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Two cases of clump formation after thawing of hematopoietic progenitor cell products for transplantation

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ABSTRACT

While rare, clump formation has been described in the thawing of cryopreserved hematopoietic progenitor cell products intended for transplantation. When this occurs, it presents a significant challenge to the transplant team, potentially influencing the viability and safety of the product. Though the exact cause of clumping in thawed products remains unknown, studies have linked clump formation with the occurrence of adverse events. This report aimed to describe two cases of clump formation in thawed apheresis products observed in our processing laboratory, detailing the procedures followed and the outcomes achieved.

Keywords: Hematopoietic Progenitor Cells. Cryopreservation. Cell Aggregation.

INTRODUCTION

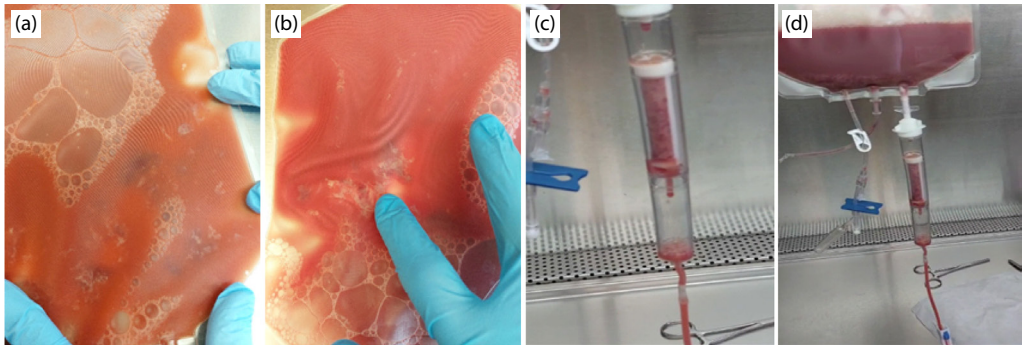
The collection of hematopoietic progenitor cells (HPC) via apheresis, its cryopreservation, and subsequent thawing are critical processes in cellular therapies. These steps enable the collection, storage, and later use of HPCs, in autologous and allogeneic transplants.

While clump formation in thawed products is uncommon, when it occurs, it poses a substantial challenge to the transplant team, potentially affecting the product's viability and safety. Studies have linked clump formation with adverse events[1]. The precise cause of clumping during the thawing of cryopreserved products is not well understood. Possible causes include the presence of cryofibrinogen[2], activation of monocytes which have procoagulant activity[3], lack of anticoagulant[4], and the presence of free DNA from granulocytes that are easily damaged during the cryopreservation and thawing process[1].

This report aimed to describe two cases of clump formation in thawed apheresis products observed over the past year, detailing the procedures followed and the outcomes achieved.

CASE 1

A HPC product obtained via apheresis from an unrelated donor, collected in another country, was received at the processing laboratory 46.5 hours after harvest. A visual inspection revealed the presence of clumps in the fresh product; the collection report detailed 176 mL of the total product sent and 18 mL of citrate anticoagulant, approximately 10% (Figs. 1a and 1b).



Source: Elaborated by the authors.

Figure 1. Case 1 - (a and b) Fresh product received 46.5 hours after harvesting. (c and d) Details of filter and filtration process.

Cryopreservation of the allogeneic product was required due to an unexpected patient complication that prevented the initiation of the conditioning regimen on the scheduled date and the impossibility of rescheduling the collection with the foreign registry that provided the cells. Given the significant time elapsed and the need to proceed with the cryopreservation, the product was gently rotated at room temperature to see if the observed clumps were due to platelet aggregation. No improvement was observed, so the decision was made to filter the product using a 180-µm infusion set, after which a sample was taken, and two equal fractions were cryopreserved without further volume reduction or additional processing (Table 1).

Table 1. Characteristics of the products.

