Transfusion and Apheresis Science xxx (xxxx) xxxx

Contents lists available at ScienceDirect

Transfusion and Apheresis Science

journal homepage: www.elsevier.com/locate/transci

Manipulation, and cryopreservation of autologous peripheral blood stem cell products in Italy: A survey by GITMO, SIDEM and GIIMA societies

F Lanza[a,*], S Mangianti[b], P Accorsi[c], L Lombardini[d], M Martino[e], R Saccardi[f], A Vassanelli[g], A Ostuni[h], F Ciceri[i]

[a] Romagna Transplant Network, Ravenna, Italy
[b] Cell Processing Laboratory- PS-Cesena, Italy
[c] Dept Onco-hematology-Transfusion Service, Italy
[d] Italian National Transplant Centre(CNT), Rome, Italy
[e] Transplantation Unit, Reggio Calabria, Italy
[f] TranplantationUnit, Florence, Italy
[g] Transfusion Medicine Dept AOUI Verona, Italy
[h] Transfusion Service, Bari, Italy
[i] IRCCS San Raffaele University Hospital- Milan, Italy

ARTICLE INFO

Keywords:
Stem cells
CD34 counting
Graft manipulation
Peripheral blood
Stem cell storage

ABSTRACT

There is considerable heterogeneity in manipulation and cryopreservation of hematopoietic stem cells (HSC) for autologous HSC transplantation across Europe and Italy. To better address this point, three Italian Scientific Societies (GITMO- Gruppo Italiano per il Trapianto di Midollo Osseo; SIDEM- Società Italiana Emaferesi e Manipolazione Cellulare; and GIIMA- Gruppo Italiano Interdisciplinare Manipolazione e Aferesi per Terapie Cellulari), in collaboration with the Competent Authority "National Transplant Center" (CNT) sent to 85 Italian transplant centers (TC) a survey, which included 12 questions related to the most critical elements in graft processing. Fifty-nine centers (70 %) responded to the questionnaire. Overall, this survey demonstrates that the majority (> 90 %) of responding TC used standardized procedures for HSC processing; however, an intercenter heterogeneity was clearly documented in several standard operating procedures adopted by different TC. These results seem to suggest that further standardization and efforts are needed to provide recommendations and guidelines on HSC manipulation, cryopreservation and functional assessment of cryopreserved material for autologous HSCT.

1. Introduction

Hematopoietic stem cell transplantation (HSCT) has been rapidly expanding worldwide over the last twenty-five years for several hematological malignancies as well as for non –hematological indications [1]. The widespread use of HSCT derived from peripheral blood (PB) in the autologous settings have evolved in the absence of consensus not only with respect to assessment of graft adequacy, but also with respect to method of collection, processing and cryopreservation. The Joint Accreditation Committee of the International Society for Cellular Therapy (ISCT) and European Society for Blood and Marrow Transplantation (EBMT) (JACIE) has provided standards which acts as minimum guidelines that, however, leave room for significant variations in practices at the individual transplantation center (TC) [2–6]. In general, the primary objectives for laboratories supporting an autologous HSCT program are to provide secure storage for leukoapheresis products, and to adequately characterize the functional properties of the graft [5].

With the increasing number of HSC transplants occurring on a global scale it is of great interest to learn more about the procedures that support HSC collection and their processing and reinfusion in the autologous setting [4–6]. To explore this issue, a survey was launched by three Italian Societies (GITMO- Gruppo Italiano per il Trapianto di Midollo Osseo; SIDEM –Società Italiana Emaferesi e Manipolazione Cellulare; and GIIMA: Gruppo Italiano Interdisciplinare Manipolazione e Aferesi per Terapie Cellulari), in collaboration with the National Transplant Center (CNT)-Italy. Survey questions were constructed to address the different facility types that perform collections, manipulation, cryopreservation and assessment of quality standards for HSCT. Multiple choices and open questions were used to determine the

* Corresponding author at: Section of Hematology and Romagna Transplant Network, Hospital of Ravenna, Via Randi 5, Ravenna, Italy.
E-mail address: francesco.lanza@auslromagna.it (F. Lanza).

https://doi.org/10.1016/j.transci.2020.102753

1473-0502/

Table 1
Italian Survey on HSC enumeration, processing, and storage.

