treatment for many SCID patients to offer proven long term cure, the development of GT may offer a safe, effective, definitive therapy in the future that diminishes the immunological complications of HSCT

NEWBORN SCREENING FOR SCID

Importance of an early diagnosis

The importance of an early diagnosis is demonstrated by studies showing improved survival of SCID patients diagnosed at birth due to a positive family history (OS 85-90%) compared to the first presenting family members (OS 40-42%) [51, 52]. These observed differences were irrespective of conditioning regimen, donor source, or underlying (genetic) diagnosis suggesting the relation between improved survival and early diagnosis. In addition, retrospective multi-center studies in larger SCID patients' cohorts have shown that patient outcomes are significantly improved when curative therapy with HSCT is performed before the age of 3.5 months and/or prior to the onset of severe and debilitating infections [32]. Survival rates were adversely impacted by active infection pre-transplantation; 81% for patients with active infection at the time of transplantation versus 95% in infection free patients [6]. These findings suggest that an early diagnosis and the prevention of infections are predominant determinants of a good transplantation outcome.

Many SCID cases are sporadic, with no positive family history leading to prompt early diagnosis. Infants with SCID appear healthy at birth and are diagnosed after frequent medical encounters for recurrent and persistent (opportunistic) infections and/or failure to thrive. These nonspecific disease manifestations can lead to delay in recognition of the underlying disease and subsequently to delay in treatment. Realistically, an early diagnosis prior to the development of life-threatening complications is only achievable by early identification of infants with SCID through newborn screening (NBS) programs.

How can you screen for SCID? T-cell receptor excision circles

Several screening strategies have been proposed to identify patients with SCID directly after birth. A complete blood count (CBC) was suggested to detect T-cell lymphopenia, but this simple laboratory test lacked sensitivity as patients with present

B-lymphocytes, maternal engraftment or oligoclonal expansion would be missed [53]. The same was the case for protein immunoassays on dried blood spots for T-cell specific markers such as CD3 [54]. Subsequently, flow cytometry to determine T-cell populations in cord blood was also considered but proved to be too time-consuming and expensive for a population screening test [55]. There was need for an extremely sensitive and specific biomarker that could identity T-cell lymphopenia in dried blood spots (DBS) while avoiding excessive costs and anxiety associated with false-positive screen results [56].

V(D)J recombination of the TCR loci is the process whereby a diverse repertoire of antigen receptors is generated. In each T-cell randomly chosen combinations of variable (V), diversity (D) and joining (J) segments are formed to synthesize a unique rearrangement in each cell. Only T-cell progenitors with in-frame rearranged locus are selected to survive and mature. The excised DNA fragments that are not destined to be incorporated into the mature TCR locus can be joined at their ends to form a great variety of circular DNA byproducts, called T-cell receptor excision circles (TRECs). Precursor T-cells in the thymus first start to rearrange their *TCRD* and *TCRG* genes. When this leads to a functional receptor, the cell exits the thymus as TCRγδ+ T-cell. Most cells, however, do not form a functional γδ TCR and start rearranging their *TCRB* and *TCRA* genes. *TCRD* deleting rearrangements therefore exist for only a short period during thymocyte differentiation [25]. The δREC–ψJα rearrangement in the *TCRA* locus excising the *TCRD* gene is initiated after unsuccessful generation of a γδ TCR. It is estimated that 70-80% of the thymocytes that ultimately express αβ TCR form a specific circular DNA TREC in this process: the δRec-ψJα signal joint TREC [57] (Figure 2). The δRec-ψJα coding joint might still be present on the nonfunctional *TCRAD* allele and by subsequent Vα–Jα rearrangements, the δREC–ψJα coding joint will be removed and placed on a novel excision circle [58]. Quantitative PCR amplification across the joined ends of the δRec-ψJα TREC reflects the number of recently formed T-cells in peripheral blood.

TRECs were found to be unique to naïve αβ T-cell and memory T-cells lack the δRec-ψJα signal joint mentioned above. In addition, TRECs were considered to be an ideal marker for naïve T-cell production as they were noted to be stable and remained in the cytoplasm of the T-cells, not replicating during mitosis. As a result, TRECs become diluted when the T-cell population expands through cell division [60]. In 2005, the first application of quantitative PCR for TREC detection as a large-scale population screening method for SCID was described [61]. SCID became the first immune disorder in the NBS program and at the same time the TREC assay became the first high-throughput DNA-based NBS test.

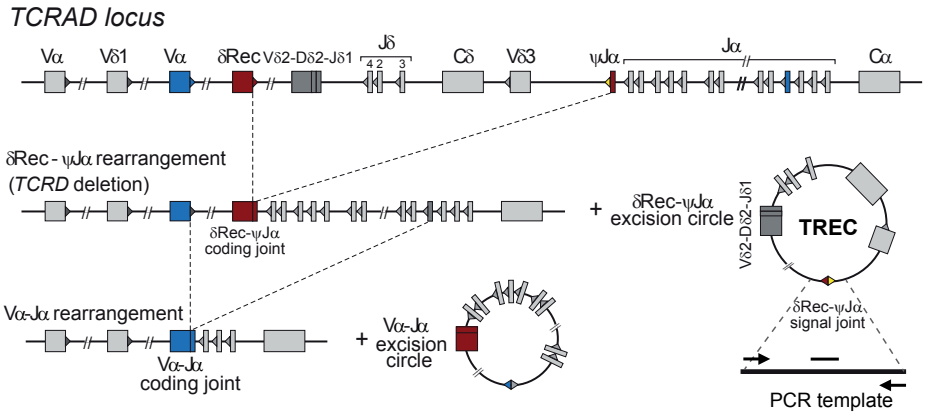

Figure 2. TRECs are stable, circular fragments of DNA formed during by excisional rearrangements of the TCR genes. During the δREC–ψJα rearrangement in the *TCRA* locus, the *TCRD* gene is excised and the δRec-ψJα signal joint TREC is formed. This specific TREC is produced by 70-80% of the αβ T-cells. With quantitative PCR amplification across the joined ends of the δRec-ψJα TREC the number of recently formed T-cells in peripheral blood can be determined [59].

Follow-up after abnormal TREC values

Most screening tests, including the TREC assay, are not designed to establish a diagnosis, but rather to signal the potential for a serious condition for which specific follow-up is required [62]. Low TREC levels indicate that a T-cell developmental problem might be present, but referral to the pediatric-immunologist is needed to confirm T-cell lymphopenia and to identify the underlying cause [63, 64]. An important part of this initial evaluation is a thorough family history and physical examination. A maternal history can reveal factors that influence T-cell numbers, such as immunosuppressive medication. A family history of unusual or fatal infectious events or unexplained infant death is important, particularly in consanguineous families. Recognizing dysmorphic features is key in physical examination.

