# Severe combined immunodeficiency in the newborn

Severe combined immunodeficiencies (SCIDs) are a group of rare diseases of T-lymphocyte or thymic failure, which comprise the most severe immunodeficiencies. Generally fatal by one year of age without treatment, they constitute a paediatric emergency and early diagnosis facilitates a better prognosis. This article will review the molecular mechanisms of SCID, clinical signs and symptoms, diagnostic investigations and review treatment modalities and outcomes, and discuss future developments.

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## Key points

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1. Severe combined immunodeficiency is a
   paediatric emergency – urgent referral
   should be made once the diagnosis is
   considered.
2. Persistent viral respiratory or enteral
   infections are hallmarks of disease.
3. A persistent low lymphocyte count
   should be investigated, particularly in
   the presence of a family history
   suggestive of immunodeficiency.
4. Newborn screening programmes to
   detect severe combined immuno-
   deficiency before symptoms appear
   have been introduced in the USA and
   may soon be introduced in the UK.

T-and B-lymphocytes constitute the cellular components of adaptive immunity. Congenital absence of T-lymphocytes with associated panhypogammaglobulinaemia (low levels of all immunoglobulins) defines the group of disorders, which lead to the most profound immunodeficiency disorders, severe combined immunodeficiencies (SCIDs). While not common, with an incidence of around 1:50,000 in the UK, the disease is usually fatal by one year of age if not treated; successful treatment is often curative. A number of different genetic defects affecting development of T-lymphocytes or thymic development give rise to the immunological phenotype and clinical picture classified as SCID (**TABLE 1**)[1]. The definition of SCID, <500 CD3+ cells/µL, is based on the absolute number of T-lymphocytes present[2]. While newly discovered, rare defects permitting T-lymphocyte development but abrogating function are described, this article will discuss classical SCID conditions. The clinical presentation of severe, persistent viral infection, with a predisposition to protozoan infection is found, whatever the underlying molecular defect, although other specific associated anomalies may be seen in certain conditions.

## History

Pregnancy and birth history may suggest possible congenital infection, intrauterine growth retardation or prematurity, all of which are associated with primary immunodeficiency. A family history of infective or unexplained infant death is important[3], particularly in consanguineous families or diagnosis of SCID in a distant family member. A history of affected male relatives suggests X-linked common gamma chain (CγC) deficiency, the most common form of SCID.

## Presentation

In the newborn period, affected infants usually appear well. The hallmark presentation of an infant with SCID is persistent viral respiratory or gastrointestinal tract infection in the first few months of life. Persistent respiratory tract infection is common, with failure of viral clearance accompanying persistent bronchiolitic-like signs. *Pneumocystis jiroveci* pneumonitis develops insidiously over several weeks, leading to a gradual hypoxia and oxygen requirement. Co-infection with respiratory viruses is common[4]. Persistent diarrhoea, usually viral, with failure to thrive is an important presentation. Although most patients with SCID are initially well and grow normally, they usually fall away from their growth centile when infection occurs, because of intestinal villous atrophy, leading to malabsorption, which in severe cases results in malnutrition and wasting. For infants vaccinated with BCG, disseminated BCG infection, including skin lesions or hepatitis may be found. Materno-fetal-engraftment, due to transplacental passage of immuno-competent maternal T-lymphocytes can give rise to materno-fetal graft versus host disease (GvHD), with a skin rash and occasionally pneumonitis, hepatitis and bone marrow involvement[5]. Transfusion with non-irradiated blood products can give rise to transfusion related GvHD due to immunocompetent

| Disorder | Disease | Phenotype | Inheritance |
|---|---|---|---|
| Cytokine signalling | CγC | T⁻B⁺ | XL |
| | JAK3 | T⁻B⁺ | AR |
| | IL7Rα | T⁻B⁺ | AR |
| Nucleotide biosynthesis salvage pathway defects | ADA deficiency | T⁻B⁻ | AR |
| Defects affecting signalling through the T-lymphocyte antigen receptor | CD45 | T⁻/lowB⁺ | AR |
| | CD3δ | T⁻/lowB⁺ | AR |
| | CD3ε | T⁻/lowB⁺ | AR |
| | CD3ζ | T⁻/lowB⁺ | AR |
| VDJ recombination defects | RAG1 and RAG2 | T⁻B⁻ | AR |
| | DCLRE1C (artemis) | T⁻B⁻ | AR |
| | DNA-PKcs | T⁻B⁻ | AR |
| | DNA ligase 4 | TlowBlow | AR |
| | NHEJ1 (Cernunnos-XLF) | TlowBlow | AR |
| Mitochondrial defect | AK2 deficiency (reticular dysgenesis) | T⁻B⁺/⁻ | AR |
| | RMRP (Cartilage hair hypoplasia) | T⁻B⁺ | AR |
| Thymic defects | DiGeorge syndrome | T⁻B⁺ | AD |
| | CHARGE syndrome | T⁻B⁺ | AD |
| | FOXN1 (winged helix) | T⁻B⁺ | AR |
| Other | Coronin-1A deficiency | T⁻B⁺ | AR |