1) Which flow cytometry method do you use for the enumeration of CD34+ HSCs in the leukoapheresisproduct?
a) Single platform ISHAGE-derived protocol: yes (…) ; no (…)
b) Dual platform ISHAGE-derived protocol yes(…) ; no (…)

2) Which protocol do you use for the identification of non viable cells in the graft?
a) ISHAGE method is based on the use of a nucleic acid dye (7-AAD) for the identification of non viable cells yes (…) ; no (…). If no please specify (……..)

3) In which samples do you perform the CD34+ cell enumeration assay in your daily practice?
a) leukoapheresis product before cryopreservation (…)
b) leukoapheresis product after cryopreservation only (…)
c) leukoapheresis product before and after cryopreservation (...)

4) At what time do you perform the cryopreservation of the apheresis product?
a) always the same day of the collection procedure (…)
b) always the day after the collection procedure (…)
c) occasionaly the day after (it depends on the timing of the end up of the collection procedure)(…)

5) Which is you policy for the overnight storage of the leukoapheresis bag?
a) The leukoapheresis bag is kept overnight in the fridge at + 4/+2°C in the collection unit (…)
b) Do you use some compounds for overnight storage: ACD? yes(...) ; no (…); How much? (…); other ……

6) Which is the TNC (total nucleated cells) concentration used for long tem storage?
a) < = 200 x 10^6/mL (…)
b) < = 100x10^6/mL (…)
c) Other ……

7) Which cryopreservation compound or medium do you use for long term storage:
a) DMSO 5 % (…)
b) DMSO 7–8 % (…)
c) DMSO 10 % (…)
d) Other (……..)

8) Long-term storage: how do you dilute the leukoapheresisbag?
a) Autologous plasma (…)
b) Steril water with albumin (…)
c) Other ……

9) Do you use a controlled rate (slow) freezing before cyopreserving the graft?
a) Yes …
b) No ….

10) Lomg-term storage: which is your policy?
a) Storage at – 80°C (mechanical freezer) (…)
b) Storage at -180°C +/- 5°C (Liquid nitrogen) (…)
c) Storage at vaporphaseliquid nitrogen (LN_2) temperatures (≤ -160°C) (…)
d) other (……..)

11) do you perform cell washing before the infusion of the cryopreserved bag?
a) yes, manual washing (…)
b) yes, automatic washing (Sepax system) (…)
c) yes, automatic washing (Lovo system) (…)
d) no (…)

12) Which policy for the disposal of autologous HSC do you use in your transplant center?
a) I use the SIDEM-GITMO recommendation (Perseghin et al, "Policy for the disposal of autologous hematopoietic progenitor cells: report from an Italian consensus panel, Transfusion, 201,454:2353-2360)
b) I do not use the SIDEM-GITMO recommendations (…)
c) I use the following procedure (……)

important elements included in the questionnaire. The survey has included 12 questions, related to the most critical stages of the processing pathway(Table 1).

2. Materials and methods

A joint panel discussion within boards of promioting societies selected a list of 12 questions and were sent to 85 TC. Fifty-seven centers (70 %) responded in 4 months to the questionnaire, for a total coverage of 81 % of autologous transplants reported to the registry in the year 2019 (Table 1). Centers participating to the Survey were JACIE-accredited in 33/57 (58 %) as compared to those not responding (14 %).

3. Results

The first question was related to the cytofluorometric method used by TC for assessing the absolute numbers of viable CD34+ cells in mobilized blood and leukoapheresis products. Surprisingly, only a minority of TC (11 %) used a single platform analysis for CD34+ cell enumeration, while in 89 % of TC the dual platform approach represented the reference protocol for HSC quantitation; the latter method is based on WBC count measurement through the use of an external hematology analyser [7,8]. Moreover, most TC (96 %) used a nucleic acid dye for a precise assessment of viable CD34+ cells in the apheresis product [7,8].

Interestingly, in 43 % of the reporting Processing Labs, the CD34 cell count was performed either in fresh or cryopreserved grafts.