NBS for SCID introduced clinical immunologists to diagnostic testing of apparently healthy newborns without any medical history of infections or other manifestations. Confirmatory testing strategies after an abnormal TREC value might differ between individual screening programs, but flow cytometry to enumerate CD3+ T-cells, CD4+ and CD8+ T-cells, CD56/16 NK-cells and CD19 B-cells and T-cell subsets CD45RA/CD45RO (%) naive T-cells is the cornerstone [65]. If naïve T-cells are low (< 200 cells/μl) SCID might be suspected and additional SCID diagnostics and management will be initiated. Even though TREC-based NBS programs are primarily aimed at the detection of SCID, low TRECs can be identified in a range of other conditions associated with impaired T-cell production or loss of T-cells from the peripheral circulation. These

non-SCID conditions can be referred to as incidental findings, secondary findings or even primary/secondary targets depending on the NBS program. For infants with low T-cells (300-1500 cells/μL), reduced but present naïve CD4 T-cells and no maternal cells, initial immune evaluation might be similar to that for SCID, but hospitalization may not be required if immunodeficiency is not profound. Specific diagnostic testing and management of these conditions will depend on the comorbidities.

Non-SCID cases identified via NBS for SCID

Low TREC levels can be identified in other forms of IEI such as less profound combined immunodeficiencies classified by the IUIS [8]. Newborns with a recognized genetic syndrome that include low T-cell numbers within its spectrum of clinical findings can also present with low TREC numbers. Examples are newborns with 22q11.2 deletion syndrome (DiGeorge syndrome), CHARGE-syndrome, trisomy 21, ataxia telangiectasia, trisomy 18 and Jacobsen syndrome. TRECs and T-cell numbers can also reversibly reduced due to secondary causes such as congenital malformations (e.g. cardiac or gastrointestinal anomalies), or disease processes without an intrinsic defect in production of circulating cells (e.g. loss into third space in hydrops or chylothorax or vascular leakages in sepsis) [2, 66]. Maternal immunosuppressant use can also be a cause of transient neonatal T-cell lymphopenia [67, 68]. In these cases, T-cell lymphopenia usually resolves once excess T-cell losses or suppression of T-cell maturation has been abrogated. In newborns with idiopathic T-cell lymphopenia, TRECs and T-cells might be low without an identified underlying cause, even after immunologic and comprehensive genetic evaluation. For infants with T-cell lymphopenia, longitudinal immunological evaluation is important to determine if the T-cell lymphopenia is transient [69].

Not all serious disorders affecting T-cell function can be identified via TREC screening. Combined immunodeficiencies such as ζ-associated protein of 70kDa (ZAP-70) deficiency or MHC class I and II gene expression deficiency, have severely impaired T-cell function but can have normal TREC levels as T-cell development is intact beyond the point of TCR gene recombination [70].

Infants with preterm birth (gestational age <37 weeks) and/or low birth weight are a disproportionate source of abnormal TREC results [50]. T-cell lymphopenia in these infants is depending on the degree of thymic maturity, although T-cells are not functionally impaired, and T-cell numbers usually normalize with increasing gestational age. Many NBS programs have incorporated adaptations in their screening algorithms (different cut-off values or second NBS cards) for preterm infants with low TREC levels to avoid high referral rates.

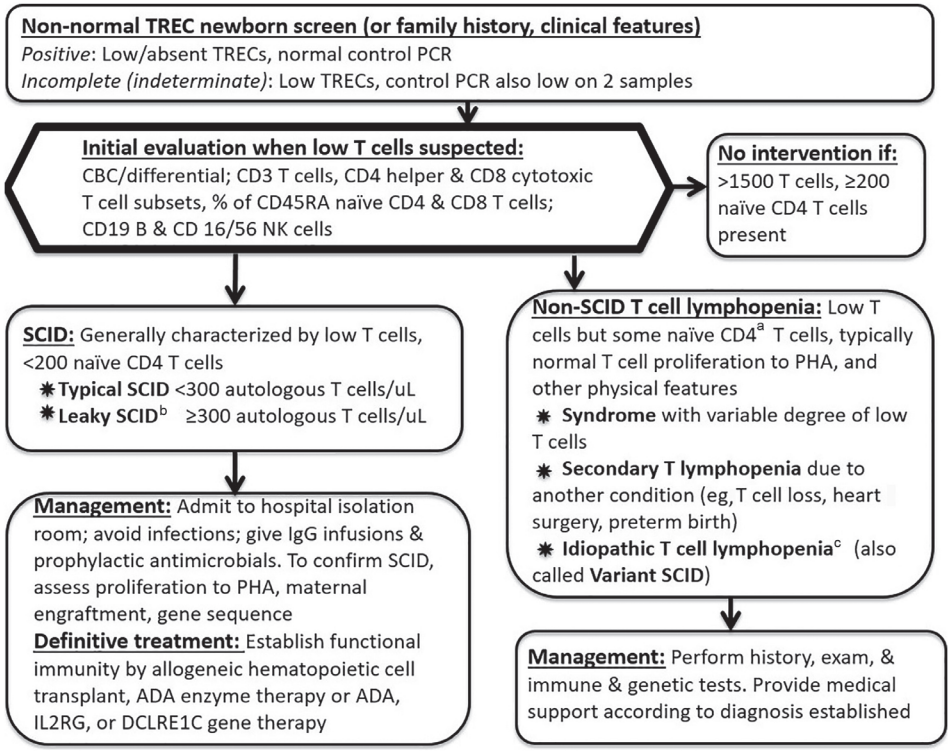

Figure 3. Example of a follow-up scheme after an abnormal TREC value in NBS for SCID. Figure from Dorsey *et al.* 2017 [65].

Finally, in the case of an abnormal TREC value, but normal levels of T-cells (> 1500/µL and > 200 naïve/µl) no further immunological work-up is required within the SCID screening context [65]. In these cases, TRECs could have been low at the time of the heel prick due to transient T-cell lymphopenia that resolved in the first weeks up to referral. TRECs could also be low due to technical test errors leading to false-positive results. Uniform follow-up protocols are required for a prompt and consistent approach to a definitive diagnosis and can provide guidance for pediatrician-immunologists when dealing with these non-SCID cases identified via NBS for SCID (Figure 3).