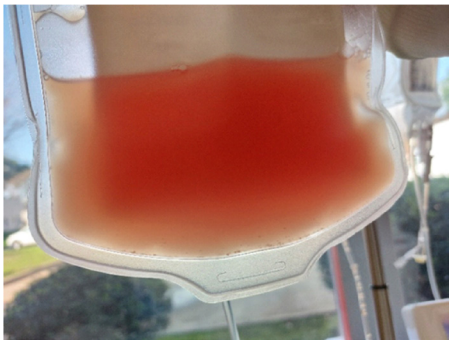
Characteristic	Case 1: Allogeneic unrelated adult donor		Case 2: Autologous pediatric patient
	Cryopreserved product	Infused product (post-wash)	Cryopreserved product
$CD34^+$ cells $\times 10^6$ / kg	7.08	5.48	2.22
TNC $\times 10^6$ / mL	210.54	72.06	334.83
Platelets $\times 10^6$ / mL	2753	618	3039
MNC %	72.1	89.3	63.9
Granulocytes $\times 10^9$	1.84	0.56	6.68
$CD45^+$ cell viability %	91.5	57.1	96.1
$CD34^+$ cell viability %	99.5	97.2	99.8
Sterility testing	Aerobic: negative	Aerobic: negative	Aerobic: negative
	Anaerobic: negative	Anaerobic: negative	Anaerobic: negative

TNC: total nucleated cells; MNC: mononucleated cells. Source: Elaborated by the authors.

Upon examining the filter, clumps were observed to be retained, blocking the sample passage, necessitating multiple filter replacements to complete the product filtration (Figs. 1c and 1d).

The product remained cryopreserved for three days and was then washed using an automated method before infusion (Sepax Smart Wash v314). Briefly, the unit was placed in a double sterile bag and thawed in a water bath at 38 ± 2°C. In a biological safety cabinet, the disposable thawing kit (Biosafe, Sepax CS 600.1) was connected, and a 5% albumin and hydroxyethyl starch (HES 6% 130 KDa) solution was used for washing. The protocol included washing and resuspending the cells in a final volume of 100 mL. After processing,

the kit was disconnected, and samples were taken to characterize the final product (Table 1). Small clumps were observed (Fig. 2), and the product was filtered in the laboratory before being sent for infusion to the transplant unit (180-µm filter). Peripheral blood HPC graft infusion was administered, using a new infusion set with a filter, without complications.



Source: Elaborated by the authors.

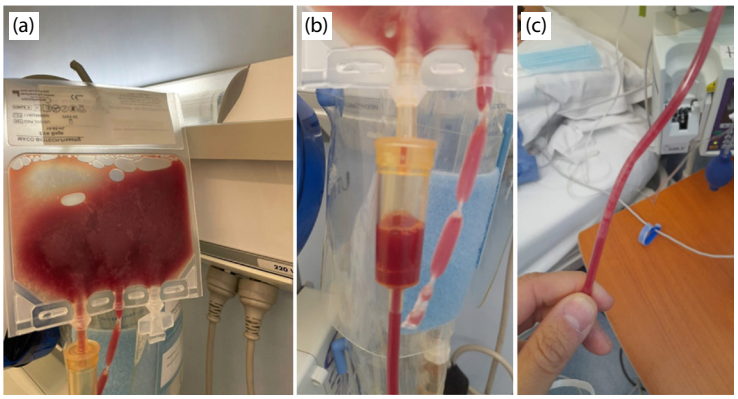
Figure 2. Case 1 - Thawed and washed product showing small white clumps at the bottom.

Colony-forming unit assay was performed on a sample of the washed product. Colony growth was observed. On day 18, neutrophil engraftment was achieved, but platelet engraftment was never attained. Chimerism reached 100% donor. The patient developed graft-*versus*-host disease and infectious complications, mainly cytomegalovirus reactivation. Over time, blood counts declined, leading to poor graft function with high transfusion needs that did not respond to thrombopoietin-receptor agonists. As a result, a second haploidentical transplant from the mother was performed, and the child is currently in recovery.

CASE 2

An autologous HPC product obtained via apheresis, collected at a nearby center, was received at the processing laboratory 2 hours after collection. A visual inspection revealed no abnormalities (120 mL of collected product and 8 mL of acid citrate dextrose, less than 7% as per the collection report notes).

The product was processed (Table 1), cryopreserved, stored for 31 days and then transported from the processing laboratory to the transplant center, located 6 km away, in the same city. Thawing was performed at the patient's bedside. When the infusion set was connected and dripping began, clumps were observed in the thawed bag, tubing, and accumulating in the filter (Fig. 3). The infusion was halted, and the decision was made to filter the product with a new infusion set before continuing. The infusion was completed without



Source: Elaborated by the authors.

Figure 3. Case 2 - Patient's bedside thawed product showing clumps at the bag, filter and tubing.

complications using a second infusion set. In all instances, a 180-µm filter was used. Due to the observed clumps, additional patient monitoring was decided upon. The patient demonstrated neutrophil engraftment on day 13 and platelet engraftment on day 17. The patient was discharged in good condition on day 21.

