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Improved immunomagnetic enrichment of CD34$^+$ cells from umbilical cord blood using the CliniMACS cell separation system

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Abstract

Background aims. CD34$^+$ enrichment from cord blood units (CBU) is used increasingly in clinical applications involving *ex vivo* expansion. The CliniMACS instrument from Miltenyi Biotec is a current good manufacturing practice (cGMP) immunomagnetic selection system primarily designed for processing larger numbers of cells: a standard tubing set (TS) can process a maximum of 60 billion cells, while the larger capacity tubing set (LS) will handle 120 billion cells. In comparison, most CBU contain only 1–2 billion cells, raising a question regarding the optimal tubing set for CBU CD34$^+$ enrichment. We compared CD34$^+$ cell recovery and overall viability after CliniMACS processing of fresh CBU with either TS or LS. *Methods.* Forty-six freshly collected CBU ($\leq$ 36 h) were processed for CD34$^+$ enrichment; 22 consecutive units were selected using TS and a subsequent 24 processed with LS. Cell counts and immunophenotyping were performed pre- and post-selection to assess total nucleated cells (TNC), viability and CD34$^+$ cell content. *Results.* Two-sample *t*-tests of mean CD34$^+$ recovery and viability revealed significant differences in favor of LS (CD34$^+$ recovery, LS = 56%, TS = 45%, $P = 0.003$; viability, LS = 74%, TS = 59%, $P = 0.011$). Stepwise linear regression, considering pre-processing unit age, viability, TNC and CD34+ purity, demonstrated statistically significant correlations only with the tubing set used and age of unit. *Conclusions.* For CD34$^+$ enrichment from fresh CBU, LS provided higher post-selection viability and more efficient recovery. In this case, a lower maximum TNC specification of TS was not predictive of better performance. The same may hold for smaller scale enrichment of other cell types with the CliniMACS instrument.

Key Words: *CD34, CliniMACS, cord blood, enrichment, immunomagnetic selection*

Introduction

While umbilical cord blood represents a readily available source of hematopoietic stem cells (HSC) for transplantation, the low cell dose available in a cord blood graft correlates with a significant delay in hematopoietic recovery and a higher risk of primary graft failure (1,2). In order to provide higher absolute numbers of HSC for infusion, significant efforts have focused on developing clinically relevant *ex vivo* expansion methodologies, many of which require an initial enrichment of the progenitor population (3–7). A number of devices and reagents are commercially available for enrichment of CD34$^+$ or CD133$^+$ populations; however, these devices must comply with current good manufacturing practices (cGMP) if they are to be used in the manufacturing process of cellular therapies (8).

With the discontinuation of the Isolex 300i Magnetic Cell Selection System by Baxter Healthcare, the CliniMACS Cell Separation System from Miltenyi Biotec is currently the only cGMP-grade cell separation system available for immunomagnetic separation of CD34$^+$ progenitor cells from blood products. The CliniMACS system was originally designed for selection of CD34$^+$ cells from peripheral blood apheresis collections. Miltenyi offers two clinically compliant tubing sets, the Tubing Set-TS (TS) and the Tubing Set-LS (LS), for use with the CliniMACS system based on the total nucleated cells (TNC) of the starting material. Following the manufacturer recommendations, TS are for processing up to 0.6×10^9 CD34$^+$ cells from a maximum TNC of 60×10^9, and LS are intended for larger scale enrichments of up to 1.2×10^9 CD34$^+$ cells from 120×10^9 TNC (9). Schumm *et al.* (10) have reported no significant differences in the enrichment performance of the different tubing sets when used to enrich CD34$^+$ cells from apheresis collections that are within the recommended TNC ranges. In contrast, cord blood units (CBU) typically contain

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only 1–2 billion cells. However, no lower limit specifications are described for such smaller scale selections from CBU or other low TNC sources with the different tubing sets, although a logical assumption would be that the TS would be more appropriate for those products that have 10–100-fold lower TNC than a typical apheresis collection.

In support of ongoing clinical trials using *ex vivo*-expanded cord blood, we have been performing CD34$^+$ cell selection with the CliniMACS system, and have evaluated enrichment performance using both TS and LS with freshly collected CBU (no older than 36 h). Here we present a consecutive cohort unpaired comparison of CD34$^+$ cell selection efficiencies from 46 CBU, the first 22 enriched using TS and the next 24 with LS, on a CliniMACS instrument.

