Established in 1871

Swiss Medical Weekly

Formerly: Schweizerische Medizinische Wochenschrift

An open access, online journal • www.smw.ch

Review article: Biomedical intelligence | Published 15 June 2020 | doi:10.4414/smw.2020.20254
Cite this as: Swiss Med Wkly. 2020;150:w20254

Swiss newborn screening for severe T and B cell deficiency with a combined TREC/KREC assay – management recommendations

Trück Johannes[a], Prader Seraina[a], Natalucci Giancarlo[b], Hagmann Cornelia[a], Brotschi Barbara[a], Kelly Janet[a], Bassler Dirk[b], Steindl Katharina[c], Rauch Anita[c], Baumgartner Matthias[a], Fingerhut Ralph[a], Hauri-Hohl Mathias[a], Güngör Tayfun[a], Schmid Jana Pachlopnik[a], Berger Christoph[a], Reichenbach Janine[a]

[a] University Children's Hospital Zurich, Switzerland
[b] Department of Neonatology, University Hospital Zurich and University of Zurich, Switzerland
[c] Institute of Medical Genetics, University of Zurich, Switzerland

Summary

The recent introduction of newborn screening for severe primary T and B cell deficiencies in Switzerland allows rapid identification of patients with severe combined immunodeficiency (SCID). Outcomes for SCID are greatly improved by early diagnosis and treatment with allogeneic haematopoietic stem cell transplantation or, in selected cases, gene therapy. National centralised newborn screening is performed in Switzerland since January 2019 using a combined T cell receptor excision circles (TREC) / κ-deleting recombination excision circles (KREC) assay, also revealing infants with non-SCID severe T and B cell disorders, who are often diagnosed with a substantial delay. Here, we outline the screening procedure currently performed in Switzerland and give recommendations for diagnostic evaluations and precautionary measures against infection in children with abnormal screening test results.

Keywords: *newborn screening, primary immunodeficiency, inborn errors of immunity, T cells, B cells, TREC, KREC, Severe combined immunodeficiency, agammaglobulinaemia*

Introduction

Severe combined immunodeficiency (SCID) is one of the most severe forms of primary immunodeficiency (PID) and is considered a paediatric emergency. SCID was first described in Switzerland in the 1950s [1, 2] by the founders of Swiss Paediatric Immunology as "Swiss-type agammaglobulinaemia". The treatment of choice for SCID is allogeneic haematopoietic stem cell transplantation (HSCT) or, in selected cases, gene therapy. Of note, one of the most important factors for achieving high HSCT cure rates is a good clinical condition of the child at time of transplant, i.e., absence of active infection. With early diagnosis and treatment of SCID patients at an experienced transplant centre prior to the onset of infection, high cure rates of 80–95% can be achieved [3–6]. However, there is a considerable number of undiagnosed patients, many of whom die before treatment can be started. Therefore, newborn screening for severe primary T and B cell deficiencies was introduced in Switzerland on the 1 January 2019. Testing is performed centrally in the Swiss Newborn Screening (NBS) Laboratory at the University Children's Hospital in Zurich.

SCID and other severe T cell diseases (here referred to as combined immunodeficiency, CID) are disorders of T cell development and can be associated with additional defects such as B and natural killer (NK) cell deficiency. Affected infants appear healthy at birth but typically develop life-threatening bacterial, viral, fungal or opportunistic infections, persistent diarrhoea and failure to thrive within the first weeks and months of life [7–9]. If left untreated, severe T cell disorders are usually fatal in the first year of life. Advances in cellular treatments, mainly HSCT, and more recently gene therapy, as well as the development

The recommendations in this guideline were endorsed by the Paediatric Infectious Disease Group of Switzerland (PIGS), the Swiss Society of Neonatology (SSN) and the Swiss Society for Allergy and Immunology (SSAI/SGAI).

Correspondence:
Johannes Trück, MD, DPhil, University Children's Hospital Zurich, Steinwiesstrasse 75, CH-8032 Zurich, Johannes.Trueck[at]kispi.uzh.ch

ABBREVIATIONS:

ADA	adenosine deaminase
CID	combined immunodeficiency
CMV	cytomegalovirus
DBS	dried blood spots
GA	gestational age
HSCT	haematopoietic stem cell transplantation
IgG	immunoglobulin G
KREC	kappa-deleting recombination excision circles
MHC	major histocompatibility complex
MMR	measles, mumps and rubella
NBS	newborn screening
PID	primary immunodeficiency
PNP	purine nucleoside phosphorylase
SCID	severe combined immunodeficiency
TREC	T cell receptor excision circles
ZAP-70	zeta-chain-associated protein kinase 70

of novel therapeutics have considerably improved the survival and quality of life of these patients [10].

