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Quality controls of cryopreserved hematopoietic stem cells

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ABSTRACT

HPC processing has been performed routinely for many years for the preparation and cryopreservation of HPC used for autologous and allogeneic transplantation. JACIE Standards (section D) regulate HPC processing and request that processing is performed within the framework of a quality management system (QMS). Implementing QMS in HPC-processing laboratories is feasible, and many processing laboratories are already accredited according to various standards.

Before hematopoietic stem cell transplantation, it is recommended that accurate quality controls be performed to assess the median number of viable CD45+/7-aminoactinomycin-D (7-AAD) and CD45+/CD34+/7-AAD cells, the presence of microbiologic contamination, and the proliferative potential of hematopoietic progenitor cells. The guidelines for the determination of the QCs have been established by FACT/JACIE standards.

To be optimal, process and quality controls have to be performed in a real-time manner in order to ensure safe product release and an immediate recognition of deviations. Furthermore, the immediate initiation of corrective measures is crucial for risk prevention.

Introduction

Hematopoietic stem cell transplantation is a field of enormous therapeutic advances and worldwide expansion of applications over the past four decades. Studies of hematopoietic progenitor cell transplantation in humans began in the 1950s, following experiments in mice that showed protection against the lethal effects of irradiation, by the intravenous infusion of donor bone marrow containing hematopoietic cells capable of colonizing the recipient's bone marrow. HSCT has historically relied upon the steep dose–response

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Abbreviations: HSCT – hematopoietic stem cell transplantation; FACT – Foundation for the Accreditation of Cellular Therapy; JACIE – Joint Accreditation Committee European Group for Blood and Marrow Transplantation-Euro-ISHAGE; HPC – hematopoietic progenitor cell; EQA – External Quality Assessment; PT – Proficiency Testing; LI – leukocyte Immunophenotyping; SCE – stem cell enumeration; CB – cord blood; BM – bone marrow; CD – cluster of differentiation; QCs – quality controls; 7-AAD – 7-aminoactinomycin-D; HPC – hematopoietic progenitor cells; QMS – quality management system; ISHAGE – International Society of Hematotherapy and Graft Engineering; PBPCPs –

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relationship of chemoradiotherapy to maximize tumor cell kill, with the subsequent infusion of hematopoietic progenitor cells in order to circumvent the myelo and immunoablative effects of the preparative regimen [1–3]. More recently less intensive conditioning regimens have been utilized, often in older individuals, in an effort to reduce transplant related morbidity and mortality, while still capturing the potent graft *versus* tumor effect of an allogeneic HSCT. HSCT can be broadly classified according to donor source: autologous and allogeneic. Autologous HSCT involves the administration of myeloablative doses of chemoradiotherapy, followed by the infusion of previously collected autologous (self-donor) cells. Allogeneic HSCT refers to the transplantation of hematopoietic cells from a donor other than the patient [4–6].

The "quality" in a transplant program

HSCT as a discipline continues to rapidly evolve through translation of discoveries in the basic and clinical aspects of immunology, oncology, and infectious diseases into the transplant clinic. The continuing evolution of clinical care and the diverse group of patients and diseases treated with HSCT have contributed to disagreement as to how to establish measures of quality in transplant programs [7]. Quality of health care remains a topic of intense interest at all levels of the health-care delivery system. Measurement of quality in no less important in HSCT than in other areas of medicine and may even be more important, for a host of reasons. These include the life-threatening nature of the diseases, the treatment, the opportunity for cure, the intensive resource utilization, the manipulation of cells and the involvement of healthy donors in HSCT. It is quite likely that results of all transplant centers do not yield equivalent outcomes. Despite the acceptance of HSCT as the standard of care, meaningful measures of program quality are still in development [8].