IMPLEMENTATION OF SCID IN NBS PROGRAMS

General background information NBS programs

The primary aim of NBS programs is to identify potentially fatal or disabling conditions in pre-symptomatic newborns for which timely intervention is available and critical to improve the outcome. These conditions might not be evident at birth, but if left undiagnosed and untreated could have fatal or severe developmental consequences for the child. With early detection and early intervention, morbidity and mortality can be reduced. In addition to individual health benefits, NBS also aims to minimize negative societal and economic impacts of life-threatening diseases [71]. Since the initiation of NBS in the 1960s with screening for phenylketonuria (PKU), innovations have led to the gradual expansion of screened conditions in NBS panels. The introduction of tandem mass spectrometry led to a boost in the late 1990s allowing the simultaneous biochemical analysis for a significant number of inborn errors of metabolism (IEM). The availability of tandem mass spectrometry led to test-driven expansions in NBS programs worldwide, with some NBS programs currently screening for more than fifty conditions [72, 73].

Most programs are structured to screen for a number of core disorders, along with secondary target disorders. The spectrum of disorders included in NBS programs greatly varies between countries. National health care politics, healthcare structures, input from patient advocacy groups and different interpretations of screening criteria have led to differences in panels of screened conditions [73, 74]. The recommendations of Wilson and Jungner (1968) for populated-based disease screening are the backbone of the screening policy [75]. Since their publication in 1968, these criteria have provided a framework against which conditions can be assessed for their suitability for screening, being of aid in decision making with regard to inclusion of new disease candidates in NBS programs. The criteria have been refined in 2008 by the WHO due to the growing interest in genetic screening and changing demands of modern times (Table 2) [76].

Table 2. Criteria used for inclusion of new conditions in NBS programs

Original Wilson and Jungner criteria (1968) [75]	**Additional WHO-criteria (2008) [76]**
1. The condition sought should be an important health problem	1. The screening program should respond to a recognized need.
2. There should be an accepted treatment for patients with recognized disease	2. The objectives of screening should be defined at the outset
3. Facilities for diagnosis and treatment should be available	3. There should be a defined target population
4. There should be a recognizable latent or early symptomatic stage	4. There should be scientific evidence of screening program effectiveness
5. There should be a suitable test or examination	5. The program should integrate education, testing, clinical services and program management
6. The test should be acceptable to the population	6. There should be quality assurance, with mechanisms to minimize potential risks of screening
7. The natural history of the condition, including development from latent to declared disease, should be adequately understood	7. The program should ensure informed choice, confidentiality and respect for autonomy
8. There should be an agreed policy on whom to treat as patients	8. The program should promote equity and access to screening for the entire target population
9. The cost of case-finding (including diagnosis and treatment of patients diagnosed) should be economically balanced in relation to possible expenditure on medical care as a whole	9. Program evaluation should be planned from the outset
10. Case-finding should be a continuing process and not a “once and for all” project	10. The overall benefits of screening should outweigh the harm

Organization of a NBS program

As previously mentioned, most screening tests are not designed to establish a diagnosis, but rather to signal the potential for a serious condition for which specific follow-up is required. Screening should therefore be thought of as an integrated program or a system and not merely a test [62]. Organization of population screening programs are complex due to the involvement of many stakeholders. It is important to realize that countries have their own distinguished manner of organizing their health care system and this also applies to the NBS system.

The NBS system includes preanalytical, testing, and postanalytical phases. The preanalytical phase includes collection of demographic data, blood sampling and shipment of NBS cards. In the preanalytical phase communication is key. Most countries verbally inform parents with the aid of written brochures or websites prior to sample collection. Information is usually provided by a neonatologist, midwife or nurse [77]. In Europe, sample collection is usually performed between 48-72 hours after birth, while NBS programs in the US have an earlier sampling window of 24-48 hours after birth. These differences are mostly due to differences in the organization of the NBS program and maternity care. Some countries perform the heel prick in the hospital before discharge, while in other countries sample collection is done by midwifes or screeners at home [78]. The testing phase usually occurs at designated department of health laboratories and includes samples preparation, test conduction, results interpretation, and report issuing. The final and most important phase is the postanalytical phase where abnormal NBS results requiring further testing are communicated and confirmed, treatment is initiated, and long-term follow-up is monitored [71]. Typically, the laboratory reports abnormal NBS results to the primary health care provider, who will subsequently notify the family and refer the infant to the pediatric-specialist. In Europe, screening results are primarily confirmed in specialized centers. Several countries make all screening results available to parents either online, by mail or by post. Other countries only inform parents if an action is required, such as a referral or a request for a second sample [79]. The Dutch NBS structure is depicted in Figure 4. Key aspects for the success of NBS programs are timelines of sample transport, quality assurance for performed tests, good and clear communication to parents, easy access to health care and continuous program evaluation. Ongoing tracking of test performance and outcomes must be part of every screening program, with regular communication and adjustments to improve sensitivity, specificity, turnaround times, follow-up care, cost effectiveness and outcomes. Sharing of information at every level makes the program efficient, but also affords opportunities for new insights [62].