**TABLE 1** Classification of SCIDs.
Key: CγC = common gamma chain, JAK3 = Janus associated kinase 3, IL7R = interleukin 7 receptor, ADA = adenosine deaminase, RAG = recombinant activating gene, DCLRE1C = DNA cross-link repair 1C, DNA-PKcs = DNA protein kinase catalytic subunit, NHEJ1 = nonhomologous end-joining factor 1, AK2 = adenylate kinase 2, RMRP = RNA component of mitochondrial RNA processing endoribonuclease, CHARGE = coloboma, heart anomalies, choanal atresia, retardation of growth and development, and genital and ear anomalies, FOXN1 = forkhead box N1, XL = X-linked, AR = autosomal recessive, AD = autosomal dominant.

cells in the blood product.

Recalcitrant cutaneous candida infection can also manifest and, more rarely, serious invasive fungal or bacterial infection. Other rare presentations are listed in **TABLE 2**.

## Examination

A newborn infant with SCID, who does not have infection, is likely to have a normal examination. A persistent napkin candidal rash or oral candidiasis may be a clinical finding. The absence of lymphoid tissue is an important sign, but detecting this is not easy, because lymph nodes and tonsils in normal infants are often very small. However, in Omenn syndrome evolving erythroderma is accompanied by large, rubbery lymphadenopathy, usually with hepatosplenomegaly. Alopecia is also prominent. An inflammatory, rather than infectious pneumonitis may also be present. Rarely, these features may be associated with other genetic causes of

SCID[6]. A similar pneumonitis may also be found in ADA (adenosine deaminase)-deficient SCID.

Specific findings are encountered in particular genetic causes of SCID. Complete DiGeorge or CHARGE (Coloboma, Heart anomalies, choanal Atresia, Retardation of growth and development, and Genital and Ear anomalies) syndrome, with associated thymic aplasia may manifest other features of the syndrome, including characteristic facial features, cleft palate, cardiac defects, hypocalcaemia and tracheo-oesophageal or urogenital anomalies. Alopecia, nail dysplasia and skin abnormalities are characteristic in patients with FOXN1 (forkhead box N1) mutations[7]. Disproportionate short stature may be seen in cartilage hair hypoplasia. Reticular dysgenesis leads to profound cytopenia including neutropenia and affected infants usually present in the first day or two of

life with omphalitis or severe bacterial sepsis. Profound sensori-neural deafness is also a specific feature in these infants[8]. Microcephaly is a feature of DNA repair defects, which may present with SCID[9].

Radiological evaluation may be diagnostically useful. Cupping and flaring of the anterior costochondral junctions and metaphyseal cupping, and irregularity at the costovertebral junction may be seen on chest radiographs in ADA deficiency as well as a 'bone-in-bone' appearance of the vertebral bodies and squaring of the scapula tip[10]. Absence of a thymic shadow on anterior-posterior and lateral chest radiographs is consistent with a combined immune defect in infants and young children. However, atrophy may also occur in response to stress including infection and as such, this finding is not diagnostic. Classic signs of interstitial pneumonitis, including lung hyperinflation and interstitial shadowing, may be seen if infection is present.

## Laboratory findings

Routine tests can be extremely helpful as an aid to diagnosing SCID. As most infants with SCID appear well, but full blood counts are performed commonly, the opportunity to make a diagnosis at this stage should not be missed. The most important laboratory finding is a persistent lymphopenia on a full blood count[11]. Other cytopenias including thrombocytopenia and neutropenia may be rarely present, particularly in reticular dysgenesis, or in some DNA repair disorders such as DNA ligase 4 deficiency. Eosinophilia is a feature of Omenn syndrome. A full blood count is the most common investigation requested, yet the absolute lymphocyte count is often overlooked. Lymphocyte counts are normally higher in infancy than in adulthood. An absolute lymphocyte count of less than $2.8 \times 10^9$/L is two standard deviations below the mean. When infants with infection have a count lower than this, especially if they are below six months of age and fail to thrive, it is likely that they have SCID. Although a normal lymphocyte count does not preclude a diagnosis of SCID, lymphopenia on two occasions, especially if there are also supportive clinical findings and/or a positive family history, should prompt detailed lymphocyte phenotyping. In Omenn syndrome, the lymphocyte count may be normal, or even above the normal range, due to proliferative expansion of a few

