**Case Commentary: Severe Combined Immune Deficiencies**

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**ABSTRACT**

This case commentary concerns a 3-month old female infant (of parents who were first cousins) who presented with persistent diarrhoea, failure to thrive, and a low lymphocyte count. The topics addressed include the typical histories for severe combined immune deficiencies (SCID), how SCID can be confirmed, and the relevance of immunophenotyping circulating lymphocytes in the diagnosis of different types of SCID. This commentary was created as part of the masters programme in Molecular and Cellular Biochemistry at the University of Oxford.

**CASE**

A 3-month old female infant (of parents who were first cousins) presented with persistent diarrhoea, following a long course of antibiotics for “chestiness” since 1 month of age. She was considerably underweight. On initial full blood count, it was noticed that she had a low lymphocyte count of $1.9 \times 10^9 \text{L}^{-1}$ and she was investigated for SCID.

**COMMENTARY**

Severe combined immunodeficiencies (SCIDs) are genetic diseases, first described in 1950, which result in impaired development and function of T lymphocytes with concomitant primary or secondary impairment of B (and sometimes NK) lymphocytes (Glanzmann and Riniker 1950; Buckley 2004). The incidence is estimated at 1 in 58,000 births and it is fatal unless treated in infancy (Kwan et al. 2014).

**IS THIS A TYPICAL HISTORY FOR SCID? WHAT OTHER FEATURES MIGHT BE RELEVANT? WHAT IS THE RELEVANCE OF THE FAMILY HISTORY?**

SCID patients can present differently depending on the type of SCID, their age, and the particular combination of infections they have experienced. However, in many ways this case is a typical SCID patient. The mean age of presentation of SCID patients without a family history and in the absence of newborn screening is usually between 2 to 6 months after birth, consistent with this case (McWilliams, Railey, and Buckley 2015; Yao et al. 2013). This is the time at which the passive immunity afforded by maternal IgG is waning and the first attempts at treatment have failed, prompting further investigations (Waaijenborg et al. 2013).

Overall more males than females are diagnosed with SCID due to the presence of an X-linked form of SCID caused by mutations in the IL2RG gene. As females have two X chromosomes and males only one, X-linked diseases almost exclusively affect males. (One exception being turner syndrome, 45X0, females and the other exception being if both parents were carriers, in which case the father would be affected.) However, there are many non X-linked forms of SCID and so the male bias is not great.
enough to describe this case as atypical.

Consanguinity of parents is also a common finding in SCID cases with rates of up to 70% in some regions (Aghamohammadi et al. 2014). This is due to the higher likelihood of inheriting homozygous mutations from related parents as they will share a higher proportion of their DNA than unrelated parents (Saggar and Bittles 2008). A more detailed family history could be relevant in diagnosing SCID. For example if the child had or has any relatives, siblings especially, with any health problems or who had died in infancy from infection (which may not have been diagnosed as SCID at the time). If any of these cases were true it would add to the suspicion of SCID. However, the majority of patients do not have a family history (Chan et al. 2011). Family ancestry can also provide a clue, for example the Navajo population have a 30 fold higher risk of SCID due to a founder mutation in DCLRE1C (protein artemis) (Kwan et al. 2015).

Persistent diarrhoea, failure to thrive (low weight for age), and respiratory infection are key clues in this case and are all common symptoms in SCID cases (McWilliams, Railey, and Buckley 2015; Yao et al. 2013; Hague et al. 1994; Gennery and Cant 2001; European Society for Immunodeficiencies 2015; Rivers and Gaspar 2015). Severe, persistent or unusual infections are indicative of an immunodeficiency and the use of a long course of antibiotics suggests a persistent infection. Identification of the infectious organisms can reveal the presence of unusual and or opportunistic pathogens. Parainfluenza, respiratory syncytial virus, candida, Pneumocystis jiroveci, and Pseudomonas aeruginosa are particularly common in SCID patients (McWilliams, Railey, and Buckley 2015). If the child was vaccinated against tuberculosis at birth BCGosis may also be present (Shahmohammadi, Saffar, and Rezai 2014). The broad range of both systemic viral and bacterial septic infection reflects the deficiencies of both the cellular and humoral arms of the immune system in SID.

If chest radiography was performed due to the “chestiness” an absent thymic shadow due to a small underdeveloped thymus (the location of T-cell development) can be indicative of SCID (Nickels et al. 2015). The lack of visible tonsils or palpable lymph nodes can likewise be rapidly assessable clues pointing towards SCID (McWilliams, Railey, and Buckley 2015).