Methods

CBU collection and processing

Fresh, non-cryopreserved, red blood cell (RBC)-replete CBU were obtained from the Puget Sound Blood Center cord blood bank (Seattle, WA, USA) after normal delivery from full-term births ($\geq$ 36 weeks gestational age) using standard collection procedures. All collections were performed with maternal informed consent and donor infectious disease marker (IDM) testing at multiple hospitals in Washington and Hawaii, USA, under institutional review board (IRB) approval. All units were transported and stored at room temperature (RT).

Upon receipt, CBU were sampled for cell counts and immunophenotyping by flow cytometry prior to processing; units that contained less than 3.5×10^6 viable CD34$^+$ cells were not used. The entire volume of a single CBU was then transferred to one of two plastic transfer packs connected by sterile tubing, and RBC depleted by gravity hydroxyethylstarch (HES) sedimentation for 60–90 min at RT. The RBC-reduced CBU was then processed for CD34$^+$ selection with CliniMACS CD34 reagent (Miltenyi Biotec GmbH, Bergisch Gladbach, Germany), following recommended Miltenyi protocols, with the following exceptions: CliniMACS buffer (Miltenyi Biotec) was supplemented with 2% human serum albumin; cell labeling was performed at RT in a 50-mL tube and cells were resuspended to a total volume of 5 mL prior to the addition of intravenous immunoglobulin G (IVIG) and CliniMACS CD34 reagent; CliniMACS CD34 reagent was added at a ratio of 20 μL reagent for every 10^8 corld blood (CB) cells; the final loading volume for CD34$^+$ selection was 100 mL. This modified labeling procedure was developed by our laboratory (Fred Hutchinson Cancer Research Center) to compensate for the reduced TNC and total viable CD34$^+$ cell numbers in CBU compared with apheresis collections; a limited number of experiments was conducted employing the Miltenyi AutoMACS selection device to verify that this procedure would result in good selection performance (data not shown). As indicated by the manufacturer, TS were run with the 'CD34 Selection 1' program, while LS were run with the 'CD34 selection 2' program (9). All selections were performed on a CliniMACS Plus instrument. After separation, the CD34$^+$-enriched fraction was transferred to a 50-mL centrifugation tube, centrifuged to remove buffer, and resuspended in a volume of 2–5 mL culture medium. Samples were then taken for cell counting and analysis by flow cytometry for CD34$^+$ purity and overall viability.

Flow cytometry

The pre-processing CBU samples were incubated with ammonium chloride red cell lysis buffer for 10 min at RT and then washed with phosphate-buffered saline (PBS) containing 2% human AB serum. Cells were then stained with fluorescein isothiocyanate (FITC)-conjugated anti-CD34, allophycocyanin (APC)-conjugated anti-CD45 (BD Biosciences, San Jose, CA, USA) and 7-aminoactinomycin D (7-AAD; Beckman Coulter, Marseille, France). The specific fluorochrome combinations used to evaluate CD34$^+$ cell content were based on protocols developed and used by our laboratory (Fred Hutchinson Cancer Research Center). The stained samples were run on Becton Dickson flow cytometers (BD Biosciences) and the data analyzed using FlowJo v9.0.1 software (Tree Star Inc., Ashland, OR, USA).

CD34$^+$ cell enumeration

TNC from samples of CBU were determined using a hemocytometer, and the percentage CD34$^+$ and viability were determined by flow cytometry analysis and used to calculate total CD34$^+$ cell numbers in the CBU before and after processing.

Statistical methods

The unmanipulated CBU characteristics age (in hours, from time of collection to time of processing), volume, TNC, viability (by 7-AAD flow cytometry), CD34 purity (defined as pre-processing viable CD34$^+$ cells divided by viable and non-viable TNC), total CD34$^+$ cell number and viable CD34$^+$ cell number were considered to be explanatory variables that could affect selection efficiency in addition to the tubing set used. The post-selection outcome measures were TNC viability, viable CD34 purity (defined as viable CD34 divided by viable and non-viable TNC) and viable CD34$^+$ cell recovery (measured as the ratio of post-selection viable CD34$^+$