Bone marrow processing for hematopoietic stem cell transplantation: recommendations of the SFGM-TC

Responsable	**Hélène Rouard**
Participants	Florence Boulanger Etienne Baudoux Boris Calmels John De Vos Eric Gautier Marie-Noëlle Lacassagne Yordanka Tirefort Pascale Turlure
Expert	
Lecteurs	Christian Chabannon Christiaine Gerard Oliver Giet Catherine Letellier Melanie Monfort Jean-Baptiste Thibert Ibrahim Yakoub-Agha
Questions posées	In which setting is bone marrow processing required? What are the techniques available and applicable for bone marrow processing? What are the critical release criteria?

ABSTRACT

Le prélèvement de moelle osseuse fait l'objet d'un ou plusieurs traitements préalablement à son injection au receveur. Ces transformations sont basées sur l'évaluation de la compatibilité érythrocytaire entre le donneur et le receveur, et sur le volume maximal acceptable du greffon. Elles doivent préserver la quantité de cellules mononuclées injectées ainsi que leur qualité. Une enquête au sein de centres de thérapie cellulaire francophones a révélé une hétérogénéité d'une part des pratiques d'ingénierie cellulaire, en partie liée au marché des automates, et d'autre part des techniques de titrage des anticorps anti-érythrocytaires et de leur absence de normalisation. Dans une démarche qui vise à uniformiser les pratiques d'allogreffe de cellules souches hématopoïétiques (CSH), la Société française de greffe de moelle et de thérapie cellulaire (SFGM-TC) a organisé les cinquièmes ateliers d'harmonisation des pratiques en septembre 2014 à Lille. Nous proposons des recommandations concernant la transformation des greffons de moelle osseuses et les critères de libération.

STATE OF THE ART

Bone marrow processing practices are well established in cell processing facilities [1]. However, these practices remain heterogeneous and are based on scarce publications and mostly single-center results. Availability of automatic devices is usually limited in each center and evolving, with some well-established devices getting withdrawn from the market, while new devices are coming into the market.

In addition, testing and quality control methods are improving, thus creating the need to revise established thresholds and release criteria.

METHODOLOGY

To evaluate practices, we conducted a survey to French speaking cell processing laboratories regarding bone marrow processing techniques. We received 23 responses: main results are summarized in Tables 1-5.

Table 1: Erythrocyte depletion methods

COBE® Spectra/SpectraOptia®	6
COBE® 2991 – FICOLL gradient	10
FICOLL gradient (Tube)	1
Biosafe Sepax	1
SEDIMENTATION with macromolecule (in tubes or bags)	2
Not available	3

Table 2: Thresholds for residual red blood cell contamination after erythrocyte depletion

Red blood cell volume < 0,2 ml/Kg	9
Red blood cell volume < 0,4ml/Kg	6
Hematocrit < 1%	1
Hematocrit < 3%	1
Not available	6

Table 3: Thresholds for plasma removal decision

Anti-A or anti-B antibodies titer > 64	11
Anti-A or anti-B antibodies titer > 32	1
Positive for anti-A or anti-B antibodies (without titration)	1
Systematic	8
Not available	2

Table 4: Plasma removal methods

COBE® 2991	10
Centrifugation (in bags)	9
Biosafe Sepax	1
Not available	3

Table 5: Substitution solutions after plasma removal

NaCl 0,9% +/- ACD-A	3
Albumine 4%	11
Albumine 4% + NaCl 0,9% +/- ACD-A	4
Plasma	1
Not available	4

RECOMMENDATIONS

Filtration

Bone marrow filtration is mandatory for removal of bone debris fat, clots and cell aggregates and should be documented. The usual filter has a mesh size of 200 micrometers. This can be done either in the operating room or in the cell processing laboratory.

Plasma removal

This technique is based on bone marrow centrifugation of the with or without additional washing steps, depending on the initial titer of anti-A or anti-B antibodies, or allo-antibodies in the donor's serum. As of today, plasma removal can be achieved by several devices such as conventional bag centrifugation, followed by manual extraction, semi-automatic devices (Cobe Cell Processor 2991, etc.) or automatic devices (Biosafe Sepax, etc.). We do not recommend plasma removal if the anti-A or anti-B antibody titer is below the threshold as defined by the cell processing system or facility. Validation of this technique should be based on anti-A, anti-B or allo-antibody titer decrease, while minimizing cell loss (usually less than 20% nucleated cell loss) and preservation of cell viability.