Severe isolated disorders of B cell development typically result in absent B cells in the peripheral blood and low or absent antibody levels (hypo- or agammaglobulinaemia). Due to materno-fetal transfer of IgG antibodies, young infants with isolated B cell deficiency are temporarily protected against infections despite a lack of endogenous antibody production. This leads to a delay in the presentation of clinical symptoms, most commonly due to infections affecting the respiratory tract, until the age of 3–6 months after maternal antibodies have waned [11, 12].

The estimated incidence of severe forms of T and B cell deficiencies requiring immediate attention is variable between different populations and settings, but is expected to be around 1:50,000 live births for SCID and 1:10,000 live births for severe T cell lymphopenia of other origin [13–16]. SCID and other T cell disorders may be associated with syndromal features or congenital heart defects, such as cartilage-hair hypoplasia, ataxia telangiectasia or DiGeorge syndrome (22q11.2 deletion syndrome), the latter representing a relatively common PID characterised by thymus hypo- or aplasia, which is treated by thymus transplantation rather than HSCT.

Screening

Switzerland's newborn screening programme for severe primary T and B cell deficiencies is based on a commercially available assay (TREC-KREC-ACTB, "SPOT-it" Kit, ImmunolVD, Stockholm, Sweden), which quantifies T cell receptor excision circles (TRECs) and kappa-deleting recombination excision circles (KRECs) by means of a real-time polymerase chain reaction (PCR) test from dried blood spots (DBS) collected as part of the national centralised newborn screening programme (Guthrie cards) [17, 18].

TRECs are small ring-shaped DNA fragments that are produced as waste products during somatic recombination of the T cell receptor gene locus upon T cell maturation in the thymus [19]. The number of TRECs in the peripheral blood correlates well with the number of freshly formed naïve T cells, making the TREC test suitable for detecting disorders in T cell development. In healthy infants, TRECs are produced in large numbers, whereas in infants with SCID or severe T cell lymphopenia TRECs are either not or only barely detectable. Similarly, KRECs develop during the maturation and somatic recombination of the B cell receptor locus, and their copy number correlates with the number of freshly formed naïve B cells [20].

The quality marker beta-actin is used as a positive test control to evaluate and ensure the methodological success of the measurement of TREC/KREC copy numbers and is always run alongside the TREC/KREC test. The thresholds for both TRECs and KRECs recommended by the manufacturer have been cautiously adapted by the Swiss NBS centre for the first year of screening, to allow sufficient sensitivity while keeping the false-positive rate as low as possible:

- TRECs: abnormal if <10 copies/punch of DBS
- KRECs: abnormal if <6 copies/punch of DBS
- (beta-actin: normal if >1000 copies/punch of DBS)

The combined TREC/KREC screening is known to miss certain potentially severe functional (as opposed to above mentioned numerical) T cell deficiencies such as zeta-chain-associated protein kinase 70 (ZAP-70) deficiency, major histocompatibility complex (MHC) class II deficiency, as well as milder forms of adenosine deaminase (ADA) or purine nucleoside phosphorylase (PNP) deficiency that clinically present with a late onset [21], and is also not sensitive enough to detect milder forms of T and B cell deficiency. In addition, the TREC/KREC assay is not an appropriate method for detecting disorders that affect other elements of the immune system, such as neutrophil or complement diseases, or disorders of innate immunity.

Diagnostic procedure in the case of abnormal screening results

TREC/KREC testing

As a screening test may only give an indication of potential diseases, confirmatory testing is required after an abnormal screening result. Testing is performed stepwise depending on the findings and the severity of possible outcomes (fig. 1). Abnormal screening results are reported to the physicians of the Department of Immunology at the University Children's Hospital Zurich during the operating hours of the Newborn Screening Laboratory (Monday to Friday 8:00–17:00). Parents and local healthcare professionals are then immediately informed and subsequent management planned.

In the case of undetectable TRECs (0–1 TREC copy per punch of DBS, i.e., a so-called "urgent-positive" result with very high suspicion of SCID), families are preferably seen within 24–48 hours in the immunology outpatient clinic at the University Children's Hospital Zurich or, exceptionally, in another immunology department highly experienced in the care of PID patients in the proximity of the family. A longer delay is acceptable in situations with low but detectable TRECs/KRECs, but patients with low TRECs should still be seen within 72 hours of notification.

In patients with low or undetectable KRECs, a follow-up NBS card measurement is performed 10–14 days later by the midwife, the primary-care paediatrician or a hospital nearby, after the family and involved healthcare professionals have been informed. In the event of continuing abnormal NBS results, families are invited for a consultation in the Immunology outpatient clinic at the University Children's Hospital Zurich. If the risk of primary B cell deficiency is low, such as in the case of low but detectable KRECs and maternal immunosuppression during pregnancy, there is the possibility to discuss with the parents whether to measure another NBS card before further investigations (fig. 1).