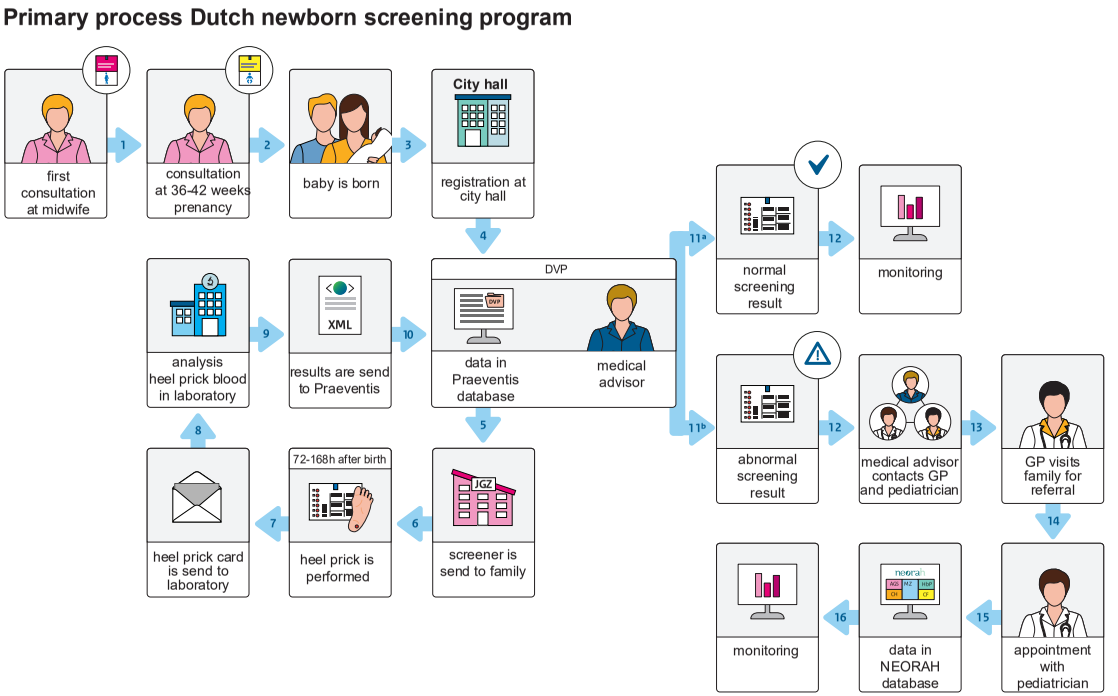

Figure 4. Primary process Dutch newborn screening program. Figure available via RIVM-website. Expecting parents will receive information about the NBS program during the first and second consultation with the midwife (1-2). During registration at city hall, an information brochure will be handed out as well (3). The screening organizations (JGZ) will be informed about the registration of the newborn, after which screeners will visit the family to perform the heel prick (6). The heel prick card is sent by post to one of the five screening laboratories (7). The heel prick cards are then analyzed, and the results are registered in the national monitoring database Praeventis (8-9). Abnormal results are forwarded to the general practitioner (GP) and pediatrician by the medical advisor (12-13). Medical advisors coordinate logistics of the referral procedure. GPs will visit the family to inform them about the referral after which the family will visit the pediatrician for follow-up diagnostics (14).

Cultural differences and expansion of NBS programs

While significant treatment- and test-driven expansions are seen in several NBS programs worldwide, other NBS programs expand at a slower rate. This illustrates that even though screening tests and treatments are available, the local context will determine the NBS program put in place [72]. The United States wields a more liberal approach when it comes to expansion of their NBS program with an increasing number of disorders being recommended for inclusion. As of July 2018, the Recommended Uniform Screening Panel (RUSP) includes 35 core conditions and 26 secondary targets [80]. In the US, public opinions can greatly influence NBS policies. Carol Ann, mother of David Vetter together with the Immune Deficiency Foundation (IDF) launched a successful advocating campaign for NBS for SCID. However, problems have also arisen from parent group advocacy campaigns pressuring individual states to screen for specific, non-recommended disorders [81-83]. Europe has a more conservative and heterogenous approach when it comes to population screening programs [73]. Healthcare has always been left to the own responsibility of the member states (principle of subsidiarity) allowing each country to make its own decision with regard to conditions that should be included in NBS programs [78]. Unlike the US where public opinions are more likely to influence NBS policies, advocacy efforts concerning health policy are limited [79]. European funding for NBS is typically organized by national health care services or health insurances, making NBS free of charge for parents. This often results in complex, time-consuming governmental financial decisions when expansion and inclusions of new conditions is considered [84].

In the past years, changes in understanding of conditions, technological developments and new treatments, have fueled the expansion of NBS. Some NBS programs have developed from programs that screen for a small number of conditions to complex programs sometimes including over 50 conditions. In the genomic era, further expansion of NBS programs will lead to new technical, clinical, ethical, and societal challenges accompanied by DNA-based screening [85].

NBS for SCID pilot programs and implementation in other countries

NBS programs are a complex, multi-faceted system and introduction of a new condition can lead to disruption if all steps of the public health policy cycle are not carefully considered. While the central idea of early detection of a disorder to facilitate treatment is simple, successful implementation of NBS for a disorder is something else. Pilot studies provide the opportunity to add new conditions and evaluate feasibility and disparities before disruptions of the program can occur. In addition, pilot studies are vital to the development of a strong evidence base to support decision-making regarding the addition of new conditions.

Specifically for SCID, pilot studies were of great aid when introducing DNA analysis as a primary screening modality in NBS laboratories. In addition, as SCID is the first immunodeficiency disorder added to the NBS program, pilot studies have helped with the gradual introducing pediatric-immunologists and clinical immunologists to the field of NBS. Clinical immunologists were less familiar with pre-symptomatic apparently healthy newborns, secondary findings and false-positive referrals. As screening is imposed upon an entire population with the goal of advancing public health, it is important to appreciate the differences between population-based screening programs versus clinical care [62]. Pilot studies and international shared learning have helped clinical immunology community with uniform follow-up protocols for a prompt and consistent approach to a definitive diagnosis.

First pilot studies for NBS for SCID were performed in the US almost a decade ago. The first state-wide SCID screening pilot study was initiated in Wisconsin in 2008 [86], with subsequently implementation of NBS for SCID in Massachusetts, Louisiana, and New York in 2009, and California, Texas, and Pennsylvania in 2010 [2]. In 2010, SCID was added to the RUSP which resulted in an acceleration in the number of states screening for SCID over the following years. By the end of 2018, NBS for SCID had been adopted by public health programs in all 50 states, the Navajo Nation, and Puerto Rico [87]. Pilot and proof-of-principle studies in Europe followed some years later in Sweden, the UK, France, the Netherlands and Spain [88-94]. Multiple nations around the world have instituted population-wide NBS for SCID, including New Zealand, Taiwan, Israel, Denmark, Sweden, Norway, Germany, Iceland and Switzerland, whereas others offer SCID screening in limited areas or have published pre-implementation analyses and pilot studies (Figure 5) [95-99].

In 2015, the Health Council of the Netherlands published the report '*Neonatal Screening, New Recommendations*' stating that SCID should be included in the Dutch NBS program [100]. The Committee believed that NBS for SCID would prevent significant, irreversible damage and yield substantial health gains for the affected child, while the disadvantage of unavoidable secondary findings did not outweigh the advantage of improved treatment by early diagnosis. The Dutch Ministry of Health adopted the advice and recommended an implementation pilot study including an exact cost-benefit analysis prior to national implementation. The pilot study would not focus on whether the TREC assay was a suitable method for the detection of SCID, as the effectiveness of NBS for SCID had already been proven in other screening programs abroad. However, as NBS for SCID is executed with a new, relatively expensive assay for the screening laboratory, an implementation pilot study was deemed instrumental for successful implementation.