DISCUSSION

The characteristics of the cryopreserved and infused products in case 1 (allogeneic, unrelated adult donor) showed notable differences, as presented in Table 1. $CD34^+$ cell count decreased from 7.08×10^6/kg in the cryopreserved product to 5.48×10^6/kg in the infused product, likely reflecting cell loss during the washing process. A similar reduction was observed in granulocyte count, which declined from 1.84×10^9 to 0.56×10^9. In terms of viability, $CD45^+$ cell viability dropped significantly post-thaw, from 91.5 to 57.1%. However, $CD34^+$ cell viability remained high (> 97%). In contrast, case 2 (autologous, pediatric patient) showed a much higher granulocyte content (6.68×10^9), slightly exceeding the threshold identified as relevant for the occurrence of adverse events in the study by Cordoba et al.[1], in which the best cutoff point for predicting adverse events–with 47% sensitivity and 89% specificity–was 6.065×10^9 granulocytes. Sterility testing was negative in all cases.

Although clumping was observed in the thawed apheresis products in both cases, in case 2, no abnormalities were reported during the visual inspection upon receipt at the processing laboratory. In contrast, in case 1, the extended time from collection (46.5 hours), combined with international transportation and the anticoagulant proportion (10%), might have contributed to clump formation in the fresh product. Therefore, in case 1, the fresh product was filtered using blood infusion sets before cryopreservation.

In case 1, the product was washed post-thaw in the same laboratory it was cryopreserved (in the same institution as the patient's transplant center). The decision to filter the product before release for infusion was made at the laboratory. Although both HPC products were received and cryopreserved in the same laboratory, infusions were conducted at two different transplant centers. In case 2, thawing occurred at the patient's bedside without washing. Upon observing large clumps, the infusion was halted, the processing laboratory staff was contacted, and it was jointly decided to filter before continuing the infusion.

In both cases, the infusion was completed using a new infusion set with a 180-µm filter without complications. The filtering procedure used in both cases proved to be effective in improving the visual inspection of the final product and enabling a complication-free infusion. In case 1, the initial filtering before cryopreservation and the additional filtering post-thaw ensured the removal of clumps and the viability of the product for infusion. It is important to notice that the CD34 dose of the collected product exceeded the patient's requirements. Therefore, after filtration, a sufficient dose was obtained for the allogeneic procedure (5.48 CD34+ cells $\times 10^6$ / kg). Otherwise, the transplant procedure could have been at risk. In case 2, filtering conducted after noting clumps during thawing ensured the safe progression of infusion. However, no data on the final dose was available as the product was not processed in the laboratory and no samples were obtainable for characterization when thawed bedside.

CONCLUSION

Clump formation in thawed apheresis products represents a significant challenge in the handling and infusion of these products. In the two reported cases, the high number of granulocytes and/or the low anticoagulant level may have contributed to clump formation, and the filtering procedure proved to be effective in mitigating this issue. It is crucial to continue investigating the factors contributing to clump formation and optimizing handling and filtering techniques to ensure the quality and safety of thawed apheresis products.

Establishing written procedures that delineate potential causes and solutions for deviations observed during infusion in the transplant unit, based on relevant literature, will enhance our ability to maintain the quality and safety of thawed apheresis products. Furthermore, vigilant patient monitoring is essential due to the heightened risk of adverse effects associated with these events.

CONFLICT OF INTEREST

Nothing to declare.

DATA AVAILABILITY STATEMENT

The data will be available upon request.

AUTHORS' CONTRIBUTIONS

Conceptualization: Gamba C, Silvestri MR, Miguel A; **Methodology:** Gamba C, Silvestri MR,Miguel A; **Resources:** Sánchez DD, Escobar NF, Staciuk R, Kuperman SL; **Investigation:** Gamba C, Silvestri MR, Miguel A, Labonia D, Escobar NF, Pizzi SM, Sánchez DD, Staciuk R, Kuperman SL; **Data curation:** Gamba C, Silvestri MR, Miguel A; **Supervision:** Labonia D, Escobar NF, Silvia Mariana Pizzi, Sánchez DD; **Original – draft writing:** Gamba C, Silvestri MR, Miguel A; **Final approval:** Gamba C, Silvestri MR, Miguel A, Danila Labonia, Escobar NF, Pizzi SM, Sánchez DD, Staciuk R, Kuperman SL.

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