Low KRECs in particular can be due to secondary causes such as maternal immunosuppression during pregnancy (e.g., azathioprine, rituximab) and therefore careful history-taking is required [11]. Information on maternal immunosuppression is requested on the new Guthrie cards, which have been available in Switzerland since the start of 2019.

Abnormal TREC results may sometimes be associated with other (not primarily immunological) diseases such as prematurity, hydrops fetalis, congenital heart disease, trisomy 21, sepsis, malformations, chylothorax or congenital syndromes, although an associated immunodeficiency must still be ruled out [22, 23].

Preterm infants with abnormal TREC/KREC testing

Preterm infants may have low TREC and/or KREC levels due to the immaturity of their immune system. Since preterm infants may nonetheless suffer from SCID, neonates with undetectable TRECs should still be urgently evaluated by a paediatric immunologist experienced in the management of PID and SCID, and relevant investigations must be performed. Preterm infants with low but detectable TRECs may be followed clinically and the TREC/KREC screening repeated every 2 weeks until results normalised or they reach 37 weeks adjusted gestational age, at which time lymphocyte enumeration via flow cytometry is performed [24].

Breastfeeding

In the case of abnormal TREC results, it is important to rapidly assess whether the infant's mother has already acquired cytomegalovirus (CMV) infection, because only those mothers who are CMV seronegative should be allowed to breastfeed (table 1). As preterm infants particularly benefit from human milk [25–27] and may remain in the evaluation phase longer while repeated TREC/KREC testing is performed, infants with low (but measurable) TREC levels may be given pasteurised human milk (Holder pasteurisation: 62.5°C for 30 min), even when the infant's mother is CMV seropositive.

