

Diagnosis of severe combined immunodeficiency

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Abstract

Early diagnosis of severe combined immunodeficiency (SCID) is important to enable prompt referral to a supraregional centre for bone marrow transplantation before the occurrence of end organ damage secondary to infective complications. This review outlines clinical, microbiological, and immunopathological clues that aid the diagnosis of SCID and emphasises the multidisciplinary approach needed to diagnose and treat these infants.

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Severe combined immunodeficiencies (SCIDs) represent the most severe forms of primary immunodeficiency and have an incidence of about 1/30 000 to 1/70 000 live births.¹ A variety of inherited defects prevent or severely impair T and B cell development and function.² Without treatment, opportunistic or otherwise self limiting infections lead to death within infancy or early childhood.³ Bone marrow transplantation (BMT) is curative with a very high chance of normal immunity, growth, and development, as long as the diagnosis is made early and the patient is transferred to a supraregional unit for urgent BMT. Children with SCID are particularly vulnerable to community acquired infection before diagnosis. Infection induced end organ damage, particularly to the lungs and liver, is associated with greatly increased morbidity and mortality.⁴ Quite rightly, SCID has been described as a paediatric emergency.⁵ It is imperative that paediatricians recognise the clinical clues, that microbiologists alert clinicians to suggestive infections, and that immunopathologists advise promptly about the best tests and the interpretation of results.

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Table 1 Classification of severe combined immunodeficiency

| Syndrome | T cells | B cells | NK cells | Inheritance |
|--|---------|---------|----------|-------------|
| Reticular dysgenesis | - | - | - | AR |
| ADA deficiency | - | - | - | AR |
| RAG 1, 2 deficiency | - | - | + | AR |
| TCR + BCR recombination gene deficiency | - | - | + | AR |
| C γ C deficiency | - | + | - | XL |
| JAK3 deficiency | - | + | - | AR |
| IL7R α deficiency | - | + | + | AR |
| Omenn's syndrome | + | - | + | AR |
| ZAP-70 kinase deficiency | CD4+ | + | + | AR |
| CD4+ lymphopenia | CD8+ | + | + | AR |
| MHC II deficiency | CD8+ | + | + | AR |
| p56lck deficiency | CD8+ | + | + | AR |
| Non-host T cells (MFE or transfusion GvHD) | + | +/- | +/- | |

The hallmark of severe combined immunodeficiency is an absence of mature T cells. Further delineation is marked by the presence or absence of B and natural killer cells, inheritance pattern, and the genetic molecular defect, where known.

ADA, adenosine deaminase; BCR, B cell receptor; C γ C, interleukin 2 receptor common γ chain; GvDH, graft versus host disease; IL7R α , the α chain of the interleukin 7 receptor; JAK3, Janus associated kinase 3; MFE, maternofetal lymphoid engraftment; MHC, major histocompatibility complex; NK, natural killer; RAG, recombination activating genes; TCR, T cell receptor.



*Figure 1 Chest radiograph of infant with severe combined immunodeficiency (SCID) showing absence of thymus, hyperinflated lungs, and interstitial pneumonitis secondary to *Pneumocystis carinii* pneumonia and parainfluenzae type 3 infection.*

Clinical pointers

A family history of infective or unexplained infant death is important, particularly in consanguineous families; a history of affected male relatives suggests X linked SCID (table 1), the most common form of SCID.⁶ By contrast, scoring systems to assess the likelihood that a child has an immunodeficiency according to the number and severity of infection⁷ appear seriously flawed and have failed to diagnose SCID patients.⁸

Infants with SCID often suffer an ongoing bronchiolitic type illness with a chronic cough and wheeze, which gradually worsens. Chest radiographs show hyperinflation, sometimes with an interstitial pneumonitis (fig 1); these children continue to exhibit symptoms, often becoming progressively worse. Persistent respiratory infection with evidence of interstitial pneumonitis on chest radiograph should raise the suspicion of *Pneumocystis carinii* pneumonia, cytomegalovirus (CMV), or aspergillus infection complicating the immunodeficiency. Persistent or recurrent oral or gastrointestinal candidiasis, or viral diarrhoea with failure to thrive are also important because, although patients with SCID are often initially well and growing normally, they fall away from the growth centile after a few months when infection occurs.⁹ Children presenting within the first 6 months of life are more likely to have severe combined immunodeficiency or a severe T cell defect. Other T cell immunodeficiencies such as major histocompatibility complex (MHC) class II deficiency and T cell activation defects often present in infancy,¹⁰⁻¹² but may present later. Antibody deficiencies in particular are masked for longer by the protective effect of transplacentally acquired IgG, and present towards the end of the 1st year of life, or later.