The procedure of autologous HSCT further requires short- and long-term storage.

Cryopreservation of cells is performed in a mixture containing dimethyl sulfoxide (DMSO), since DMSO is necessary to secure cell viability. Overnight storage of the apheresis product before freezing is a quite common procedure in Italian TC (85 %); in particular, leukoapheresis bag is kept overnight in the fridge at + 4/+2 °C in the collection unit. However, the protocol employed by TC for diluting the graft to be kept overnight before cryopreservation resulted quite heterogeneous in terms of types of compound used and ACD concentration.

Long-term storage was performed mainly either in liquid nitrogen or in the vapor phase of liquid nitrogen with 10 % DMSO (87 % of the TC). In the remaining 13 % cases, DMSO was used at a lower concentration (4 % used DMSO at 7–8 %, and further 7 % of TC used DMSO at 5 %).

As far as DMSO removal is concerned, in only a minority (11 %) of the responding centers washing of the graft was performed before its infusion, either manually by centrifugation (11 %) or with automated methods (Sepax system, 7 %). No TC used the Lovo system for cell washing.

The graft was thawed at the bedside (89 %) in a water bath.

Most of the TC (82 %) used a controlled rate (slow) freezing before the long term storage at vapor phase liquid nitrogen (LN_2) temperatures (≤ −160 °C).

With regard to the question related to the TNC (total nucleated cells) concentration used for long tem storage, the protocol used by TC resulted highly heterogeneous. A cell concentration < = 100 × 10^6/mL was used in 9 % of the centers, < = 200 × 10^6/mL in 59 % of TC, and higher cell concentration in the remaining cases.

The last issue examined in this survey was the policy for the disposal of autologous HSC in the various transplant centers. Most of the TC (85 %) used the SIDEM-GITMO recommendation published by Perseghin et al. ("Policy for the disposal of autologous hematopoietic progenitor cells: report from an Italian consensus panel"- Transfusion, 2014) [9]. In contrast, 15 % of the Processing Lab used local guidelines for the disposal of cryopreserved bags.

4. Discussion

A successful HSCT is dependent on transplantation of a sufficient amount of CD34+ cells to ensure prompt and durable engraftment. The preferred source of HSC for autologous HSCT is represented by mobilized PB stem cells (PBSC), since they are easier to collect and engraft faster than BM-derived cells [10]. Quality assessment of stem cell graft plays a pivotal role in the autologous setting [10,11]. The development and worldwide use of the so called "single platform ISHAGE methodology" allows direct quantification of the numbers of viable HSC transplanted [8]. The required number of CD34+ cells is still under discussion, but most investigators accept a minimum of 2.0 × 10^6 CD34+ cells/kg body weight (b.w.) for transplantation, although cell doses of 4–5.0 × 10^6 CD34+ cells/kg b.w. are associated with faster engraftment of neutrophils and platelets, reduced incidence of infection and reduced need for transfusions [12,13]. Viability and potency of the

stem cell product constitute formal release criteria from cryopreservation facilities; furthermore rigorous quality parameters and control measures are required by regulatory agencies [14]. The major objectives were to promote quality of medical and laboratory practice in HSCT. Moreover, cell processing hold heavy clinical implications, since are strictly related to transplant safety and patient outcome [10,11].

However, the large majority of the results so far published in the literature deals with the assessment of the graft before cryopreservation [8]. The effects of graft handling, cryopreservation, storage and thawing on the recovery of CD34+ cells needs to be carefully analysed and standardized on a global level. Pretransplant quality assessment of reinfused autografts may be an important step in the prediction of posttransplant support, complications and safety [10,14]. As a general rule, we do believe that post-thaw viability assay should be routinely performed as a measure of graft quality for autologous transplant. Enumeration of viable CD34+ cells at the time of infusion becomes particularly relevant in patients in whom stem cell mobilization has been problematic and/or collected a total amount of stem cells which is borderline to ensure a safe transplant procedure (i.e. 1.5–2.5 $\times 10^6$ CD34+/kg) [15].