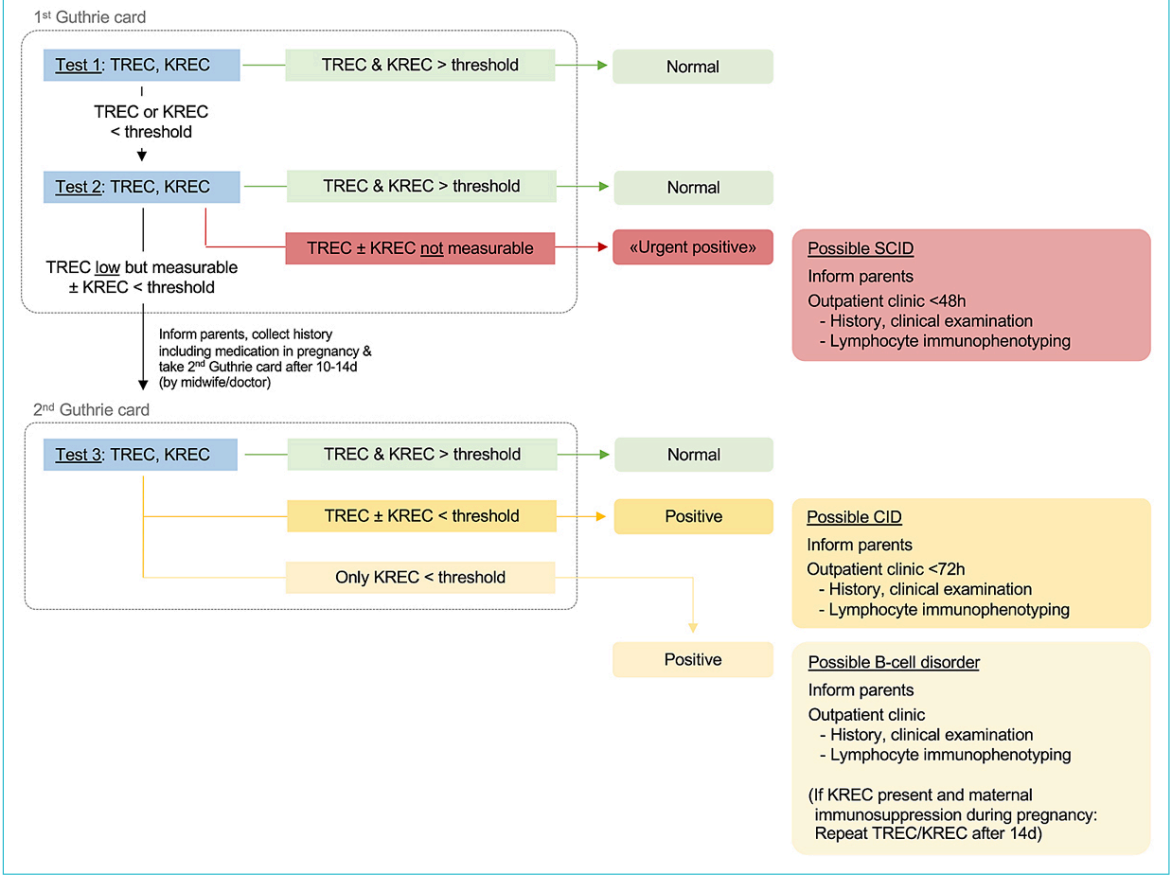
Diagnostic follow-up investigations

Whenever possible, the initial clinical assessment is performed by a SCID-experienced paediatric immunologist at the Swiss national HSM (highly specialised medicine) reference centre for Paediatric Immunology at the University Children's Hospital Zurich. If this is not possible (e.g., for premature or non-transportable infants), an on-call immunologist / HSCT specialist is available round the clock to discuss the necessary measures (via the hospital switchboard +41 44 266 7111).

The evaluation of an infant with an abnormal TREC result includes a thorough medical history to determine if there were any prenatal exposures or perinatal illnesses impacting the screening. For well-appearing full-term infants, the family history is reviewed specifically for deaths in early childhood or fetal loss during pregnancy, significant infectious history in siblings and close family members, as well as consanguinity. A thorough physical examination is performed, whenever possible together with a clinical geneticist, including evaluation for dysmorphic features, skin rashes, absence of lymphoid tissue, and somatic growth.

In addition to the clinical evaluation, a fresh blood sample for lymphocyte immunophenotyping including naïve $CD4^+CD31^+$ T cells (representing recent thymic emigrants) is taken at the initial consultation (table 2 top panel). Whenever possible, this is performed in the Immunology Diagnostics Laboratory of the HSM centre for

Figure 1: Screening algorithm –TREC/KREC screening and initial diagnostic evaluation.



Paediatric Immunology at the University Children's Hospital Zurich to ensure comparability of the results. In addition to lymphocyte immunophenotyping, further work-up is required to narrow down the differential diagnosis and determine the severity of the disease (table 2 second panel).

Genetic testing

In the case of suspected SCID, genetic testing is performed through whole-exome sequencing and additional Sanger sequencing of the two RNA genes *RMRP* and *RNU4ATAC* at an accredited laboratory of medical genetics with experience in the analysis of variants in PID genes to identify the underlying mutation [12]. Results are usually available within 2–3 weeks. Generally, blood samples are also taken from both parents in addition to the blood sample from the infant in order to carry out family segregation analysis. If microdeletion syndromes (such as microdeletion 22q11.2 / DiGeorge syndrome) are suspected, a targeted multiplex ligation-dependent probe amplification (MLPA) assay or a genome-wide microarray analysis is also performed.

In the case of isolated abnormal KREC levels suggesting a B cell disorder, genetic investigations through whole-exome sequencing may be used to identify mutations in immune genes that are known to cause agammaglobulinaemia or severe hypogammaglobulinaemia [12].

Management of infants with severe T cell lymphopenia (suspected SCID)

Infants with severe T cell lymphopenia should be placed in protective isolation while in hospital and blood products, if required, should be leucocyte-depleted and irradiated [8, 9]. If at home, families of affected patients should be appropriately instructed on how they can reduce the risk of infection, including good hand hygiene and the avoidance of public places and day-care, limiting contact with young children and unvaccinated persons, and boiling drinking water [24]. To further reduce the risk of infection, infants are given antimicrobial prophylaxis, intravenous or subcutaneous immunoglobulin replacement, and prophylaxis with palivizumab during the respiratory syncytial virus season (table 2 third panel).

Live vaccines are contraindicated for infants with severe T cell lymphopenia, and inactivated vaccines are unlikely to be effective and are therefore not recommended. Family members, however, should be actively assessed for incomplete vaccine histories, and catch-up or booster vaccines administered if needed (table 2 bottom panel). This includes the annual influenza, pertussis, live attenuated measles, mumps and rubella (MMR) and varicella zoster vaccines. A recent extensive analysis has found no evidence of human-to-human transmission of the measles vaccine virus, so this vaccine can be given safely to people who are in contact with immunocompromised patients [28]. The transmission risk for varicella vaccine strain is extremely low and published evidence shows that transmission is only possible when skin lesions occur [29]. Hence, varicella vaccination is recommended for all non-immune contact persons of severely immunocompromised patients. If a vaccinated person develops a rash (local or systemic), contact with immunocompromised patients should be avoided until the rash has resolved to further decrease the chance of vaccine strain transmission. If close contacts (such as siblings) receive the live rotavirus vaccine, contact with their diapers should be avoided for 2–4 weeks after vaccination [30, 31].

