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Analysis of CD34 Viability: Fresh is Best?

Judy Stevens¹, Pam Dyson¹, Trevor Rawling¹, Gosia Badowicz¹, Ian Lewis^{1,2}

1. SA Pathology 2. Royal Adelaide Hospital

Enumeration of viable CD34⁺ cell numbers by flow cytometry is routinely performed on peripheral blood stem cells, using 7AAD to identify dead cells. Comparison of estimates from assays performed on the product prior to cryopreservation and after thawing provides a measure of the number of stem cells that survive the cryopreservation/thaw process.

In this retrospective study of viability assays performed between January 2003 and May 2008, we reviewed the recovery data for patients infused with varying doses of CD34 cells, with the aim of determining if the post-thaw CD34/kg estimate was a more useful indicator of patient recovery than the pre-cryopreservation estimate.

The haemopoietic recovery timepoints used were days to neutrophil counts of 0.1×10^6 , 0.5×10^6 and 2.0×10^6 , and platelet counts of 50,000 and 150,000. The median recovery timepoints for patients receiving $<2.0 \times 10^6$ CD34/kg, $2-3 \times 10^6$ CD34/kg, $3-4 \times 10^6$ CD34/kg, $4-5 \times 10^6$ CD34/kg and $>5.0 \times 10^6$ CD34/kg, based on pre-cryopreservation estimates, were calculated. Within these groups, the patient data was sorted on the basis of post-thaw CD34/kg and the median recovery timepoints were compared.

We found that where patients had received an infusion of $<2 \times 10^6$ CD34/kg based on the post-thaw estimate there was an increase in the time taken for neutrophils to reach each timepoint compared to patients receiving $2-3 \times 10^6$ CD34/kg. However this increase was not statistically significant. The time taken for platelet recovery was not increased for patients receiving $<2 \times 10^6$ CD34/kg based on the post-thaw estimate.

We also compared the median recovery timepoints for each group, based on the pre-cryopreservation CD34/kg dose. We noted a significant increase in the time to neutrophil recovery to 2×10^6 ($p = 0.0054$) and for the platelet count to reach 50,000 ($p = 0.0410$) in the $2-3 \times 10^6$ CD34/kg group when compared to the $3-4 \times 10^6$ CD34/kg group. However we noted no increase in neutrophil or platelet recovery timepoints when $3-4 \times 10^6$ CD34/kg were infused compared to when $4-5 \times 10^6$ CD34/kg were transplanted.

We conclude from these data that infusing greater than $3-4 \times 10^6$ CD34/kg has no significant effect on patient recovery timepoints.

No conflict of interest to disclose

P075**Collection of Cells, One Step Further****David Collins***Bone Marrow Transplant Network (New South Wales)*

The world has become a smaller place, with stem cells for transplantation coming from all over the world. Haemopoietic Progenitor Cells (HPC) collected either by apheresis (HPC-A) or by bone marrow donation (HPC-M) are best used as fresh as possible, however, in Australia, most cells from overseas are 24 hours or more away. It is even possible that cells collected in Australia can be 12 or more hours away. We know that there is deterioration the longer the cells are in storage. So how do we accommodate getting the cells safely to our patients? This paper looks at how donated HPC's make it from donor to patient. It will look at the role of the courier, how cells are stored for their journey, and what can go wrong. There will be discussion on who is best to care for the cells for the passage from the donation centre to the patient.

No conflict of interest

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Impact of Gating Strategy on Enumeration of Viable CD34 in Cryopreserved Haemopoietic Progenitor Cells

Annabella Chang¹ and David Ma²

On behalf of Katherine Marsden³, the RCPA Haematology QAP staff³ and Participants of the RCPA CD34 QAP

¹Department of Haematology, PaLMS, Royal North Shore Hospital. Sydney, Australia. ²Department of Haematology, SydPath, St. Vincent's Hospital. Sydney, Australia. ³RCPA Haematology QAP

Aim

Viable CD34 (V-CD34) enumeration based on membrane integrity is one of the proposed assays for quality control of processing, cryopreservation or storage of haemopoietic progenitor cells (HPC), and for assessment of engraftment potential. This pilot study is to assess the logistics for introducing a V-CD34 QAP, and the impact of gating strategy on V-CD34 enumeration, independently of reagents and method.

Methods

56 participants in the current RCPA CD34 QAP were asked for feedback for a V-CD34 QAP using cryopreserved samples. For list mode data (LMD) analysis, apheresed HPC were cryopreserved, thawed, and then stained with CD45-FITC, CD34-PE and 7-AAD in Trucount tubes. Parameters acquired by Cellquest/FacsCalibur were FL1, FL2, FL3, FL4, FSC and SSC. LMD files were uploaded on the RCPA QAP website for participants to analyse by any BD software, Coulter CXP or Expo32. Data were collected on the gating strategy, events acquired for various populations, total and V-CD34 per ul. The acceptable limits of performance were median \pm 25%.

Results

21 of 25 respondents were interested in a V-CD34 QAP. 17 centres submitted results for LMD analysis, showing 2-19% CV for total number of events acquired, bead events, total CD34 events, total CD45 events and total CD34/ul. The V-CD34 events and V-CD34/ul showed 32-65% CV. Comparing the different gating strategies, the number of results within acceptable limits of performance were 6 of 6 for NSW BMT Network template, 3-5 of 7 for single platform and 1 of 4 for dual platform.

Conclusion

A majority of respondents showed interest in a V-CD34 QAP. The gating strategy is a contributing factor to variations in enumeration of total and V-CD34. This pilot study demonstrated that the adoption of a common gating strategy such as the NSW BMT Network template would contribute towards standardisation of V-CD34 enumeration in cryopreserved/thawed samples.

P077**Haemopoietic Progenitor Cell Infusion: What Could Possibly Go Wrong?****Andrew McCutchan**^{1,2}, Jose Isnard¹*1 Pathology Queensland, Townsville, Queensland, Australia**2 Townsville Cancer Centre, Townsville, Queensland, Australia***Aim**

To investigate (and hopefully validate) a worst-case scenario in the infusion of Haemopoietic Progenitor Cell's (HPC). Specifically, the patient being medically unable to receive already thawed HPC.

Method

Thawed HPC were handled according to 3 different proposals.

Proposal 1: Store thawed bag in the refrigerator (4°C) until required.

Proposal 2: Store thawed bag at room temp (23°C) until required.

Proposal 3: Refreeze the bag.

The viability of the HPC were assessed by determining viable CD34 and CFU numbers.

Result

The percentage of viable CD34 cells remained relatively stable.

Clumping of nucleated cells was observed in stored samples not treated with DNase. The clumping had a significant impact on the viable CD34 numbers, with a dramatic drop over time mirroring the increase in observed clumping. Conversely, the DNase treated samples showed no visible clumping and appeared to show no adverse effect on viable CD34 numbers or CFU's.

The stored samples showed a small decrease in viable CD34's after overnight storage.

The difference between storage at 4°C and RT was small, but 4°C consistently yielded better results.

The refrozen sample showed only a slight decrease in number of viable CD34 cells and CFU's from the compared to the control.

Conclusion

Faced with a scenario where a thawed bag of HPC was unable to be infused, these results suggest two possible paths to preserve the HPC clonogenic ability and viable CD34 population;

Where the infusion is expected to proceed within hours, store the diluted, DNase-treated cells at 4°C.

Where the infusion is likely to be delayed overnight, immediately refreeze the cells.

No conflict of interest to disclose

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