Although the absence of lymphoid tissue is often cited as an important sign, detecting this in young infants is not easy, because lymph nodes and tonsils in normal infants are often

very small. Clinical examination of the lymphoid system is important, however, because Omenn's syndrome (a form of SCID characterised by erythroderma, alopecia, and hepatosplenomegaly¹³) and SCID with maternofetal lymphoid engraftment (MFE) are characterised by lymphadenopathy and a thickened infiltrative skin rash owing to massive expansion of lymphocyte clones,¹⁴ although MFE is frequently clinically silent.¹⁵ Skin sepsis is an often overlooked feature of immunodeficiency, particularly in patients whose skin is already abnormal (fig 2).

Routine tests are often overlooked as an aid to diagnosing SCID. A full blood count is the most common investigation requested, yet the absolute lymphocyte count is often overlooked. Lymphocyte counts are higher in infancy than in adulthood,¹⁶ and it is not widely appreciated that an absolute lymphocyte count of less than $2.8 \times 10^9/\text{litre}$ is 2 SD below the mean. When infants with infection have a count lower than this, it is highly likely that they have SCID.¹⁷ Although a normal lymphocyte count does not preclude a diagnosis of SCID, lymphopenia on two occasions should prompt lymphocyte phenotyping. In contrast, the neutrophil count is usually normal.¹⁷

There may also be clues on initial admission chest radiographs including the absence of a thymic shadow, hyperinflation of lungs, interstitial pneumonitis (fig 1) and, in adenosine deaminase (ADA) deficient SCID, typical cupping and flaring of the costochondral junction.

Infective clues

Unusually persistent, severe, or opportunistic infection is the most common presentation of SCID, particularly with viruses, fungi, or intracellular bacteria, and should prompt immunological investigation. A high degree of suspicion of an immunodeficiency is necessary to ensure that appropriate microbiological and virological investigations are performed. Specimens should be processed in a specialist laboratory with adequate facilities for looking for opportunistic and viral pathogens. Infants presenting with *Pneumocystis carinii* pneumonia, CMV pneumonitis, disseminated BCG infection, or atypical mycobacterial infection require further investigation. Prolonged or recurrent candidiasis or persistent respiratory or gastrointestinal symptoms should prompt investigation for continued viral excretion, which is highly suggestive of SCID, particularly when associated with failure to thrive. Infections with common organisms in unusual sites,¹⁸ or invasive fungal infection warrant further investigation.

Pneumocystis carinii is the most common respiratory infection in SCID, and is often found as a co-pathogen with a respiratory virus. It is rarely detected in nasopharyngeal secretions and, although usually found in bronchoalveolar lavage specimens, it is sometimes only detected in lung biopsy¹⁹ (fig 3). Persistent respiratory infection with respiratory syncytial virus or parainfluenza viruses is also common, but adenoviruses and enteroviruses can also be responsible.^{10 20 21} Rapid diagnostic techniques are invaluable; at present, immunofluorescence

is the most widely available for respiratory virus diagnosis, but genome detection methods, such as the polymerase chain reaction (PCR), are becoming increasingly important. Although immunofluorescence gives rapid results, good specimens with plentiful cellular material are crucial to making a diagnosis; it is important that they are taken by an expert nurse or physiotherapist. Poor specimens should not be accepted as giving negative results and it might take several attempts to achieve a positive diagnosis; thus, liaison between laboratory and clinical staff is essential. Parainfluenza type 4 should also be looked for as well as types 1–3. CMV infection is a less common but serious problem requiring investigation by the detection of early antigen fluorescent foci or PCR methods. Serological tests for virus detection are of no use whatsoever, because patients cannot make antibody.

Gastrointestinal infection causing failure to thrive in SCID is usually viral; common viruses include rotavirus and adenovirus,^{10 20 21} but small round structured virus, astrovirus, and enteroviruses may also be found. After routine immunisation, polio vaccine strains may be persistently excreted but only very rarely cause disease.²² Some viruses cannot be detected by virus isolation and require detection by enzyme immunoassay or electron microscopy. Failure to detect these enteric viruses may have serious clinical consequences and so liaison with a virologist experienced with these problems is essential.