Conclusions

Inclusion of the screening test for severe primary T and B cell disorders in the Swiss routine newborn screening panel represents a major step forward in improving the clinical management of these rare conditions, which are otherwise associated with significant morbidity and mortality. Early detection of SCID patients allows rapid implementation of precautions to reduce acquisition of infectious organisms by avoidance of viral vaccines and initiation of antimicrobial prophylaxis as well as IgG replacement therapy. In addition, pathogen transmission by maternal milk, family members and close contacts to immunodeficient patients can be reduced through pre-emptive vaccination and other relevant measures.

Table 1: Breastfeeding in infants with abnormal screening for severe primary immunodeficiencies.

Investigations (as soon as possible) *Mother*: CMV serology from peripheral blood (or from serum collected during pregnancy) *Infant*: CMV-PCR from urine (or peripheral blood), not Guthrie card			
KREC low/undetectable and TREC normal and ADA/PNP normal Breast milk can be given (irrespective of the mother's CMV status and the infant's gestational age)			
TREC low / undetectable			
1. Evaluation phase			
	Gestational age	*Mother CMV seropositive**	*Mother CMV seronegative*
TREC low but measurable	<37 weeks	No breastfeeding, pasteurised breast milk possible	Breast milk possible‡
	≥37 weeks	No breast milk†	
TREC not measurable	Any gestational age	No breast milk†	Breast milk possible‡
2. Suspected SCID			
		Child CMV+	*Child* CMV−
Mother CMV$^+$		No breast milk	No breast milk
Mother CMV$^-$		Breast milk possible	Breast milk possible‡

ADA = adenosine deaminase; CMV = cytomegalovirus; KREC = kappa-deleting recombination excision circles; PCR = polymerase chain-reaction; PNP = purine nucleoside phosphorylase; SCID = severe combined immunodeficiency; TREC = T cell receptor excision circles; * Parents should be informed about all routes of CMV transmission (e.g., saliva) and clinical signs of CMV infection. † During the evaluation phase, breast milk can be discarded or frozen. ‡ Parents should be informed about possible CMV transmission routes and precautions to avoid CMV infection; regular testing for CMV through CMV-PCR in urine is recommended for these children (e.g. weekly in the first 4 weeks of life and 2-weekly thereafter). The mother should also be regularly tested for CMV if initially seronegative.

Early diagnosis of SCID results not only in a better clinical outcome since uninfected SCID patients exhibit superior cure rates after HSCT [4, 32], but also increases our understanding of the epidemiology of these rare disorders. The

Table 2: Follow-up investigations for infants with suspected severe primary T and B cell deficiencies.