The low lymphocyte count is a hallmark of SCID patients, the majority of which have counts lower than $3 \times 10^9 \text{L}^{-1}$ (McWilliams, Railey, and Buckley 2015; Gennery and Cant 2001; Hague et al. 1994; Conley, Notarangelo, and Etzioni 1999). This is due to the lack of development of T cells (and sometimes B cells) the former of which account for 70% of all lymphocytes (J. M. Puck 2012).

**WHAT INVESTIGATIONS COULD BE DONE TO CONFIRM SCID AND HOW?**

The lymphocyte count should be repeated possibly with the addition of a qPCR based test for T-cell receptor excision circles (TRECs); byproducts of V(D)J recombination of TCR genes which do not replicate with cell division and are therefore a marker of newly formed T cells (figure 1) (J. M. Puck 2012). This assay is now being used in over 23 states in America as a newborn screen for SCID(Kwan et al. 2014). A TREC count of less than 25/µL occurs in almost all SCID patients (Spek et al. 2015).
Figure 1: The deletion of the $\delta$ locus to form a TREC (J. M. Puck 2012).

Absolute counts of T cells specifically can be obtained through flow cytometry; a CD3+ T-cell count of less than $3 \times 10^8 \text{L}^{-1}$ would classify as typical SCID and a count of less than $1 \times 10^9 \text{L}^{-1}$ as leaky SCID or Omenn syndrome (Shearer et al. 2014).

As well as being confirmed with a low number of T lymphocytes the lack of function of these cells should also be tested as in some cases a low T-cell count is not due to SCID for example in intestinal lymphangiectasia and cartilage hair hypoplasia. Additionally in other SCID cases T-cell count may be nearly normal due to the presence of maternal T cells from trans-placental transfer (McWilliams, Railey, and Buckley 2015). T-cell function can be assayed by stimulating lymphocytes with mitogens such as phytohemagglutinin, concanavalin, or pokeweed mitogen and measuring proliferation by $[^{3}{H}]$ thymidine incorporation. A value of less than 10% compared to the control is indicative of SCID (McWilliams, Railey, and Buckley 2015; Conley, Notarangelo, and Etzioni 1999; Buckley 2004). HIV infection should also be ruled out by a RT-qPCR test of HIV RNA as HIV infection can also cause a low T-cell count and opportunistic infections. Note that as a SCID positive patient would have an impaired ability to produce antibodies in response to an infection, an ELISA to test for anti-HIV antibodies would be inappropriate (as it could give a false negative). Other similar diseases to rule out would include “less profound combined immunodeficiencies” such as complete DiGeorge syndrome, ZAP70 deficiency, CD3y deficiency, MHC Class II deficiency and PNP deficiency (Al-Herz et al. 2014; Conley, Notarangelo, and Etzioni 1999).

A lack of antibody production is also seen in SCID patients. This can be the result of either a lack of B cells, for example in adenosine deaminase (ADA) deficiency where the cells undergo apoptosis, or as a consequence of lacking T-cell help. Hence, in some cases immunoglobulin testing can support the diagnosis of SCID. However, the presence of maternal IgG and naturally low levels of other immunoglobulins at this age can hinder the identification of a deviation from the norm (Gennery and Cant 2001; Hague et al. 1994; McWilliams, Railey, and Buckley 2015).
Mutation analysis of genes known to cause SCID, guided by flow cytometry to identify candidates, can help to provide a definitive diagnosis by comparison to mutations known to cause loss of function. However, the results may return variants of unknown significance and in rare cases the mutation could be in areas currently not associated with SCID which may require whole exome sequencing to identify (Patel et al. 2015; Kwan et al. 2014).

One form of SCID, ADA deficiency, can additionally be confirmed by low (<2% of normal) adenosine deaminase plasma activity and high (>300 fold increase from normal) concentrations of deoxyadenosine metabolites which cause the apoptosis of lymphocytes in this condition (Ozdemir 2006). ADA deficient patients also commonly have chondro-osseous dysplasia which would be apparent in a chest radiograph (Manson et al. 2013).

**DISCUSS THE RELEVANCE OF IMMUNOPHENOTYPING CIRCULATING LYMPHOCYTES FOR THE DIAGNOSIS OF THE DIFFERENT TYPES OF SCID**

Immunophenotyping is a method of determining the phenotype of cells using antibodies which bind to specific surface proteins and are linked to a detection marker for example a fluorophore. In this context phenotype refers to whether a lymphocyte is a T cell, B cell or NK cell. Each of these phenotypes express different surface molecules to which antibodies can bind and hence the type of cell can be deduced. For example T cells express CD3, with helper T cells also expressing CD4 and effector T cells expressing CD8. B cells are CD3 negative but express CD19, and NK cells are CD3 negative but express CD16. The bound antibodies are often measured using a flow cytometer due to the ability to rapidly measure multiple fluorophores at once (Maecker, McCoy, and Nussenblatt 2012).
SCIDs can be classified based on which gene contains the causal mutation. Each of these mutations acts at a different place in the lymphocyte development pathway (figure 2) and so each class of SCID has a different profile of circulating lymphocytes. For example, ADA deficiency results in a build up of S-adenosylhomocysteine and dATP due to the lack of deoxyadenosine deamination by ADA. These lymphotoxic precursors build up and cause apoptosis in all three types of lymphocytes and so these patients have a T-B-NK- profile (Ozdemir 2006).