Bacterial infection is less common but is often overlooked. Skin sepsis, particularly in Omenn's syndrome, when broken skin allows colonisation and invasion by staphylococci, streptococci, or enterococci, and Gram negative bacteria, such as *Pseudomonas* spp. can rapidly become life threatening.²³ Infants given BCG vaccination may develop disseminated infection, with organisms isolated from bone marrow or liver tissue by auramine staining.^{24 25} PCR may identify mycobacterial species more rapidly.²⁶ Respiratory tract bacterial infection with organisms of relatively low pathogenicity, such as non-typeable *Haemophilus influenzae* or moraxella, often coexist with viral or pneumocystis infection, and when isolated from nasopharyngeal secretions they should be considered important because treatment can lead to the resolution of signs and symptoms. Confirmatory testing can take time, and transfer to one of the two supraregional units in London or Newcastle, nationally designated for treatment of SCID in the UK, should not be delayed while results are awaited.

Candidiasis is a frequent finding and colonisation of skin, oropharynx, and gut is common in SCID. Invasive candidiasis is less common but might be a presenting feature and tissue biopsy specimens should always be processed for fungi. Renal and biliary tract candidiasis, and invasive aspergillosis, are surprisingly rare but are seen occasionally. As well as fungal culture, aspergillus and candida PCR and histopathological examination of tissue for fungi might be important in establishing a diagnosis of opportunistic infection.



Figure 2 Infant with Omenn's syndrome, showing alopecia, erythroderma, and varicella zoster lesions.

In addition to stool and respiratory secretions, other samples might be required to stage the extent of infection, including urine and blood, as well as bronchoalveolar washings, cerebrospinal fluid (rarely), and lung, bowel, liver, lymph node, or skin biopsy material. Histopathology has a role to play and histochemical staining of biopsy material may confirm a diagnosis—for example, CMV or human herpesvirus 6 infection. As a general principle, the stronger the suspicion of SCID with an undiagnosed infection, the more persistent and invasive investigations should be, and these should probably be performed in a regional centre with expertise in the investigation for opportunistic pathogens.

Immunopathology

Immunoglobulin measurement rarely helps in the diagnosis of SCID, particularly in early infancy. The IgG concentration is often normal because it is of maternal origin, providing false reassurance. IgM and IgA values are normally low in early infancy and might be difficult to

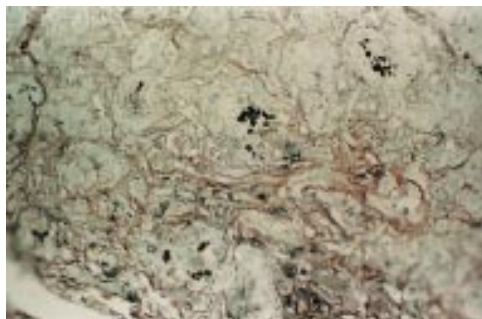


Figure 3 Pneumocysts demonstrated by silver staining of alveolar tissue from a lung biopsy.

distinguish as being abnormal. Values must be compared with age specific reference ranges, ideally correlated for racial background. Isohaemagglutinins are a useful measure of specific IgM production, but specific titres must be requested: absence is an important indicator.

If a low absolute lymphocyte count is the most important clue to diagnosis, then lymphocyte phenotyping using monoclonal antibodies and fluorescent activated cell sorter (FACS) analysis is the most important confirmatory test. Absolute numbers of lymphocyte subtypes are more useful than percentages, and each phenotype pattern suggests a specific diagnosis. The presence or absence of B cells or natural killer (NK) cells is suggestive of the specific molecular defect (table 1), and also influences prognosis after BMT.²⁷ The measurement of lymphocyte activation (CD3/HLA-DR+, CD45 RA/RO ratio) and T cell receptor (TCR) phenotype (TCR $\alpha\beta/\gamma\delta$) can give further information; high numbers of TCR $\gamma\delta$ + cells or CD45 RO+ lymphocytes suggest Omenn's syndrome or MFE with restricted TCR gene clonality also being seen,^{28 29} whereas absent HLA-DR expression indicates bare lymphocyte syndrome (MHC class II deficiency). MHC class I deficiency has been described, but pathological manifestations do not usually appear until later in life.³⁰