The main factors recognized to affect cryopreserved HSCs viability are latency time between stem cell collection and cryopreservation, WBC contamination of the graft, method of cryopreservation (passive or controlled freezing), freezing speed and, after thawing, delay in HSCs reinfusion to the patient [14,15]. However, quality control measures are challenging as there is not standardized assay for such test, little is known on the correlation between viable CD34+ count and graft clonogenic potential, and progenitor cells responsible for hematopoietic recovery are actually only a minority of the HSC infused [16]. Different authors suggested that post-thaw assessment of CD34+ cell viability may be a more accurate method to predict hematopoietic engraftment rather than CD34+ cell enumeration at the time of harvest [17–19].

In order to better understand the extent of heterogeneity in graft storage conditions, quality controls, PBSC processing and disposal across Italy, GITMO, SIDEM and GIIMA societies wanted to collect data regarding the procedures used by the various TC in Italy. To this purpose a questionnaire composed by 12 questions was sent to 85 TC. Some of the Cell Processing laboratories served multiple TC (Roma transplant Network, Romagna Transplant network, Turin transplant network).

As far as CD34+ cell count is concerned, the use of CD45 antibodies, and fluorescent counting beads have given a contribution in this area of investigation [8]. This so called “single platform” approach allows the determination of the absolute CD34+ cell count directly from the cytometer and should be used in combination with a viability stain such as 7-AAD and, possibly, additional markers for further immunophenotypic characterisation of CD34+ cells [16]. It represents the reference method for the enumeration of CD34+ HSCs in fresh samples such as mobilized blood and leukoapheresis products. As far as the analysis of cryopreserved/thawed samples is concerned, we must say that some technical unresolved issues still limits the application of the ISHAGE derived single platform protocol. A study trial is needed through the consecutive control of the fresh sample- a cryopreserved and thawed reference ampoule- the cryopreserved thawed transplant. The selection of reference standards for standardisation trials are critical steps in this area.

Based on a few data so far published in the literature as well as personal data collected in Italy, we do believe that the flow-cytometry analysis of thawed samples requires an adaptation of both the acquisition setting and the gating strategy, with reference to the standard ISHAGE technique.

Results from the survey has shown that 43 % of the Italian institutions perform a post-thaw viability assessment on a retain vial or on the bag, using 7-AAD in combination with CD34 and CD45 antibodies, as part of their product release procedure for cryopreserved products. Although 43 % of the TC do perform CD34 enumeration on cryopreserved material, it is unknown whether this analysis may have had an impact on the clinical decision to transplant patients having an high proportion of non viable CD34+ cells in the thawed material.

With regards with other questions, most cryopreservation protocols could involve volume reduction, addition of a cryoprotectant solution, the use of a controlled rate (slow) freezing and storage at vapor phase liquid nitrogen (LN_2) temperatures ($\leq -160\,^{\circ}C$) [20].

In our daily practice, it is crucial that the space containing the liquid nitrogen storage tanks and supply Dewars should be separate from the processing facility, and needs to have sufficient air handling capacity to maintain safe levels of oxygen during the times when the LN_2 tanks are filling. An oxygen sensor that alarms when levels are dangerously low is needed.

Looking at the results derived from TNC (total nucleated cell) concentration and neutrophil contamination in the bag, we must say that the influence of this parameter on platelet and granulocyte engraftment following HSCT is still object of debate. It is wise to keep a TNC concentration below 200 $\times 10^6$/ml in order to limit cell death at the time of graft infusion.

Interestingly, in only a minority (11 %) of the responding centers washing of the graft was performed in order to remove DMSO from the cryopreserved bag before its infusion, either manually by centrifugation or with automated methods. Recent reports in the literature have clearly shown that lower or no DMSO contamination of the graft is associated with fewer adverse reactions to infusion [21]. However, we must say that DMSO removal is associated with additional resources and costs, which limits the application of this procedure worldwide. Generally speaking, manual removal is easier and cheaper and, despite automated methods being highly efficient, they are more expensive and time consuming.