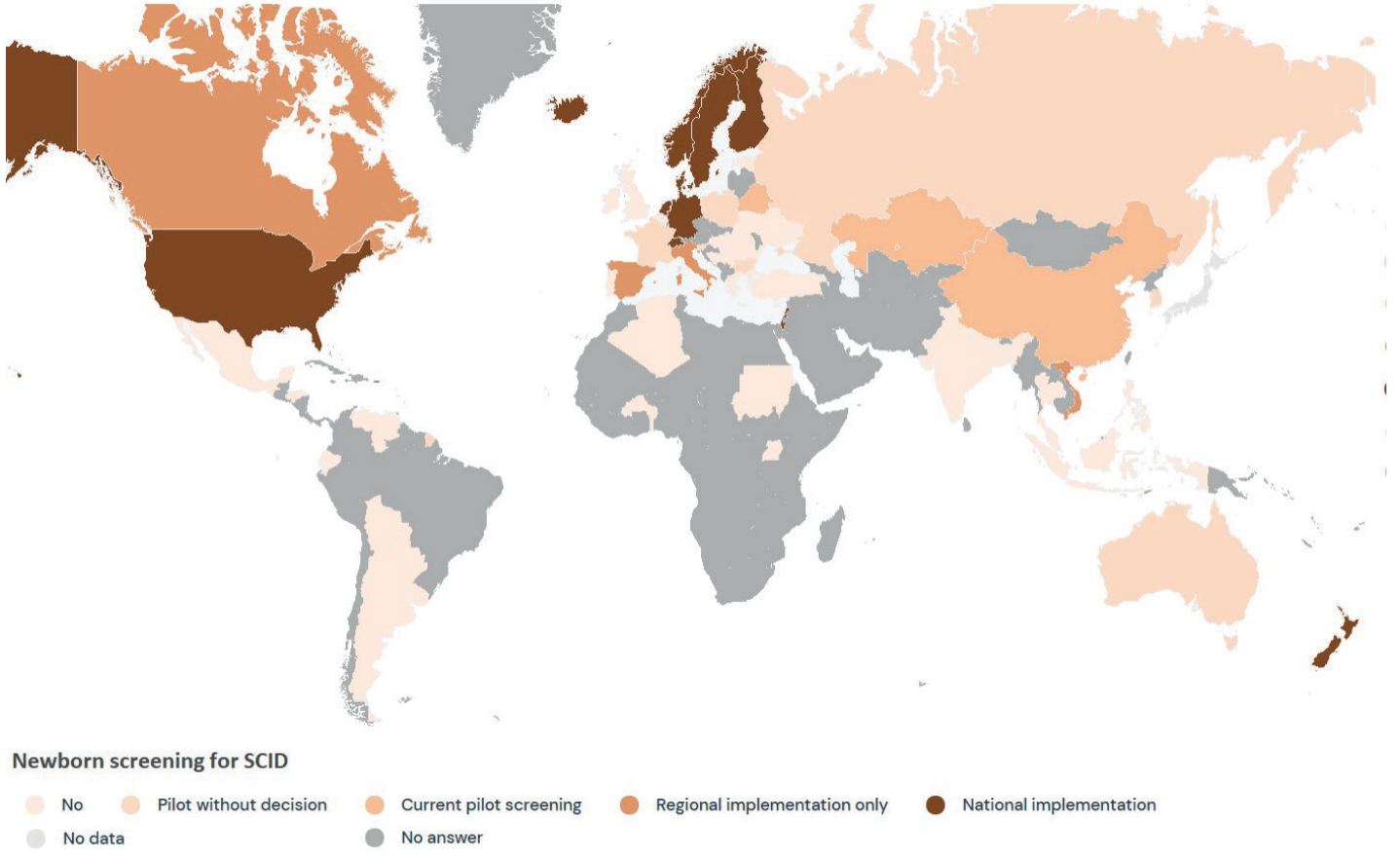

Figure 5. Overview of worldwide implementation of NBS for SCID in different countries. Figure adapted from PID Life Index (IPOPI).

AIM OF THIS THESIS

Introducing a new disorder into a screening program is a multifaceted process that needs to be carefully done without disruption of the program. This thesis therefore aimed to evaluate the many aspects that are associated with NBS for SCID, assessing feasibility and disparities prior to national implementation. NBS for SCID based on TREC detection has been implemented in many countries, with initial pilot studies dating back to 2008. The aim of this thesis was therefore not to prove the effectiveness of TREC quantification for the detection of SCID positive cases, but to obtain knowledge about practical implications, test qualities, costs and ethical and social implications of NBS for SCID. Practical implications included test modalities as NBS for SCID is associated with a new screening method, while also covering diagnostic and clinical follow-up aspects including unexpected screening outcomes and secondary findings. With a concise cost-effectiveness analysis, this thesis tried to provide an overview of costs and benefits associated with NBS for SCID, aiding in the final decisions to include SCID in the NBS program. Unique to this thesis was the inclusion of societal and ethical implications of NBS for SCID, aspects that had never been studied before. By assessing the perspectives of parents as key stakeholders in NBS, potential benefits and harms of NBS for newborns and their families could be identified. Moreover, societal acceptance is a major criterion when introducing new disorders in NBS programs and as parents are important stakeholders, their support is paramount. The ultimate aim of this thesis was to enable a flawless implementation of NBS for SCID in the Netherlands, while providing valuable recommendations for other countries that are considering SCID screening and for countries that want to optimize their implemented NBS SCID program. NBS for SCID in the Netherlands will contribute to improved outcomes for future SCID patients after HSCT: "*helping to break the protective bubble in the best possible way*".

THESIS OUTLINE

This thesis will address the many aspects of NBS for SCID from preparatory steps to pilot study to optimizing after implementation. **Chapter 2** focuses on the first preparatory steps by exploring test modalities and evaluating a commercially available TREC assay in the NBS laboratory. In **Chapter 3**, the structure of the Dutch NBS program is further specified, while different aspects needed for a pilot study are assessed and results of a comparison study between test methods are discussed. All preparatory steps led to a prospective implementation pilot called the SONNET-study (SCID screening Onderzoek in Nederland met TRECs) of which the results are discussed in **Chapter 4.** This pilot study did not only focus on the technical aspects of NBS for SCID, but also evaluated the perspectives of parents as public uptake and parental acceptance of a test method are not guaranteed. **Chapter 5** explores different second tier test options and screening strategies showing that even after implementation, NBS programs should continue to optimize their programs aiming for the highest sensitivity while limiting the number of false-positive referrals. Some outcomes of NBS are unanticipated as showed in the case report of **Chapter 6** in which newborns with abnormal NBS SCID results and profound T-cell lymphopenia due to maternal immunosuppressant use are presented. More ethical aspects are addressed as this thesis delves deeper into the dilemma of an early diagnosis of the incurable condition ataxia telangiectasia (A-T) as a secondary finding of NBS for SCID. Parents of children with A-T provide their opinion on this quandary in **Chapter 7,** while the perspectives of parents of healthy newborns are presented in **Chapter 8**. Cost-effectiveness is key when adding new conditions to a NBS program and **Chapter 9** provides a cost-effectiveness analysis for NBS for SCID in Netherlands based on real-life data from the SONNET-study. In **Chapter 10** recommendations are provided for uniform definitions of screening terminology and case definitions after follow-up in NBS for SCID. These guidelines will unite the NBS community and the clinical immunological community by bridging the gaps in language and perspective between these disciplines. Expansion of NBS with new disorders is driven by development of new test modalities and treatment options, therefore **Chapter 11** will discuss the future perspectives on NBS for SCID and other IEI that could benefit from an early diagnosis and intervention. This thesis ends with a general discussion describing new points of debate, recommendations and future directions coupled with my personal perspective in **Chapter 12** and a summary of all work presented in this thesis in **Chapter 13.**