1. Initial diagnostic work-up History, clinical examination Full blood count, lymphocyte immunophenotyping, ADA/PNP, repeat DBS (Guthrie card), collect DNA (from blood) Always or at least in case of known syndromal aspects: aim to perform first consultation together with a clinical geneticist				
Definitions used for categorisation of T-cell disorders:				
Total $CD3^+$ T cells	**Naïve $CD4^+$ T cells**	**Disorder**	**Action**	
>1500/µl and	>200/µl	Healthy	No further testing	
≤1500/µl	>200/µl	Suspected CID	Partial workup according to findings (see ticks below in column far right)	
≤1500/µl*	≤200/µl	Suspected SCID	Full workup (see below)	
* In some cases, total T cell numbers can be higher (even normal) due to maternal engraftment or incomplete SCID with peripheral expansion of autologous T cells				
2. Diagnostic workup of patients with suspected SCID				
Investigation	**Details**	**Sample**	**Comments**	**CID**
Step 1				
Haematology	Full blood count with differential	0.5 ml EDTA	Eosinophilia in (some) SCID; lymphopenia	x
Chemistry	Electrolytes incl. ionised Ca^{2+}, total bilirubin, ASAT, ALAT, albumin	0.5 ml Li-Hep	Hypocalcaemia in (some) DiGeorge patients	x
Lymphocyte immunophenotyping	B, T, NK cells, T-cell subpopulations	1–2 ml EDTA	T cells ± B/NK cells low in classical SCID; suspect maternal engraftment if T cells higher than expected and/or expanded memory T-cell pool and low/absent naive T cells	x
Antibody concentrations	Total IgA, IgG, IgM (and IgE) concentrations	0.5–1 ml serum	IgG can be normal (maternal transfer to the fetus)	x
Step 2				
ADA, PNP	Enzyme activity	0.5 ml EDTA	Low in ADA/PNP deficiency	x
T-cell function	*In vitro* T-cell proliferation (mitogen) assay	1–2 ml Li-Hep	Can be normal in incomplete SCID	x
Gamma-H2AX test	Radiosensitivity assay	2 ml	Abnormal in ataxia telangiectasia	
TCRvβ repertoire	CDR3 spectratyping (or flow cytometry)	DNA	Especially in incomplete SCID and Omenn syndrome	
Blood group	AB0 blood group testing	0.5 ml EDTA	In preparation for HSCT	
HLA typing	Prefer on sorted myeloid cells if maternal engraftment suspected	4.5 ml ACD	On patient and siblings/parents; in preparation for HCST	
Maternal engraftment	When suspected (e.g., discrepancy between low TREC and higher-than-expected T-cell counts or low/absent naive CD4 T cells combined with an expanded memory T-cell pool)	2 ml EDTA 5 ml EDTA (mother)	HLA phenotyping by flow cytometry on infant/mother pair VNTR (girls) or XY-FISH (boys) on sorted infant cells and DNA/cells of the mother	
Genetics	Whole exome sequencing ± DNA microarray ± other targeted tests	0.5 ml EDTA	In consultation with the clinical geneticist; blood/DNA also from parents (and siblings if indicated)	(x)
Microbiological investigations				
Serology (maternal blood)	HIV combo-screen, HBV (HbS, anti-HbS, anti-HbC) HCV Screen (anti-HCV IgG/M), CMV IgG		Can be requested from stored serum collected during pregnancy	x
CMV status of child	CMV PCR	Urine (from bag)		x
Additional testing if clinically indicated				x
3. Recommended antimicrobial prophylaxis for patients with SCID				
Aim	**Medication**	**Comments**		
PJP prophylaxis	Cotrimoxazole 18 mg/kg q 12 h (on 3 days per week) Leucovorin 15 mg/m^2 q 1 week	Start when total bilirubin below 2× the upper limit of the reference range Regularly check CBC and liver function tests incl. total bilirubin		(x)
Fungal prophylaxis	Amphotericin B p.o. 100 mg q 8 h OR Fluconazole p.o. 6 mg/kg (q 48 h <1 mo old, q 24 h ≥1 mo)	Amphotericin B p.o.: little or no absorption from the gastrointestinal tract		(x)
RSV prophylaxis	Palivizumab 15 mg/kg i.m. q 4 weeks	Prophylaxis during RSV season		
IgG replacement	i.v. Ig q 3-4 weeks or s.c. Ig q 1 week	Adapted to IgG serum concentration		(x)
4. Vaccinations				
Live vaccines (MMR, VZV, rotavirus, yellow fever, BCG, oral typhoid, nasal influenza)				
SCID patient	Contraindicated			(x)
Family members / close contacts	Check vaccination status and test serology if unclear; a complete course of MMR and VZV vaccination recommended; MMR vaccine can be given any time; there should be no contact to a SCID patient if a VZV immunised person develops a rash as long as the rash persists			x
Inactivated vaccines				
SCID patient	Unlikely of benefit – not recommended			
Family members / close contacts	Annual influenza vaccine and other routine vaccines as per national immunisation schedule			x

ACD = acid citrate dextrose; ADA = adenosine deaminase; ALAT = alanine aminotransferase; ASAT = aspartate aminotransferase; BCG = bacillus Calmette-Guérin; SBS = complete blood count; CID = combined immunodeficiency; CMV = cytomegalovirus; DBS = dried blood spots; EDTA = ethylenediamine tetraacetic acid; FISH = fluorescence in situ hybridisation; HIV = human immunodeficiency virus; HbC = HBV core antigen; HbS = HBV surface antigen; HBV = hepatitis B virus; HCV = hepatitis C virus; HLA = human leucocyte antigen; HSCT = haematopoietic stem cell transplantation; Ig = immunoglobulin; Li-Hep = lithium heparin; MMR = measles, mumps, rubella; NK = natural killer; PCR = polymerase chain-reaction; PJP = *Pneumocystis jirovecii* pneumonia; PNP = purine nucleoside phosphorylase; RSV = respiratory syncytial virus; SCID = severe combined immunodeficiency; TREC = T cell receptor excision circles; VNTR = variable number tandem repeat; VZV = varicella-zoster virus

establishment of the Swiss SCID newborn screening programme has also helped in the refinement of better and more efficient management and follow-up guidelines of affected patients and their families by a dedicated team of healthcare professionals including midwives, social workers, psychologists, specialised nurse-practitioners, HSCT specialists, neonatologists, paediatric intensivists, general paediatricians, medical geneticists, and infectious disease and immunology specialists.

Disclosure statement

No financial support and no other potential conflict of interest relevant to this article was reported.