| Common presentations | Rare presentations |
|---|---|
| Persistent or recurrent viral gastroenteritis | Bacterial septicaemia |
| Persistent or recurrent viral lower respiratory tract infection | Disseminated BCG infection |
| *Pneumocystis jiroveci* pneumonitis | Haemophagocytosis |
| Recurrent or recalcitrant candidiasis | Lymphoid malignancy |
| Fungal abscess | Autoimmune cytopenias |
| Recurrent bacterial lymphadenitis | Materno-fetal GvHD |
| Persistent cutaneous human papillomavirus warts | |
| Persistent molluscum contagiosum | |
| Failure to thrive | |

**TABLE 2** Disease-defining illness in SCID.

abnormal clones of T-lymphocytes.

Proportions of different lymphocytes and absolute numbers vary with age, and age-related normal ranges should be consulted[12]. Lymphocyte phenotyping by flow cytometry will demonstrate absence or very low numbers (<500 cells/μL) of CD3+ T lymphocyes and absent or low numbers of CD4+ and CD8+ lymphocytes. If T-lymphocytes are present, maternal engraftment should be excluded by molecular genetic analysis. Depending on the genetic defect, B-lymphocytes and natural killer (NK) cells may be present or absent. Conventionally, SCID is classified as T$^-$B$^+$ or T$^-$B$^-$ SCID with further subdivision based on the presence or absence of NK cells. The presentation may not be classic, and the presence or absence of NK cells may be misleading. Therefore, a phenotype describing presence or absence of NK cells no longer forms a part of the current classification system (**TABLE 1**)[1]. The absence of recent thymic T-lymphocyte emigrants is highly suggestive of SCID and, if demonstrated, should prompt careful clinical and laboratory evaluation. Results are best interpreted in a laboratory that has experience of regularly processing these investigations and, if there is any doubt, specialist advice should be sought.

Immunoglobulin measurements will reveal very low or absent IgM, as well as absent IgA and IgG, although in young infants IgG is usually within the normal range due to transplacental transfer of maternal IgG. Vaccine antigen responses, if vaccines have been administered, will usually be absent. It is important that values are interpreted in the light of correct age-related reference values and care is taken in the interpretation of the IgG result, which may seem normal in neonates due to the presence of maternal IgG. The

detailed analysis of lymphocyte phenotype, including assessing the presence of recent thymic emigrants is most useful in this situation, particularly in preterm infants, where measurement of immunoglobulins may be difficult to interpret, especially if the infant is born before the transplacental transfer of maternal IgG during the third trimester. The absence of IgM should always be noted and investigated. IgE is usually raised in Omenn syndrome.

Culturing lymphocytes *in vitro* for a defined time with an appropriate non-specific stimulus (eg phytohemagglutinin) and using the incorporation of radioactive or non-radioactive markers (eg triitiated thymidine or bromodeoxyuridine) into the DNA of dividing cells, acts as a surrogate measure of lymphocyte proliferation. An alternative method utilises the stable incor-poration of an intracellular fluorescent dye (5-carboxyfluorescein diacetate succinimidyl ester) into cells to quantify cell division, because of the sequential decrease in fluorescent labelling in daughter cells. In patients with SCID, proliferation assays give a negative or markedly depressed result although there may be some background activity. Similarly, SCID patients in whom a genetic karyotype is requested, fail to proliferate because the assay normally examines chromosomes in lymphocytes stimulated into metaphase.

Identifying the molecular defect in specific patients with combined immunodeficiency or SCID is important for prognosis, treatment and genetic counselling. The genetic basis of most SCIDs is well defined. Usually, the genetic defect coding for the protein results in no protein expression, expression of low amounts, or expression of abnormally sized protein, and can be detected by western blotting and flow cytometry[13]. In

the presence of an appropriate history or abnormal protein expression, genetic analysis may be undertaken.

## Management

Liaison with and referral to a specialist centre should be made as soon as the diagnosis is suspected, and should not await the initiation or results of more specialist or detailed laboratory investigations. In the UK, two centres are nationally designated for the treatment of SCID and related disorders: Great Ormond Street Hospital, London and the Great North Children's Hospital, Newcastle upon Tyne. Patients with suspected SCID should be discussed with one of these centres, or with the local paediatric immunology team.