T cells and NK cells require IL-7 and IL-15 respectively for development. The IL-2 receptor common gamma chain (IL2RG) and JAK3 kinase transduce IL-2 family signals (including IL-7 and IL-15). Hence mutations in these genes can prevent development of T and NK cells so these patients have a T- B+NK- profile.

The RAG1, RAG2, and DCLRE1C genes are required for V(D)J rearrangement of the heavy chain and β chain locus in B and T cells respectively, as part of the process of creating a diverse set of antigen receptors. However, they are not required in NK cells and so mutations in these genes leads to a T-B- NK+ profile. Note that a positive B or NK cell phenotype denotes that this cell type is present but not necessarily that those cells are functional.

Overall there are four different lymphocyte profiles (figure 3): all SCIDs are T cell negative but they can be either negative or positive with respect to the presence of B or NK cells. Hence by immunophenotyping circulating lymphocytes you can measure the levels of T, B, and NK cells and therefore deduce which genes may be causing SCID in the patient (and therefore the types of SCID the patient may have). It is also possible to determine more specifically the mutation by analysing more markers, but as B and T-cell development occur in the bone marrow and thymus respectively this is only possible with a biopsy and not by using circulating lymphocytes (Saint Basile et al. 2004). Hence to determine the exact type of SCID without biopsy the candidate genes must be sequenced and analysed for mutations.
Figure 3: Division of SCIDs based on lymphocyte profile (Al-Herz et al. 2014)

Hence immunophenotyping circulating lymphocytes is highly relevant to diagnosing the different types of SCID as the types can be deduced by the lymphocyte profile. However, a definitive diagnosis is likely to require mutational analysis as multiple types of SCID can have the same profile due to having mutations in different genes within the same pathway. Exceptions include ADA deficiency which can be diagnosed serologically as previously mentioned. Although this may not be necessary as AK2 deficient patients typically die within the first few weeks of life, and so in the absence of newborn screening ADA is likely the only T-B-NK- SCID to be suspected after symptoms develop. Additionally coronin-1A deficient patients have a detectable thymus as a mutation in this gene causes defects in the egress of T cells from the thymus rather than their development per se (Shiw et al. 2008). However, even sequencing may return variants of unknown significance and hence only a probable diagnosis. Furthermore, as sequencing costs decrease it becomes more feasible to check all known SCID linked genes which reduces the utility of immunophenotyping. Although it will still be useful in the case that multiple mutations of unknown significance are found or in the case that the mutation is in a novel region which could require whole exome sequencing to identify.

Currently the main treatment of SCID patients is hematopoietic stem cell transplantation (HSCT) and prognosis is generally good if the procedure is undertaken in the first few months of life and a HLA matched donor is available. However recent advances in gene therapy approaches (for which, unlike HSCT, the identity of the causative mutated gene must be known) could result in this being a more desirable treatment due to faster T-cell development and the ability to avoid problems such as graft versus host disease and graft rejection due to non HLA identical donors (Touzot et al. 2015). Furthermore, as newborn screening programs expand, diagnosis can be made earlier and therefore treatment can be started before infections develop which will also increase the percentage of favourable outcomes for these diseases.

MANUSCRIPT HISTORY

This case commentary was originally produced as part of the masters programme in Molecular and
Cellular Biochemistry at the University of Oxford (MBiochem, Part II). The case, and the commentary questions were set by the course organisers. Additionally, the following limitations were imposed: The word count must not exceed 2000 words (excluding the case, questions, citations, and figure captions), the number of references must not exceed 30, and no more than three figures or tables may be used.

REFERENCES


McWilliams, Laurie M., Mary Dell Railey, and Rebecca H. Buckley. 2015. “Positive Family History, Infection, Low Absolute Lymphocyte Count (ALC), and Absent Thymic Shadow: Diagnostic Clues for All Molecular Forms of Severe Combined Immunodeficiency (SCID).” The Journal of Allergy and Clinical Immunology: In Practice 3 (4): 585–91. doi:10.1016/j.jaip.2015.01.026.


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