Canadian Blood Services
BLOOD PLASMA STEM CELLS ORGANS & TISSUES

Research Unit no. 54
Feb 2020

Improving quality testing of stem cells for patients

Stem cell transplants are used to treat more than 80 diseases and disorders, including blood cell cancers such as leukemia. Cord blood — the blood left in the umbilical cord after a baby is born — is a rich and important source of stem cells for transplantation. The national Canadian Blood Services' Cord Blood Bank collects, processes and freezes cord blood units. These units are available to any patient worldwide who needs a stem cell transplant and finds a match in the bank.

Before a cord blood unit that matches a patient can be released for transplantation, it must be tested for quality. To do these tests, the Cord Blood Bank uses a segment of the unit. Segments are small portions of the main unit that can be thawed separately. The results of tests conducted on thawed segments are used to make decisions about whether the cord blood unit will be suitable for transplantation. The tests count the numbers of certain types of cells. The tests also measure cell viability — the percentage of cells that can grow and divide into healthy cells to replace a patient's damaged cells. Regulations and standards provide criteria for the number and viability of certain cells in cord blood units that must be met for the unit to be considered suitable for transplantation.

In this study, the researchers set out to improve the pre-transplant quality tests of thawed cord blood. The aim was to develop a standard approach that minimizes cell loss while maintaining cell viability, thus increasing the chance that units meet the standards and are found suitable for transplantation.

IN BRIEF: An improved procedure for testing thawed cord blood samples produces results that better reflect the quality of the cord blood unit and may increase the number of cord blood units that can be released for transplantation.

What did the researchers do?

Cell viability is measured using a technique called flow cytometry, which detects and counts cells based on antibody staining of markers on the cell surface, or on the cells' physical properties, such as size and membrane integrity. To meet regulated standards, the viability of CD34+ and CD45+ cells in the thawed cord blood sample must be ≥ 70 per cent and ≥ 40 per cent, respectively. The researchers sought to find sample preparation and flow cytometry conditions that maximize both CD34+ and CD45+ cell viability levels. Their main focus, however, was to find conditions that would improve CD45+ cell viability in thawed samples, since CD34+ cells typically have high viabilities and very rarely fail to meet regulated standards.

What did the researchers find?

- Viability of CD45+ cells was improved when staining with antibodies took place 20 minutes after the samples were thawed (vs. 30 minutes).
- The sample preparation procedure for quality testing includes a red blood cell lysis step. This step destroys red blood cells in the sample by bursting their membranes. A shorter red blood cell lysis step, or leaving out the lysis step completely, improved CD45+ viability. While these conditions did also decrease measures of CD34+ viability, this was thought to be a technical outcome and could be overcome by adjusting the settings on the flow cytometer.
- Ultimately, a shorter lysis step, under room temperature or cold conditions, improved CD45+ cell viability without any negative impact on CD34+ viability.

How can you use this research?

This study showed that CD45+ cells in thawed cord blood are sensitive to the red blood cell lysis step. Shortening this step provides better CD45+ cell viability measures. Ultimately, the improved procedure produces results that better reflect the quality of the cord blood unit to be transplanted. This could reduce the number of cord blood units that will fail quality tests before being released for transplantation and potentially increase availability of cord blood units for patients who need them.

Although standards for cord blood banks have been defined, there are concerns about how consistent flow cytometry analysis of samples is across instruments and laboratories. The Canadian Blood Services' Cord Blood Bank and Centre for Innovation are involved in international studies to address the lack of a standard approach to measure viability in thawed samples, to develop recommendations, and to gather the scientific evidence needed to revise and improve standards.

About the research team: This research was led by **Dr. Nicolas Pineault**, a development scientist at Canadian Blood Services and a professor in the department of biochemistry, microbiology and immunology at the University of Ottawa, ON. **Roya Pasha** is a senior research assistant in Dr. Pineault's laboratory. **Mike Halpenny** is Canadian Blood Services manager of Cord Blood Bank & stem cell manufacturing, also based in Ottawa, ON.

This Research Unit is derived from the following publications:

[1] Pasha R, Halpenny M, Pineault N. Overcoming the deceptively low viability of CD45+ cells in thawed cord blood unit segments. Vox Sang. 2019 Nov;114(8):876-883. doi: 10.1111/vox.12844.

[2] Fournier D, Lewin A, Simard C, Trépanier P, Néron S, Ballerini L, Codinach M, Elmoazzen H, Halpenny M, Kogler G, Liedtke S, Louis I, Azqueta Molluna C, Pineault N, Prasath A, Querol S, Saccardi R, Sutherland DR, Thérien C, Urbani S. Multi-laboratory assay for harmonization of enumeration of viable CD34+ and CD45+ cells in frozen cord blood units. Cytotherapy. 2019 Dec 27. pii: S1465-3249(19)30870-9. doi: 10.1016/j.jcyt.2019.10.009.

Acknowledgements: This research received funding support from Canadian Blood Services, funded by the federal government (Health Canada) and the provincial and territorial ministries of health. The views herein do not necessarily reflect the views of Canadian Blood Services or the federal, provincial or territorial governments of Canada. Canadian Blood Services is grateful to cord blood donors for making this research possible.

Keywords: cord blood, stem cell, transplant, assay, quality, test

Want to know more? Contact Dr. Nicolas Pineault at Nicolas.pineault@blood.ca