Newborns suspected of having a severe immunodeficiency disorder should be protected using isolation techniques, including limitation of the numbers of persons involved with care. Individuals with respiratory or gastrointestinal symptoms of infection should avoid contact. If the mother is cytomegalovirus (CMV) negative, breastfeeding should be encouraged – otherwise it should be discontinued to prevent neonatal CMV infection transmitted through the milk[14]. Wherever the child is managed, strict hand-washing procedures are paramount. Blood products, if required, should be CMV negative and irradiated to avoid the risk of transfusion GvHD. Prevention and treatment of infections is the mainstay of supportive care. Co-trimoxazole as prophylaxis against *Pneumocystis jiroveci* should be given on two or three days a week. If poor nutrition is present, such as associated with prematurity, weekly folinic acid supplements should be given to decrease the risk of bone marrow depression without compromising the antimicrobial efficacy. Antifungal prophylaxis with fluconazole should be instituted. Antiviral prophylaxis with aciclovir is recommended. Immuno-globulin replacement may be required, but only after diagnostic investigations have been performed, and only in consultation with a paediatric immunologist. In suspected cases, live vaccines, including BCG, must be avoided.

Currently, the standard curative treatment is haematopoietic stem cell transplantation (HSCT), using stem cells from bone marrow, or peripheral blood after granulocyte colony-stimulating factor (G-CSF) mobilisation from family donors.

Family or unrelated donor umbilical cord blood stem cells are increasingly used as an alternative source of stem cells. Best results are obtained using HLA-matched sibling donors[15], but these are available for only about 20% of patients. Since the early 1980s, techniques have been developed to facilitate HLA-mismatched parent-to-child (haploidentical) grafts, by removing mature T-lymphocytes that would otherwise cause fatal GvHD. New methods of manipulating different cell populations within the graft have improved; with better outcomes, all patients with SCID should undergo transplantation as quickly as possible[16]. In experienced centres, survival following transplantation approaches 90%, although the outcome depends on the underlying genetic condition, the type of donor available and the presence of infections[15]. For patients with ADA deficiency, the clinical status can be stabilised using infusions of glycosylated polyethylene glycol-ADA, until a suitable donor is identified[17].

In a few centres worldwide, clinical gene therapy trials for common gamma chain and ADA deficiency are in progress[18,19]. Using modified viral vectors, which contain a copy of the corrected gene, this method uses vector-transduced autologous haematopoietic stem cells to achieve correction of the immunodeficiency. Advantages include the ability to infuse cells without giving prior chemotherapy to create marrow space. Chemotherapy increases the risk of performing a transplant, particularly in the presence of infection. Additionally, patients with no well-matched donor can be safely treated, with HSCT or gene therapy as possible options. The initial trials demonstrated good immune reconstitution following treatment, but some patients developed leukaemia as a result of the viral vector inserting adjacent to an oncogene[20]. Newly developed vectors should reduce this risk, but close monitoring will be necessary.

For rare patients with complete DiGeorge or CHARGE syndrome, or FOXN1-deficient SCID, the primary defect is a failure of thymic development rather than an intrinsic defect in the haemato-poietic stem cell. Haematopoietic stem cell transplantation in this situation leads to a high incidence of GvHD and mortality[21]. For selected patients who lack a matched family donor, thymic transplantation is an attractive alternative[22].

## Future developments

The outcome of stem cell transplantation is superior for patients with SCID transplanted in the neonatal period when infection-free, than for older infants[23]. Screening programmes measuring molecular markers of T-lymphocyte production, absent in SCID, have been successfully developed from the Newborn Bloodspot Screening Programme in the USA[24]. Similar screening programmes are likely to be introduced in the UK over the next few years, but careful implementation with appropriate supporting infrastructure will be needed. Preterm infants pose a particular challenge in this respect, as false negative results are frequently observed and may introduce unnecessary familial anxiety while awaiting confirmatory tests[25]. However, given the much better results of transplanting asymptomatic patients, newborn screening is likely to significantly improve outcome of treatment. Indeed, this will be the first disease detected by newborn screening that can be cured, rather than simply have symptoms alleviated. The approach to transplantation may need to be modified, as the long-term effects of chemotherapy conditioning on immature and very young infants is not clear. Improved treatment regimens will need to parallel diagnostic advances to deliver the best outcomes for this group of patients.

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