

Correspondence

Flow cytometric assessment of cord blood as an alternative strategy for population-based screening of severe combined immunodeficiency

To the Editor:

In the March issue of the *Journal*, Puck¹ reviewed a number of technologies for newborn screening of severe combined immunodeficiency (SCID) and concluded that DNA detection of T-cell receptor excision circles (TRECs) from newborn dried blood spots (DBSs) was the preferred approach because of its successful integration into various US states and overall efficacy.¹ However, there are several disadvantages to TREC analysis of newborn DBSs as a screening strategy for SCID. These include the fact that DBSs can yield poor-quality DNA, resulting in false-positive results¹⁻³; that less than 10% of infants with low TREC counts are subsequently shown to have SCID on further testing; and that time from initial detection of low TREC counts in a DBS to final confirmatory lymphocyte phenotyping of peripheral blood can take up to 3 weeks.^{4,5}

As part of a new Australian birth cohort study, the Barwon Infant Study, we analyzed lymphocyte populations in fresh

samples of cord blood (CB) within 24 hours of birth and incidentally identified a case of SCID. This finding and the reproducibility of our analysis led us to consider the merit of such an approach as an alternative screening strategy not considered in the article by Puck.¹ Specifically, in the Barwon Infant Study, as a means of evaluating early-life immune programming and allergic disease,⁶ CB samples are collected from each participant into a heparinized solution (to prevent clotting). A small sample is then used for measurement of lymphocyte populations by using flow cytometry (anti-CD3/CD4/CD45). In the case of the infant with SCID, the lymphocyte percentage was only 5.9%, and there was a complete absence of the CD3⁺ T-cell subset (Fig 1). The infant was referred on to the immunology service at Royal Children's Hospital in Melbourne, Australia. Subsequent fluorescence-activated cell sorting assessment of a peripheral blood sample demonstrated a T⁻B⁺NK⁻ phenotype. Given that the infant was female, the T⁻B⁺NK⁻ phenotype suggested Janus kinase 3 deficiency, and this was subsequently confirmed on genetic testing. The child then received a CB hematopoietic stem cell transplant.

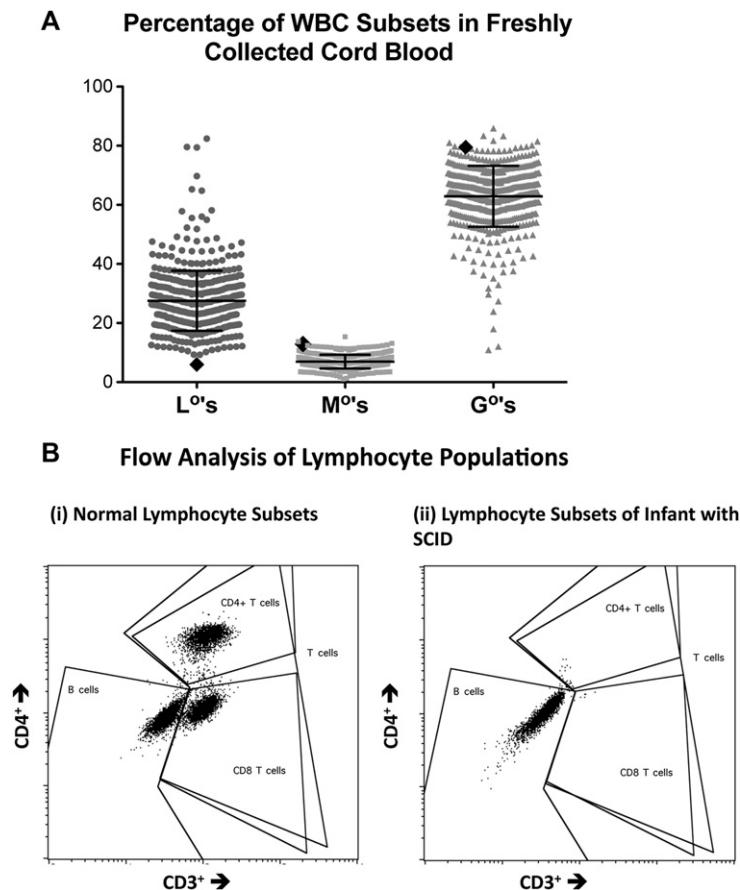


FIG 1. A, Percentages of white blood cell (WBC) subsets, as assessed by using side-scatter flow cytometric analysis of CD45⁺ populations (n = 478 cord samples, mean $\pm$ SD). Values for the infant with SCID are marked with a solid diamond. L's, Lymphocytes; M's, monocytes; G's, granulocytes. B, Flow analysis of CB CD45⁺ lymphocytes stained with antibodies for CD3 and CD4. Plot (i) shows 3 lymphocyte subpopulations: CD3⁺CD4⁺ T_H cells, CD3⁺CD4⁻ cytotoxic T cells, and CD3⁻CD4⁻ cells. Plot (ii) shows that the infant with SCID had no visible CD3⁺ T cells.

This is the first reported identification of SCID after flow cytometric analysis of lymphocytes in freshly collected CB. In our hands the processing, antibody staining, lysing, and analysis cost approximately AUD\$4.00 per sample for both labor and reagents. This amount is considerably less than quoted by a commercial pathology service but realistic. TREC screening has been estimated to cost less than US\$5.00 per infant,^{7,8} and thus the cost of the 2 strategies is similar. Flow cytometric analysis of CB would not only circumvent the various steps in the TREC analysis but could also assist in identification of other primary immunodeficiency conditions (eg, severe congenital neutropenia). The major disadvantages include transport of fresh CB samples and reliance on the obstetric/midwifery teams to collect CB, both of which might be surmountable issues. Given the importance of expediting the diagnosis of SCID and the fact that lymphocyte phenotyping is used for ultimate diagnosis of low TREC counts, the rapid flow analysis of CB samples in a centralized laboratory could be considered an alternative screening strategy, particularly in countries in which there is limited access to complex molecular biology expertise.

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