REFERENCES

1. Fischer, A., Severe combined immunodeficiencies (SCID). Clinical & Experimental Immunology, 2000. 122(2): p. 143-149.
2. Kwan, A., et al., Newborn screening for severe combined immunodeficiency in 11 screening programs in the United States. Jama, 2014. 312(7): p. 729-38.
3. de Pagter, A.P., et al., Overview of 15-year severe combined immunodeficiency in the Netherlands: towards newborn blood spot screening. Eur J Pediatr, 2015. 174(9): p. 1183-8.
4. Al-Muhsen, S. and Z. Alsum, Primary immunodeficiency diseases in the Middle East. Ann N Y Acad Sci, 2012. 1250: p. 56-61.
5. Haddad, E. and M. Hoenig, Hematopoietic Stem Cell Transplantation for Severe Combined Immunodeficiency (SCID). Front Pediatr, 2019. 7: p. 481.
6. Heimall, J., et al., Immune reconstitution and survival of 100 SCID patients post-hematopoietic cell transplant: a PIDTC natural history study. Blood, 2017. 130(25): p. 2718-2727.
7. Tangye, S.G., et al., Human Inborn Errors of Immunity: 2019 Update on the Classification from the International Union of Immunological Societies Expert Committee. J Clin Immunol, 2020. 40(1): p. 24-64.
8. Bousfiha, A., et al., Human Inborn Errors of Immunity: 2019 Update of the IUIS Phenotypical Classification. Journal of Clinical Immunology, 2020. 40(1): p. 66-81.
9. Tangye, S.G., et al., The Ever-Increasing Array of Novel Inborn Errors of Immunity: an Interim Update by the IUIS Committee. J Clin Immunol, 2021. 41(3): p. 666-679.
10. White, H., et al., Intrinsic defects of B cell function in X-linked severe combined immunodeficiency. Eur J Immunol, 2000. 30(3): p. 732-7.
11. Miggelbrink, A.M., et al., B-cell differentiation and IL-21 response in IL2RG/JAK3 SCID patients after hematopoietic stem cell transplantation. Blood, 2018. 131(26): p. 2967-2977.
12. Buckley, R.H., Molecular Defects in Human Severe Combined Immunodeficiency and Approaches to Immune Reconstitution. Annual Review of Immunology, 2004. 22(1): p. 625-655.
13. Kung, C., et al., Mutations in the tyrosine phosphatase CD45 gene in a child with severe combined immunodeficiency disease. Nat Med, 2000. 6(3): p. 343-5.
14. Notarangelo, L.D., Primary immunodeficiencies. Journal of Allergy and Clinical Immunology, 2010. 125(2, Supplement 2): p. S182-S194.
15. van der Burg, M. and A.R. Gennery, Educational paper. The expanding clinical and immunological spectrum of severe combined immunodeficiency. European journal of pediatrics, 2011. 170(5): p. 561-571.
16. Nyhan, W.L., Disorders of purine and pyrimidine metabolism. Molecular Genetics and Metabolism, 2005. 86(1): p. 25-33.
17. Gaspar, H.B., et al., How I treat ADA deficiency. Blood, 2009. 114(17): p. 3524-32.
18. Pannicke, U., et al., Reticular dysgenesis (aleukocytosis) is caused by mutations in the gene encoding mitochondrial adenylate kinase 2. Nat Genet, 2009. 41(1): p. 101-5.
19. Delmonte, O.M., A. Villa, and L.D. Notarangelo, Immune dysregulation in patients with RAG deficiency and other forms of combined immune deficiency. Blood, 2020. 135(9): p. 610-619.
20. Reeve, L., et al., Do not let them slip through the net: Catching a case of leaky severe combined immunodeficiency. J Paediatr Child Health, 2020. 56(5): p. 809-811.

21. Villa, A., L.D. Notarangelo, and C.M. Roifman, Omenn syndrome: inflammation in leaky severe combined immunodeficiency. J Allergy Clin Immunol, 2008. 122(6): p. 1082-6.
22. Shearer, W.T., et al., Establishing diagnostic criteria for severe combined immunodeficiency disease (SCID), leaky SCID, and Omenn syndrome: the Primary Immune Deficiency Treatment Consortium experience. The Journal of allergy and clinical immunology, 2014. 133(4): p. 1092-1098.
23. Seleman, M., et al., Uses of Next-Generation Sequencing Technologies for the Diagnosis of Primary Immunodeficiencies. Frontiers in Immunology, 2017. 8(847).
24. Mousallem, T., et al., Clinical application of whole-genome sequencing in patients with primary immunodeficiency. The Journal of allergy and clinical immunology, 2015. 136(2): p. 476-9.e6.
25. Griffith, L.M., et al., Improving cellular therapy for primary immune deficiency diseases: Recognition, diagnosis, and management. Journal of Allergy and Clinical Immunology, 2009. 124(6): p. 1152-1160.e12.
26. Kelty, W.J., et al., The role of breast-feeding in cytomegalovirus transmission and hematopoietic stem cell transplant outcomes in infants with severe combined immunodeficiency. The Journal of Allergy and Clinical Immunology: In Practice, 2019. 7(8): p. 2863-2865.e3.
27. Dorsey, M.J., et al., Infections in Infants with SCID: Isolation, Infection Screening, and Prophylaxis in PIDTC Centers. Journal of Clinical Immunology, 2021. 41(1): p. 38-50.
28. Gatti, R.A., et al., Immunological reconstitution of sex-linked lymphopenic immunological deficiency. Lancet, 1968. 2(7583): p. 1366-9.
29. De Koning, J., et al., Transplantation of bone-marrow cells and fetal thymus in an infant with lymphopenic immunological deficiency. Lancet, 1969. 1(7608): p. 1223-7.
30. Griffith, L.M., et al., Allogeneic hematopoietic cell transplantation for primary immune deficiency diseases: current status and critical needs. The Journal of allergy and clinical immunology, 2008. 122(6): p. 1087-1096.
31. Dvorak, C.C., et al., The natural history of children with severe combined immunodeficiency: baseline features of the first fifty patients of the primary immune deficiency treatment consortium prospective study 6901. J Clin Immunol, 2013. 33(7): p. 1156-64.
32. Pai, S.-Y., et al., Transplantation outcomes for severe combined immunodeficiency, 2000-2009. The New England journal of medicine, 2014. 371(5): p. 434-446.
33. Cavazzana-Calvo, M., et al., Long-term T-cell reconstitution after hematopoietic stem-cell transplantation in primary T-cell-immunodeficient patients is associated with myeloid chimerism and possibly the primary disease phenotype. Blood, 2007. 109(10): p. 4575-81.
34. Shaw, P., et al., Conditioning Perspectives for Primary Immunodeficiency Stem Cell Transplants. Front Pediatr, 2019. 7: p. 434.
35. Haddad, E., S. Leroy, and R.H. Buckley, B-cell reconstitution for SCID: should a conditioning regimen be used in SCID treatment? J Allergy Clin Immunol, 2013. 131(4): p. 994-1000.
36. Abd Hamid, I.J., et al., Long-term outcome of hematopoietic stem cell transplantation for IL2RG/JAK3 SCID: a cohort report. Blood, 2017. 129(15): p. 2198-2201.
37. Gennery, A.R., et al., Long Term Outcome and Immune Function After Hematopoietic Stem Cell Transplantation for Primary Immunodeficiency. Frontiers in pediatrics, 2019. 7: p. 381-381.
38. Schuetz, C., et al., SCID patients with ARTEMIS vs RAG deficiencies following HCT: increased risk of late toxicity in ARTEMIS-deficient SCID. Blood, 2014. 123(2): p. 281-9.

