Seven-year study of viability and absolute counts in cord blood units by flow cytometry in Greece

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Letter to the Editor

Assessing $CD34^+$ cell viability and absolute counts, at different stages of processing allows for accurate quality control. Alterations in the cryopreservation or collection technique can be spotted in their effect on $CD34^+$ cell viability and absolute counts. Products that are cryopreserved for lengthy periods, such as cord blood samples, can be assessed for quality using the single platform flow cytometry methodology. [1-2]

Flow cytometric analysis was performed on post freeze cord blood samples. Samples were analyzed, using the double-colour single platform variant of the ISHAGE guidelines with the addition of the viability dye 7-AAD. Prior to sample preparation, we ensured that the white blood cells (WBC) concentration was not greater than $30x10^9$ WBC cells/L.

Umbilical cord blood samples (n = 4000) were collected (collection volume > 120ml) into a modified blood collection pack and processed within 24h of collection. An aliquot of the cellular fraction was used for $CD34^+$ cell absolute counts, WBC, and viability. All procedures used for collecting and processing cord blood samples were based on the standard operating procedures of the American Cryobanks International with accreditation from the AABB (American Association of Blood Banks).

To assess thawed cord blood countenance in $CD34^+$ cells absolute numbers and their viability, in every step of processing, three-colour flow cytometry was used. In our study, we used multi-parameter flow cytometry to determine the absolute number and viability of 4000 cryopreserved cord blood samples stored in liquid phase nitrogen of -192^0C. We divided the samples, prior to counting $CD34^+$ cells absolute numbers and their viability, as follow: 1) 2500 cryopreserved cord blood samples stored in liquid phase nitrogen of -192^0C for seven years, 2) 1000 cryopreserved cord blood samples stored in liquid phase nitrogen of -192^0C for 6 months, 3) 250 cryopreserved cord blood samples stored in liquid phase nitrogen of -192^0C for 3 months and 4) 250 cryopreserved cord blood samples stored in liquid phase nitrogen of -192^0C for 24 hours.

With a flow cytometer designed to provide absolute counts [3], we directly measured the actual number of $CD34^+$ cells present ($x10^{\wedge 6}$ cells/μL) in the sample and their viability (%). The mean number of absolute $CD34^+$ counts was found to be $3.00x10^{\wedge 6}$ when the accepted value for a transplantation is 200.000 cells per kilogram of body weight [4-5]. Also, the mean viability value was 78% which shows the good cryopreservation and storage conditions of the samples (table 1).

Viability was also checked by triptan blue exclusion method, and was found to be 82% on average (data not show). These results can serve as good quality control markers for our method.

The mean absolute counts of $CD34^+$ in each time group, as well as their viability fluctuating with +/ - 1 suggest that as time passes the samples absolute counts and their viability remains stable. This conclusion is very promising for the future but more studies in longer periods of time must be done in order to make safe assumptions.

Also, we show that the actual WBC numbers in post-freeze from a haematology analyzer and at thawed samples from a flow cytometer, in each time group, were comparable (+ / - 1). It is known that erythroblast are present in high quantities in cord blood samples, therefore could be a factor that affect the WBC counts in a haematology analyzer, but this assumption was not observed. This is happening because of the erythroblast depletion during the cord blood procedure.

To elucidate this, the samples were assessed for WBC in a haematology analyzer before and after cryopreservation stage. The WBC were compared in the pre- freeze samples and was found that the point of entrance is on the vertical axes, below 35Fl, clearly proving the WBC interference. In the post-freeze samples, the point of entrance was in the axes cross point which shows that the WBC interference was absent in those samples. To further investigate the erythroblast interference, we used CD71 antibody in stem cells counts in order to find the percentage of remaining erythroblasts in our samples. We observed that the amount of erythroblasts present in the sample is 5% (+ /- 1). This finding suggests that the differences between the flow cytometer and the haematology analyzer could not exceed this limit. This verifies our initial observation of a precise correlation between the flow cytometer and the haematology analyzer.

Cryopreservation and processing of autologous stem cells collection, assessing $CD34^+$ cell viability and absolute counts, found to remain the same during periods of time. Measuring $CD34^+$ cell viability allows us the quality of control assessments of stem cells processing.

Table 1: Mean counts of CD34+ cells at various time points post thaw

Post thawed samples	Time groups							
	24 hours		3 months		6 months		7 years	
	Mean	Standard Error of Mean	Mean	Standard Error of Mean	Mean	Standard Error of Mean	Mean	Standard Error of Mean
Absolute counts of cd34+ cells ($x10^6$)	2,99	0,16	2,99	0,16	2,82	0,07	2,80	0,04
WBC viability (%)	0,78	0,00450	0,78	,00466	0,77	0,00239	0,77	0,00148

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