References

1 Glanzmann E, Riniker P. Essentielle lymphocytophthise. Ein neues Krankheitsbild aus der Säuglingspathologie [Essential lymphocytophthisis; new clinical aspect of infant pathology]. Ann Paediatr. 1950;175:1–32. PubMed.

2 Hitzig WH, Biro Z, Bosch H, Huser HJ. [Agammaglobulinemia & alymphocytosis with atrophy of lymphatic tissue]. Helv Paediatr Acta. 1958;13(6):551–85. PubMed.

3 Pai S-Y, Logan BR, Griffith LM, Buckley RH, Parrott RE, Dvorak CC, et al. Transplantation outcomes for severe combined immunodeficiency, 2000-2009. N Engl J Med. 2014;371(5):434–46. doi: http://dx.doi.org/10.1056/NEJMoa1401177. PubMed.

4 Buckley RH. Transplantation of hematopoietic stem cells in human severe combined immunodeficiency: longterm outcomes. Immunol Res. 2011;49(1-3):25–43. doi: http://dx.doi.org/10.1007/s12026-010-8191-9. PubMed.

5 Myers LA, Patel DD, Puck JM, Buckley RH. Hematopoietic stem cell transplantation for severe combined immunodeficiency in the neonatal period leads to superior thymic output and improved survival. Blood. 2002;99(3):872–8. doi: http://dx.doi.org/10.1182/blood.V99.3.872. PubMed.

6 Brown L, Xu-Bayford J, Allwood Z, Slatter M, Cant A, Davies EG, et al. Neonatal diagnosis of severe combined immunodeficiency leads to significantly improved survival outcome: the case for newborn screening. Blood. 2011;117(11):3243–6. doi: http://dx.doi.org/10.1182/blood-2010-08-300384. PubMed.

7 Dvorak CC, Cowan MJ, Logan BR, Notarangelo LD, Griffith LM, Puck JM, 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):1156–64. doi: http://dx.doi.org/10.1007/s10875-013-9917-y. PubMed.

8 Marquardt L, Lacour M, Hoernes M, Opitz L, Lecca R, Volkmer B, et al. Unusual dermatological presentation and immune phenotype in SCID due to an *IL7R* mutation: the value of whole-exome sequencing and the potential benefit of newborn screening. J Eur Acad Dermatol Venereol. 2017;31(3):e147–8. doi: http://dx.doi.org/10.1111/jdv.13888. PubMed.

9 Pachlopnik Schmid J, Güngör T, Seger R. Modern management of primary T-cell immunodeficiencies. Pediatr Allergy Immunol. 2014;25(4):300–13. doi: http://dx.doi.org/10.1111/pai.12179. PubMed.

10 Puck JM. Newborn screening for severe combined immunodeficiency and T-cell lymphopenia. Immunol Rev. 2019;287(1):241–52. doi: http://dx.doi.org/10.1111/imr.12729. PubMed.

11 King JR, Hammarström L. Newborn Screening for Primary Immunodeficiency Diseases: History, Current and Future Practice. J Clin Immunol. 2018;38(1):56–66. doi: http://dx.doi.org/10.1007/s10875-017-0455-x. PubMed.

12 Picard C, Bobby Gaspar H, Al-Herz W, Bousfiha A, Casanova JL, Chatila T, et al. International Union of Immunological Societies: 2017 Primary Immunodeficiency Diseases Committee Report on Inborn Errors of Immunity. J Clin Immunol. 2018;38(1):96–128. doi: http://dx.doi.org/10.1007/s10875-017-0464-9. PubMed.

13 Modell V, Knaus M, Modell F. An analysis and decision tool to measure cost benefit of newborn screening for severe combined immunodeficiency (SCID) and related T-cell lymphopenia. Immunol Res. 2014;60(1):145–52. doi: http://dx.doi.org/10.1007/s12026-014-8485-4. PubMed.

14 Gaspar HB, Hammarström L, Mahlaoui N, Borte M, Borte S. The case for mandatory newborn screening for severe combined immunodeficiency (SCID). J Clin Immunol. 2014;34(4):393–7. doi: http://dx.doi.org/10.1007/s10875-014-0029-0. PubMed.

15 Amatuni GS, Currier RJ, Church JA, Bishop T, Grimbacher E, Nguyen AA, et al. Newborn Screening for Severe Combined Immunodeficiency and T-cell Lymphopenia in California, 2010-2017. Pediatrics. 2019;143(2):e20182300. doi: http://dx.doi.org/10.1542/peds.2018-2300. PubMed.

16 van der Burg M, Mahlaoui N, Gaspar HB, Pai S-Y. Universal Newborn Screening for Severe Combined Immunodeficiency (SCID). Front Pediatr. 2019;7:373. doi: http://dx.doi.org/10.3389/fped.2019.00373. PubMed.