Lymphocyte proliferation studies confirm that lymphocytes are anergic and if there is anergy to certain mitogens, but not others, T cell activation defects may be demonstrated. For example, ZAP-70 deficient patients have defective calcium mobilisation and lack in vitro T cell proliferation to TCR mediated stimuli, such as phytohaemagglutinin (PHA), but proliferate normally when stimulated with phorbol myristate acetate and ionomycin,³¹ whereas lymphocytes from patients with interleukin 2 (IL-2) deficiency only proliferate on addition of exogenous IL-2.³² Requesting cytogenetic analysis unwittingly initiates a proliferation study because PHA is used to drive lymphocytes into metaphase for chromosome enumeration; thus, failure to induce metaphase might indicate a failure to proliferate in response to PHA.³³ Cytogenetics also demonstrates MFE when female lymphocytes are found in male infants. In female infants, or when transfusion related graft versus host disease is suspected, molecular DNA analysis on separated lymphocytes will determine the origin of a cell.³⁴ These techniques distinguish MFE from Omenn's syndrome, which is important because it influences the conditioning regimen for BMT. Histostaining of skin or lymph node biopsy specimens may indicate graft versus host disease secondary to maternal cells or following transfusion with non-irradiated blood, or show typical disrupted lymph node architecture in Omenn's syndrome with the absence of germinal centres.³⁵ In this situation, immunostaining might show abundant S100 positive interdigitating reticulum cells (fig 4). Metabolic investigations also have a role in diagnosis—for example, raised erythrocyte deoxy ATP levels in ADA deficiency, or

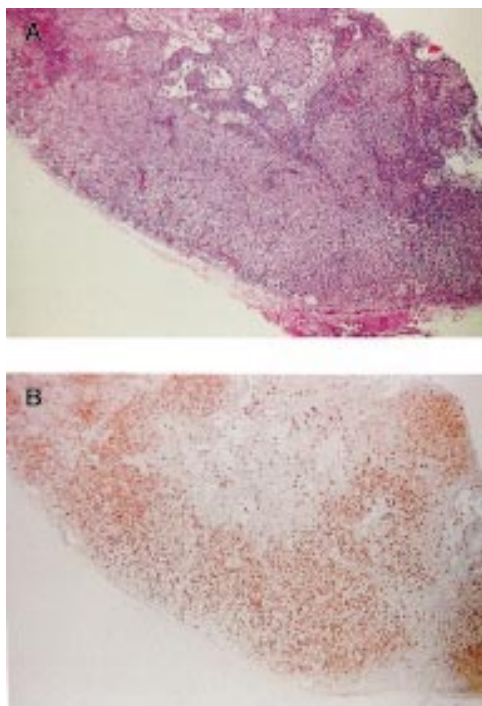


Figure 4 Lymph node biopsy in Omenn's syndrome (courtesy of B Angus). (A) Haematoxylin and eosin stained preparation. Note the absence of germinal centres and the replacement of the paracortex by a diffuse infiltrate of large cells with abundant pale cytoplasm. (B) Immunostaining for S100 protein. Note the positive labelling of almost all the cells in the cortex. This indicates infiltration of the node by a population of interdigitating reticulum cells.

decreased erythrocyte purine nucleoside phosphorylase (PNP) activity in PNP deficiency. These tests are performed at few reference laboratories in the UK, and transfer to a supraregional unit should not be delayed while these results are awaited.

Molecular genetic diagnosis

There has been an explosion of knowledge of the underlying molecular pathogenesis of many forms of SCID within the past decade (table 1), enabling SCID syndromes to be described by lymphocyte phenotype and molecular basis. Although some of these diagnostic tests are research tools, others are now routinely available as part of a diagnostic service, albeit in a limited number of centres. In the UK, gene analysis for common γ chain deficiency and Janus associated kinase 3 (JAK3) deficiency is possible and, although results are not always available to help with the initial diagnosis and management, they allow first trimester diagnosis in further pregnancies. Precise molecular diagnosis aids counselling on chances of successful BMT.

Outcome

SCID is an eminently curable condition, and after successful BMT most patients lead entirely normal lives with a fully functioning immune system, or at worst need three weekly intravenous immunoglobulin replacement. Early diagnosis is vital, because otherwise patients rapidly succumb to infection before curative BMT can be performed. Good

collaboration between local paediatricians and microbiologists on the one hand, and regional virologists and immunologists on the other is crucial to facilitate rapid diagnosis and transfer to a supraregional unit. Pre-existing infective end organ damage, particularly to lungs and liver, must be carefully assessed because BMT is much more hazardous and the chances of success much lower than in the absence of end organ damage.²⁷ Outcome is best when expertise is concentrated in a few units to maximise experience in treating these very rare conditions, a pattern followed across Europe. Survival is improving with specialist centres within Europe working together to pool information on presentation, to standardise treatment protocols, and review outcome in pan-European registries.²⁷ Such collaboration facilitates the evaluation of new treatment strategies, such as better T cell depletion techniques by positive selection for CD34+ cells, as well as umbilical cord stem cell transplantation. A European programme to evaluate gene therapy in common γ chain deficiency is currently under way but, to date, progress towards gene therapy has been much slower than expected.

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