39. Hershfield, M.S., Enzyme replacement therapy of adenosine deaminase deficiency with polyethylene glycol-modified adenosine deaminase (PEG-ADA). Immunodeficiency, 1993. 4(1-4): p. 93-7.
40. Fox, T.A. and C. Booth, Gene therapy for primary immunodeficiencies. British Journal of Haematology, 2021. 193(6): p. 1044-1059.
41. Kohn, D.B. and C.Y. Kuo, New frontiers in the therapy of primary immunodeficiency: From gene addition to gene editing. J Allergy Clin Immunol, 2017. 139(3): p. 726-732.
42. Gaspar, H.B., et al., Gene therapy of X-linked severe combined immunodeficiency by use of a pseudotyped gammaretroviral vector. Lancet, 2004. 364(9452): p. 2181-7.
43. Cavazzana-Calvo, M., et al., Gene therapy for severe combined immunodeficiency. Annu Rev Med, 2005. 56: p. 585-602.
44. Gaspar, H.B., et al., Successful reconstitution of immunity in ADA-SCID by stem cell gene therapy following cessation of PEG-ADA and use of mild preconditioning. Mol Ther, 2006. 14(4): p. 505-13.
45. Aiuti, A., et al., Correction of ADA-SCID by stem cell gene therapy combined with nonmyeloablative conditioning. Science, 2002. 296(5577): p. 2410-3.
46. Howe, S.J., et al., Insertional mutagenesis combined with acquired somatic mutations causes leukemogenesis following gene therapy of SCID-X1 patients. The Journal of Clinical Investigation, 2008. 118(9): p. 3143-3150.
47. Hacein-Bey-Abina, S., et al., Insertional oncogenesis in 4 patients after retrovirus-mediated gene therapy of SCID-X1. The Journal of Clinical Investigation, 2008. 118(9): p. 3132-3142.
48. Kohn, D.B., et al., Autologous Ex Vivo Lentiviral Gene Therapy for Adenosine Deaminase Deficiency. New England Journal of Medicine, 2021. 384(21): p. 2002-2013.
49. Kuo, C.Y. and D.B. Kohn, Gene Therapy for the Treatment of Primary Immune Deficiencies. Curr Allergy Asthma Rep, 2016. 16(5): p. 39.
50. Aiuti, A., M.G. Roncarolo, and L. Naldini, Gene therapy for ADA-SCID, the first marketing approval of an ex vivo gene therapy in Europe: paving the road for the next generation of advanced therapy medicinal products. EMBO Mol Med, 2017. 9(6): p. 737-740.
51. Brown, L., et al., Neonatal diagnosis of severe combined immunodeficiency leads to significantly improved survival outcome: the case for newborn screening. Blood, 2011. 117(11): p. 3243-3246.
52. Chan, A., et al., Early vs. delayed diagnosis of severe combined immunodeficiency: a family perspective survey. Clinical immunology (Orlando, Fla.), 2011. 138(1): p. 3-8.
53. Buckley, R.H., et al., Human severe combined immunodeficiency: genetic, phenotypic, and functional diversity in one hundred eight infants. J Pediatr, 1997. 130(3): p. 378-87.
54. Janik, D.K., et al., A multiplex immunoassay using the Guthrie specimen to detect T-cell deficiencies including severe combined immunodeficiency disease. Clinical chemistry, 2010. 56(9): p. 1460-1465.
55. Collier, F., et al., Flow cytometric assessment of cord blood as an alternative strategy for population-based screening of severe combined immunodeficiency. J Allergy Clin Immunol, 2013. 131(4): p. 1251-2.
56. Buckley, R.H., The long quest for neonatal screening for severe combined immunodeficiency. Journal of Allergy and Clinical Immunology, 2012. 129(3): p. 597-604.
57. Verschuren, M.C., et al., Preferential rearrangements of the T cell receptor-delta-deleting elements in human T cells. J Immunol, 1997. 158(3): p. 1208-16.