Regarding the main processing principles, we do believe that the primary role in supporting an autologous HSCT is to preserve stem cell viability during storage and to prevent the introduction of microbial contamination at all stages of graft processing. Apheresis products should be cryopreserved in the shortest time interval; however, overnight storage is acceptable if the graft is kept refrigerated at $+4\,^{\circ}C$.

The last issue to be dealt with is represented by the best practice for the disposal of autologous HSC. A formal consensus process allowed SIDEM and GITMO to propose a policy for autologous HSC disposal that fulfils clinical, ethical, and economic criteria [9]. Based on these considerations, two criteria for prompt disposal of freshly collected HPCs were identified by the working panel: an abnormal freezing procedure causing highly reduced viability or major microbiology contamination. Moreover, six major criteria were further proposed, each one of them allowing for the disposal of stored HPC units: patient death, withdrawal of consent to ASCT, contraindications or loss of indications to HSCT, a damaged label that prevents correct identification of the unit, and time elapsed since harvest longer than 10 years [9]. Results from the survey have shown that 15 % of the Processing Labs have used local guidelines instead of the SIDEM-GITMO recommendation for the disposal of cryopreserved bags.

In conclusion, these findings underlines the need for advanced processing principles, quality management guidelines and references guidance documents to better assist the HSC laboratory on a global level.

Acknowledgments

We would like to thank the following transplant centers for taking part the survey : Monza, Brescia (n.2), Busto Arsizio, Milano (n.3), Bergamo (n2), Varese, Reggio Emilia, Modena, Parma, Rete Trapianti Romagna, Bologna (n2), Ferrara, Piacenza, Padova, Mestre, Vicenza, Verona, Cuneo, Alessandria, Torino (n.2, Genova (n.2), Bolzano, Aviano, Trieste, Udine, Roma (n 3), Latina, Pisa, Firenze (n 2), Siena, Ascoli Piceno, Pesaro, Cagliari, Sassari, Napoli (3), Avellino, Salerno, Brindisi, Foggia, Bari, S Giovanni Rotondo, Tricase, Palermo (n. 3),

Reggio Calabria, Rionero in Vulture.

References

[1] Passweg JR, Baldomero H, Bader P, Basak GW, Bonini C, Duarte R, et al. Is the use of unrelated donor transplantation leveling off in Europe? The 2016 European Society for Blood and Marrow Transplant activity survey report. Bone Marrow Transplant 2018(March). https://doi.org/10.1038/s41409-018-0153-1.

[2] Chabannon C, Pamphilon D, Vermylen C, Gratwohl A, Niederwieser D, McGrath E, et al. Ten years after the first inspection of a candidate European centre, an EBMT registry analysis suggests that clinical outcome is improved when hematopoietic SCT is performed in a Jacie accredited program. Bone Marrow Transplant 2012;47:15–7.

[3] Snowden JA, Saccardi R, Orchard K, Ljungman P, Duarte RF, Labopin M, et al. Benchmarking of survival outcomes following haematopoietic stem cell transplantation: a review of existing processes and the introduction of an international system from EBMT and ISCT (JACIE). Bone Marrow Transplant 2019(October). https://doi.org/10.1038/s41409-019-0718-7.

[4] Holbro A, Baldomero H, Lanza F, Chabannon C, Snowden JA, Buser L, et al. Handling, processing and disposal of stem cell products in Europe: a survey by the cellular therapy and immunobiology working party of the European Society for Blood and Marrow Transplantation. Cytotherapy 2017. https://doi.org/10.1016/j.jcyt.2017.12.005.

[5] Leemhuis T, Padley D, Keever-Taylor C, Niederwieser D, Teshima T, Lanza F, et al. Essential requirements for setting up a stem cell processing laboratory. Bone Marrow Transplant 2014;49(8). https://doi.org/10.1038/bmt.2014.104.

[6] Perseghin P, Laszlò D, Bonifazi F, Introna M, Accorsi P, Petrini C, et al. Disposal of the residual autologous HSC units: Results of a survey carried out two years after the publication of a national policy in Italy. Transfus Apher Sci 2018;57(April (2)):197–200. https://doi.org/10.1016/j.transci.2018.01.007. Epub 2018 Jan 31.