17 Morinishi Y, Imai K, Nakagawa N, Sato H, Horiuchi K, Ohtsuka Y, et al. Identification of severe combined immunodeficiency by T-cell receptor excision circles quantification using neonatal guthrie cards. J Pediatr. 2009;155(6):829–33. doi: http://dx.doi.org/10.1016/j.jpeds.2009.05.026. PubMed.

18 Nakagawa N, Imai K, Kanegane H, Sato H, Yamada M, Kondoh K, et al. Quantification of κ-deleting recombination excision circles in Guthrie cards for the identification of early B-cell maturation defects. J Allergy Clin Immunol. 2011;128(1):223–225.e2. doi: http://dx.doi.org/10.1016/j.jaci.2011.01.052. PubMed.

19 Verschuren MC, Wolvers-Tettero IL, Breit TM, Noordzij J, van Wering ER, van Dongen JJ. Preferential rearrangements of the T cell receptor-delta-deleting elements in human T cells. J Immunol. 1997;158(3):1208–16. PubMed.

20 van Zelm MC, Szczepański T, van der Burg M, van Dongen JJM. Replication history of B lymphocytes reveals homeostatic proliferation and extensive antigen-induced B cell expansion. J Exp Med. 2007;204(3):645–55. doi: http://dx.doi.org/10.1084/jem.20060964. PubMed.

21 Barbaro M, Ohlsson A, Borte S, Jonsson S, Zetterström RH, King J, 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):51–60. doi: http://dx.doi.org/10.1007/s10875-016-0347-5. PubMed.

22 Kwan A, Abraham RS, Currier R, Brower A, Andruszewski K, Abbott JK, et al. Newborn screening for severe combined immunodeficiency in 11 screening programs in the United States. JAMA. 2014;312(7):729–38. doi: http://dx.doi.org/10.1001/jama.2014.9132. PubMed.

23 Mauracher AA, Pagliarulo F, Faes L, Vavassori S, Güngör T, Bachmann LM, et al. Causes of low neonatal T-cell receptor excision circles: A systematic review. J Allergy Clin Immunol Pract. 2017;5(5):1457–1460.e22. doi: http://dx.doi.org/10.1016/j.jaip.2017.02.009. PubMed.

24 Thakar MS, Hintermeyer MK, Gries MG, Routes JM, Verbsky JW. A practical approach to newborn screening for severe combined immunodeficiency using the T cell receptor excision circle assay. Front Immunol. 2017;8:1470. doi: http://dx.doi.org/10.3389/fimmu.2017.01470. PubMed.

25 American Acadamy of Pediatrics. Breastfeeding and the Use of Human Milk. Pediatrics. 2012;129(3):e827–41. doi: http://dx.doi.org/10.1542/peds.2011-3552.

26 Underwood MA. Human milk for the premature infant. Pediatr Clin North Am. 2013;60(1):189–207. doi: http://dx.doi.org/10.1016/j.pcl.2012.09.008. PubMed.

27 Bhatia J. Human milk and the premature infant. Ann Nutr Metab. 2013;62(s3, Suppl 3):8–14. doi: http://dx.doi.org/10.1159/000351537. PubMed.

28 Greenwood KP, Hafiz R, Ware RS, Lambert SB. A systematic review of human-to-human transmission of measles vaccine virus. Vaccine. 2016;34(23):2531–6. doi: http://dx.doi.org/10.1016/j.vaccine.2016.03.092. PubMed.

29 Gershon AA, Gershon MD. Perspectives on vaccines against varicella-zoster virus infections. Curr Top Microbiol Immunol. 2010;342:359–72. doi: http://dx.doi.org/10.1007/82_2010_12. PubMed.

30 Wiedermann U, Sitte HH, Burgmann H, Eser A, Falb P, Holzmann H, et al. Impfungen bei Immundefekten/Immunsuppression - Expertenstatement und Empfehlungen [Guidelines for vaccination of immunocompromised individuals]. Wien Klin Wochenschr. 2016;128(S4, Suppl 4):337–76. doi: http://dx.doi.org/10.1007/s00508-016-1033-6. PubMed.

31 Ehl S, Bogdan C, Niehues T, Burchard G, Baumann U, Hecht J, et al. Impfen bei Immundefizienz. Bundesgesundheitsblatt Gesundheitsforschung Gesundheitsschutz. 2018;61(8):1034–51. doi: http://dx.doi.org/10.1007/s00103-018-2761-8.

32 Heimall J, Cowan MJ. Long term outcomes of severe combined immunodeficiency: therapy implications. Expert Rev Clin Immunol. 2017;13(11):1029–40. Jdoi: http://dx.doi.org/10.1080/1744666X.2017.1381558. PubMed.