58. Hazenberg, M.D., et al., T cell receptor excision circles as markers for recent thymic emigrants: basic aspects, technical approach, and guidelines for interpretation. Journal of Molecular Medicine, 2001. 79(11): p. 631-640.
59. Institute, C.a.L.S., Newborn Bloot Spot Screening for Severe Combined Immunodeficiency by Measurement of T-cell receptor Excision Circles (new version, unpublished). 2021.
60. Douek, D.C., et al., Changes in thymic function with age and during the treatment of HIV infection. Nature, 1998. 396(6712): p. 690-695.
61. Chan, K. and J.M. Puck, Development of population-based newborn screening for severe combined immunodeficiency. Journal of Allergy and Clinical Immunology, 2005. 115(2): p. 391-398.
62. Puck, J.M., Newborn screening for severe combined immunodeficiency and T-cell lymphopenia. Immunological Reviews, 2019. 287(1): p. 241-252.
63. Knight, V., et al., Follow-Up for an Abnormal Newborn Screen for Severe Combined Immunodeficiencies (NBS SCID): A Clinical Immunology Society (CIS) Survey of Current Practices. International journal of neonatal screening, 2020. 6(3): p. 52.
64. Chong, H.J., S. Maurer, and J. Heimall, What to Do with an Abnormal Newborn Screen for Severe Combined Immune Deficiency. Immunol Allergy Clin North Am, 2019. 39(4): p. 535-546.
65. Dorsey, M.J., et al., Treatment of infants identified as having severe combined immunodeficiency by means of newborn screening. The Journal of allergy and clinical immunology, 2017. 139(3): p. 733-742.
66. Patrawala, M. and L. Kobrynski, Nonsevere combined immunodeficiency T-cell lymphopenia identified through newborn screening. Curr Opin Allergy Clin Immunol, 2019. 19(6): p. 586-593.
67. Thomas, C., et al., A Severe Neonatal Lymphopenia Associated With Administration of Azathioprine to the Mother in a Context of Crohn's Disease. J Crohns Colitis, 2018. 12(2): p. 258-261.
68. Kuo, C.Y., et al., Profound T-cell lymphopenia associated with prenatal exposure to purine antagonists detected by TREC newborn screening. The Journal of Allergy and Clinical Immunology: In Practice, 2017. 5(1): p. 198-200.
69. Mauracher, A.A., et al., Causes of low neonatal T-cell receptor excision circles: A systematic review. J Allergy Clin Immunol Pract, 2017. 5(5): p. 1457-1460.e22.
70. Dorsey, M. and J. Puck, Newborn Screening for Severe Combined Immunodeficiency in the US: Current Status and Approach to Management. International journal of neonatal screening, 2017. 3(2): p. 15.
71. El-Hattab, A.W., M. Almannai, and V.R. Sutton, Newborn Screening: History, Current Status, and Future Directions. Pediatric Clinics of North America, 2018. 65(2): p. 389-405.
72. Jansen, M.E., et al., Expanded Neonatal Bloodspot Screening Programmes: An Evaluation Framework to Discuss New Conditions With Stakeholders. Frontiers in pediatrics, 2021. 9: p. 635353-635353.
73. Therrell, B.L., et al., Current status of newborn screening worldwide: 2015. Seminars in perinatology, 2015. 39(3): p. 171-187.
74. Jansen, M.E., S.C. Metternick-Jones, and K.J. Lister, International differences in the evaluation of conditions for newborn bloodspot screening: a review of scientific literature and policy documents. European journal of human genetics : EJHG, 2016. 25(1): p. 10-16.
75. Wilson, J.M.G., G. Jungner, and O. World Health, Principles and practice of screening for disease / J. M. G. Wilson, G. Jungner. 1968, World Health Organization: Geneva.

76. Andermann, A., et al., Revisiting Wilson and Jungner in the genomic age: a review of screening criteria over the past 40 years. Bull World Health Organ, 2008. 86(4): p. 317-9.
77. Franková, V., et al., Regulatory landscape of providing information on newborn screening to parents across Europe. European Journal of Human Genetics, 2021. 29(1): p. 67-78.
78. Loeber, J.G., et al., Newborn screening programmes in Europe; arguments and efforts regarding harmonization. Part 1. From blood spot to screening result. Journal of inherited metabolic disease, 2012. 35(4): p. 603-611.
79. Burgard, P., et al., Newborn screening programmes in Europe; arguments and efforts regarding harmonization. Part 2. From screening laboratory results to treatment, follow-up and quality assurance. Journal of inherited metabolic disease, 2012. 35(4): p. 613-625.
80. Recommended Uniform Screening Panel. 2018 February 2019 [cited 2020 8 January]; Recommended Uniform Screening Panel]. Available from: https://www.hrsa.gov/advisory-committees/heritable-disorders/rusp/index.html.
81. Wilcken, B. and V. Wiley, Fifty years of newborn screening. Journal of paediatrics and child health, 2015. 51(1): p. 103-107.
82. Kwon, J.M., et al., Consensus guidelines for newborn screening, diagnosis and treatment of infantile Krabbe disease. Orphanet journal of rare diseases, 2018. 13(1): p. 30-30.
83. Orsini, J.J., Newborn screening for Krabbe disease: perceived and current ethical issues. Developmental medicine and child neurology, 2019. 61(12): p. 1354-1354.
84. Fischer, K.E. and W.H. Rogowski, Funding decisions for newborn screening: a comparative review of 22 decision processes in Europe. International journal of environmental research and public health, 2014. 11(5): p. 5403-5430.
85. King, J.R. and L. Hammarström, Newborn Screening for Primary Immunodeficiency Diseases: History, Current and Future Practice. Journal of clinical immunology, 2018. 38(1): p. 56-66.
86. Routes, J.M., et al., Statewide newborn screening for severe T-cell lymphopenia. Jama, 2009. 302(22): p. 2465-70.
87. Routes, J. and J. Verbsky, Newborn Screening for Severe Combined Immunodeficiency. Curr Allergy Asthma Rep, 2018. 18(6): p. 34.
88. Borte, S., et al., Neonatal screening for severe primary immunodeficiency diseases using high-throughput triplex real-time PCR. Blood, 2012. 119(11): p. 2552-5.
89. Adams, S.P., et al., Screening of neonatal UK dried blood spots using a duplex TREC screening assay. J Clin Immunol, 2014. 34(3): p. 323-30.
90. Audrain, M., et al., Evaluation of the T-cell receptor excision circle assay performances for severe combined immunodeficiency neonatal screening on Guthrie cards in a French single centre study. Clin Immunol, 2014. 150(2): p. 137-9.
91. de Felipe, B., et al., Prospective neonatal screening for severe T- and B-lymphocyte deficiencies in Seville. Pediatr Allergy Immunol, 2016. 27(1): p. 70-7.
92. Blom, M., et al., An evaluation of the TREC assay with regard to the integration of SCID screening into the Dutch newborn screening program. Clin Immunol, 2017. 180: p. 106-110.
93. Barbaro, M., et al., Newborn Screening for Severe Primary Immunodeficiency Diseases in Sweden-a 2-Year Pilot TREC and KREC Screening Study. J Clin Immunol, 2017. 37(1): p. 51-60.
94. Thomas, C., et al., Clinical and economic aspects of newborn screening for severe combined immunodeficiency: DEPISTREC study results. Clin Immunol, 2019. 202: p. 33-39.
95. Richards, S., et al., Diagnosis and management of severe combined immunodeficiency in Australia and New Zealand. J Paediatr Child Health, 2020. 56(10): p. 1508-1513.

96. Chien, Y.-H., et al., Newborn Screening for Severe Combined Immunodeficiency in Taiwan. International Journal of Neonatal Screening, 2017. 3(3): p. 16.
97. Rechavi, E., et al., First Year of Israeli Newborn Screening for Severe Combined Immunodeficiency-Clinical Achievements and Insights. Front Immunol, 2017. 8: p. 1448.
98. Strand, J., et al., Second-Tier Next Generation Sequencing Integrated in Nationwide Newborn Screening Provides Rapid Molecular Diagnostics of Severe Combined Immunodeficiency. Front Immunol, 2020. 11: p. 1417.
99. Trück, J., et al., Swiss newborn screening for severe T and B cell deficiency with a combined TREC/KREC assay - management recommendations. Swiss Med Wkly, 2020. 150: p. w20254.
100. Health Council of the Netherlands, Neonatal screening: new recommendations. 2015, Health Council of the Netherlands: The Hague.