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*, Puck[1] 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 results[1-3]; 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

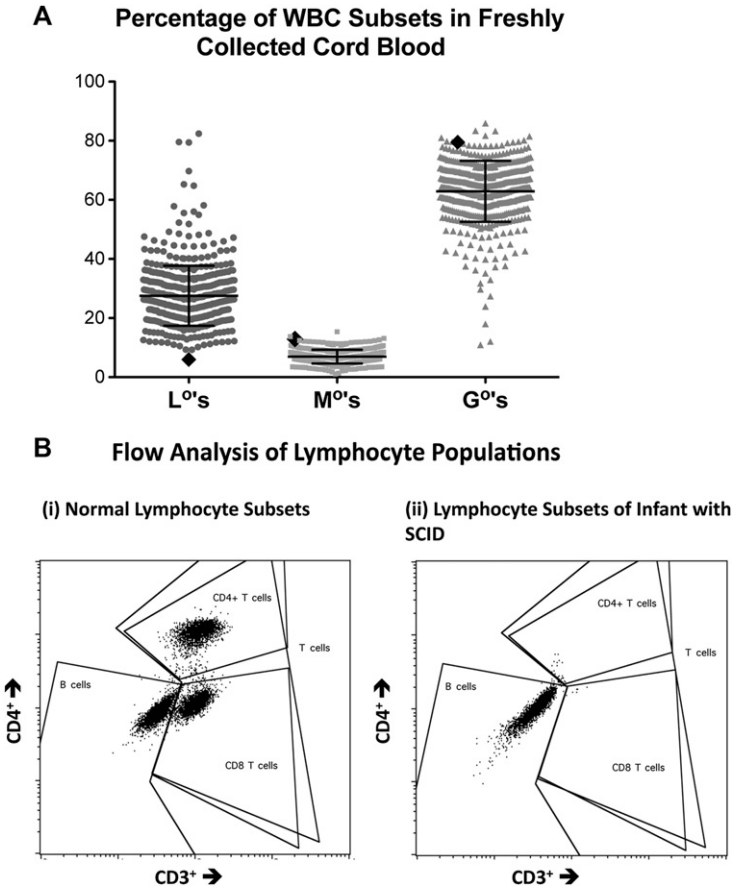

FIG 1. A, Percentages of white blood cell *(WBC)* subsets, as assessed by using side-scatter flow cytometric analysis of $CD45^+$ populations (n = 478 cord samples, mean ± SD). Values for the infant with SCID are marked with a *solid diamond*. *L^o's,* Lymphocytes; *M^o's,* monocytes; *G^o's,* granulocytes. **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.

genetic testing. The child then received a CB hematopoietic stem cell transplant.

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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Reply

To the Editor:

Collier et al[1] suggest an alternative method to screen newborn populations for severe combined immunodeficiency (SCID). They point out that collection of cord blood for flow cytometric analysis, which incidentally identified a case of SCID in their hands, could be an alternative to the assay of T-cell receptor excision circles (TRECs) in DNA isolated from dried blood spots, as described in my article.[2] They allude to the Barwon Infant Study, in which (according to the study Web site) a total of 1000 cord blood samples are planned to be collected eventually at 2 hospitals over 5 years.[3,4] They gloss over the challenges of scaling up the Barwon protocol, including transporting liquid blood from all deliveries in a population-based screening program to centers that would perform flow cytometry. Moreover, neither their correspondence nor the reference they cite contains any data on the number of infants enrolled to date; the number of samples analyzed; the proportion of infants born whose samples were not collected, were unsatisfactory, or both; or other information required to compare the flow cytometric method with TREC analysis.[3] Such data would be essential to formulate a meaningful comparison, and tens of thousands of term and preterm births across the entire range of birthing centers would need to be screened to establish whether cord blood flow cytometry is in fact amenable to large-scale universal testing. The limited markers mentioned, CD3, CD4, and CD45, would not take the place of a more detailed lymphocyte subset analysis for definitive diagnosis and could lead to false-negative results (ie, missing true cases of SCID) if maternal blood or lymphocytes were present in the samples. Because flow cytometry is not currently used by any newborn screening laboratories, there might be no advantage to addition of this platform versus addition of DNA-based testing, which promises to be useful not only for TREC measurement but also for tests in the future for a wide array of disorders.

Now that more than one million newborns in California alone have been screened with the TREC assay for SCID and other clinically significant T lymphocytopenic disorders, it is clear that this method has an extraordinarily low rate of false-positive results on the initial sample (<0.1%). Furthermore, despite the statement by Collier et al[1] regarding TREC dried blood spot tests that "less than 10% of infants with low TREC counts are subsequently shown to have SCID on further testing," in California 40% of infants referred to flow cytometry have had clinically significant T lymphocytopenia (<1500 CD3 T cells/μL), requiring intervention to avoid live rotavirus vaccination and other complications, even if typical SCID was not confirmed.

Finally, readers might be skeptical that a cost of $4 per sample would cover collection, processing, analysis, and data management of large numbers of liquid cord blood samples in their own localities.

Nonetheless, newborn screening for immunodeficiency is in its infancy, and it might not be the case that the TREC test is the only one that will succeed in every setting. Ideas for assays that could be used for the diagnosis of a wider range of diseases, such as combining TREC and B-cell excision circle testing,[5,6] or that offer greater clinical efficacy at lower cost deserve consideration.