[7] Lanza F, Healy L, Sutherland R. Structural and functional features of the CD34 antigen: an update. J Biol Regul Homeost Agents 2001;15:1–13.

[8] Keeney M, Brown W, Gratama J, Papa S, Lanza F, Sutherland R. Single platform enumeration of viable CD34+ cells. J Biol Regul Homeost Agents 2003;17:247–53.

[9] Perseghin P, Marchetti M, Pierelli L, Olivieri A, Introna M, Lombardini L, et al. A policy for the disposal of autologous hematopoietic progenitor cells: report from an Italian consensus panel. Transfusion 2014;54(September (9)):2353–60. https://doi.org/10.1111/trf.12619. Epub 2014 Mar 24.

[10] Lanza F, Campioni D, Hellmann A, Milone G, Wahlin G, Walewski Jan, et al. Individual quality assessment of autografting by probability estimation for clinical endpoints: a prospective validation study from the European group for blood and marrow transplantation. Biol Blood Marrow Transplant 2013;19(12). https://doi.org/10.1016/j.bbmt.2013.08.005.

[11] Mohty M, Duarte R, Kuball J, Bader P, Basak GW, Bonini C, et al. Recommendations from the European society for Blood and Marrow Transplantation (EBMT) for a global curriculum in hematopoietic cell transplantation. Bone Marrow Transplant 2018(May). https://doi.org/10.1038/s41409-018-0190-9.

[12] Lanza F, Lemoli RM, Olivieri A, Laszlo D, Martino M, Specchia G, et al. Factors affecting successful mobilization with plerixafor: an Italian prospective survey in 215 patients with multiple myeloma and lymphoma. Transfusion 2014;54(February (2)):331–9. https://doi.org/10.1111/trf.12265.

[13] Lanza F, Saraceni F, Pezzi A, Martino M, Bosi A, Cascavilla N, et al. A comparative analysis of biosimilar vs originator filgrastim in combination with plerixafor for stem cell mobilization in lymphoma and multiple myeloma; a propensity-score weighted multicenter approach. Am J Hematol 2017(June). https://doi.org/10.1002/ajh.24817.

[14] LeMaistre CF, Loberiza Jr FR. What is quality in a transplant program? Biol Blood Marrow Transplant 2005;11:241–6.

[15] Watts MJ, Ings SJ, Balsa C. Re-evaluation of progenitor thresholds and expectations for haematopoietic recovery based on an analysis of 810 autologous transplants: implications for quality assurance. Br J Haematol 2016;175:673–6.

[16] Pecora AL, Preti RA, Gleim GW. CD34+CD33- cells influence days to engraftment and transfusion requirements in autologous blood stem-cell recipients. J Clin Oncol 1998;16:2093–104.

[17] Allan DS, Keeney M, Howson-Jan K. Number of viable CD34(+) cells reinfused predicts engraftment in autologous hematopoietic stem cell transplantation. Bone Marrow Transplant 2002;29:967–72.

[18] D'Rozario J, Parisotto R, Stapleton J. Pre infusion, post thaw CD34+ peripheral blood stem cell enumeration as a predictor of haematopoietic engraftment in autologous haematopoietic cell transplantation. Transfus Apher Sci 2014;50:443–50.

[19] Yang H, Acker JP, Cabuhat M. Association of post-thaw viable CD34+ cells and CFU-GM with time to hematopoietic engraftment. Bone Marrow Transplant 2005;35:881–7.

[20] Montanari M, Capelli D, Poloni A, et al. Long-term hematologic reconstitution after autologous peripheral blood progenitor cell transplantation: a comparison between controlled-rate freezing and uncontrolled-rate freezing at 80 degrees C. Transfusion 2003;43:42–9.

[21] Mitrus I, Smagur A, Fidyk W, Czech M, Prokop M, Chwieduk A, et al. Reduction of DMSO concentration in cryopreservation mixture from 10% to 7.5% and 5% has no impact on engraftment after autologous peripheral blood stem cell transplantation: results of a prospective, randomized study. Bone Marrow Transplant 2018;53:274–80. https://doi.org/10.1038/s41409-017-0056-6. Epub 2017 Dec 21.