Buffy coat preparation

Buffy coat preparation is a technique intended to increase nucleated cell concentration by removing plasma and red blood cells. This can be achieved by semi-automatic devices (Cobe Cell Processor 2991, etc.) or automatic devices (Biosafe Sepax, etc.) [2]. Buffy coat preparation should minimize cell loss (usually less than 20% nucleated cell loss) and preserve cell viability.

Use of manual techniques for plasma and red blood cell removal are not recommended.

Erythrocyte depletion

This technique aims at removing most of red blood cells from the bone marrow. This can be can achieved either by semi-automatic devices (Ficoll 1.077 density gradient on Cobe Cell Processor 2991 with peristaltic pump, etc.) or automatic devices (Terumo/Cobe Spectra Optia Apheresis System, etc.) ([3], [4], [5], [6], unpublished data from Boulanger).

Validation of this technique should be focused on reducing the red blood cell contamination below 0,2 mL/kg while minimizing mononucleated cell loss and preserve cell viability.
Using manual methods for this techniques is not recommended.

Cryopreservation and thawing

In general bone marrow cryopreservation in the allogeneic setting should not be part of standard procedures. Before cryopreservation, the bone marrow should at least be processed by Buffy coat preparation and ideally erythrocyte depletion in order to minimize hemolysis and recipient exposure to erythrocyte lysate during infusion.
Standard cryopreservation, thawing and washing techniques, such as those used for peripheral blood stem cells, can be applied.

Release criteria

In case of minor ABO blood group incompatibility each processing facility should validate its own release threshold of acceptable anti-A and/or anti-B antibodies titers.

In case of major ABO incompatibility, residual red blood cells should not exceed a threshold of 0,2 mL/kg at the time of graft release. In case of bidirectional ABO incompatibility, both of the above mentioned criteria should be met.

Of note:

* The volume of the infused cell product should not exceed 20 mL/kg, depending on the clinical status of the recipient (cardiac status and kidney function, to be discussed with the clinician). For example, in the allogeneic setting with ABO identity and donor /recipient weight imbalance (i.e. adult donor/child recipient), plasma removal, buffy coat preparation or erythrocyte depletion can be used to reduce the bone marrow volume before infusion and to not surpass the limit mentioned above.

* After processing and adequate quality controls, the bone marrow graft should be delivered and infused without delay. According to the EU directive 2006/86/EC, an expiry date and time are mandatory (ref : EU directive 2006/86/EC).

ISSUES TO BE ADDRESSED IN THE FUTURE

- Given the heterogeneity of anticoagulation harvesting methods, harmonization of bone marrow harvesting procedures is needed.

- Harmonization of hematopoietic stem cell graft washing procedures after thawing is necessary.

- A multicenter study would be helpful to evaluate the efficiency of new automatic devices for red blood cell bone marrow depletion.

- A multi-centric study aimed at determining bone marrow CD34+ cell target dose (using single platform technique) is of interest.

- Techniques and threshold for anti-A and anti-B antibody titration should be harmonized between immuno-hematology laboratories. To begin with, at least each center should define its own internal standards.

DECLARATION D'INTERET

La SFGM-TC reçoit l'aide financière des laboratoires Amgen, Astellas, Biosafe, Celgene, Chugai, Jazz Pharmaceuticals, Gentium, Gilead, Janssen, Keocyt, Macopharma, MSD, Mundipharma, OrpheliPharm, Pfizer, Pierre Fabre, Sandoz, Sanofi, Spectrum, Takeda, Teva, Therakos, Vifor pharma.

Références

1. 2011, H.r.
2. Aktas, M., et al., *Separation of adult bone marrow mononuclear cells using the automated closed separation system Sepax.* Cytotherapy, 2008. **10**(2): p. 203-11.
3. Larghero, J., et al., *ABO-mismatched marrow processing for transplantation: results of 114 procedures and analysis of immediate adverse events and hematopoietic recovery.* Transfusion, 2006. **46**(3): p. 398-402.
4. Cappellesso-Fleury, S., et al., *[Erythrocyte removal from bone marrow by density gradient separation using the COBE 2991 cell processor with the triple-bag processing set]*. Transfus Clin Biol, 2009. **16**(1): p. 43-9.
5. Witt, V., E. Beiglbock, and G. Fritsch, *Bone marrow processing with the AMICUS separator system.* J Clin Apher. **26**(4): p. 195-9.
6. Guttridge, M.G., et al., *Factors affecting volume reduction and red blood cell depletion of bone marrow on the COBE Spectra cell separator before haematopoietic stem cell transplantation.* Bone Marrow Transplant, 2006. **38**(3): p. 175-81.
7. Daniele, N., et al., *The processing of stem cell concentrates from the bone marrow in ABO-incompatible transplants: how and when.* Blood Transfus. **12**(2): p. 150-8.