The JACIE standards

Early after the initiation of FACT accreditation, the Joint Accreditation Committee European Group for Blood and Marrow Transplantation-Euro-ISHAGE (JACIE) was established. JACIE standards aim to promote and maintain the quality of medical and laboratory practice in HPC transplantation and to ensure harmonization between JACIE standards and other national/international standards. JACIE accreditation is voluntary, but provides a means whereby transplant facilities can demonstrate that they are working with a quality system covering all aspects of the transplantation process [9, 10]. The JACIE standards cover all aspects of clinical transplant programs, collection facilities and processing. The JACIE standards also apply to the use of therapeutic cells derived from blood or marrow including donor lymphocytes and mesenchymal stem cells. The JACIE accreditation system is now firmly established in Europe, and the experience of centers that have been inspected are that implementation of the JACIE standards has led to significant improvements in different aspects of their transplant programs. JACIE has further assisted with a number of training courses for preparing centers for accreditation and has issued a practical guide for quality management. JACIE has developed a close working relationship with other organizations involved in cellular therapy, which form the basis for a new global approach to harmonization of standards and accreditation systems worldwide [11, 12].

A milestone: the "Directive 2004/23/EC" of the European Parliament

In March 2004, Directive 2004/23/EC set standards for the donation, procurement, testing, processing, preservation, storage, and distribution of human tissues and cells. For the first time in the area of tissues and cells, a binding supranational, transparent, and sound regulatory framework had arisen, providing all citizens with the same minimum guarantees of quality and safety [13]. It is well specified that each tissue center must put in place a quality control system, which must include at least the following information: guidelines; operating procedures; training and reference manuals; donor records (to be kept for at least 30 years); information on the final destination of tissues or cells. Moreover, tissue establishments must include in their operating procedures all the processes that affect quality and safety. They must ensure that the equipment used, the working environment and process monitoring conditions comply with the requirements regarding the processing, storage and distribution of tissues and cells.

The obligations for Member States dictated in Directive 2004/23/EC are:

(1) designation of a Competent Authority;
(2) supervision of human tissue and cell procurement;
(3) accreditation, designation, authorization, or licensing of Tissue Establishments and tissue and cell preparation process;
(4) implementation of a system of inspections and control measures;
(5) implementation of a system of traceability;
(6) guarantee on quality and safety of imported/exported human tissues and cells;
(7) Register of Tissue Establishments and reporting obligations;
(8) notification of serious adverse events and reactions [13, 14].

Aspects of donors selection

Donor and patient HLA match status should be used to assess the risk of transplantation and to plan treatment based on those risks. The benefits of high-resolution HLA class I and II typing have been well demonstrated, particularly in post-transplant survival [15].

Moreover, the possibility of infection transmission by infusion of cryopreserved peripheral blood stem cells concentrates (PBPC) or bone marrow (BM) is well known. For this reason, the European Blood and Marrow Transplantation

Group (EBMT) and International Society for Haemotherapy and Graft Engineering (ISHAGE) standards include a panel of serological tests to be performed in donors with the aim of lowering the likelihood of infection transmission.

In addition, choice of donor source is dependent on the indication for HSCT, its urgency, the age of the patient, and the expertise and resources of the center [16].

Although the donation process is generally considered safe, side effects are a known risk, and care must be taken to minimize the potential of harm to donors.

In a prospective study, Billen et al. found that predonation health-related quality of life markers were the most important factors associated with recovery and the development of side effects, more so than any demographic variable [17].

The importance of "quality controls"

In hematopoietic stem cell transplantation, the final quality control of cryopreserved progenitor cells is a successful and persistent three lineage engraftment after transplantation. The stem cell providing institution is obliged to have a program for controlling and monitoring the manufacturing of cellular therapy products before the patients' conditioning therapy is started. The FACT–JACIE standards prescribe that the director of the institute shall define tests and procedures for measuring and assaying cellular therapy products to ensure their safety, viability and integrity and shall also ensure that products meet predetermined release specifications [18]. This requires specifications of assays and the definition of thresholds to allow release. The most common cell viability test is still trypan blue dye exclusion, although its predictive value is low and it does not seem to be a substitute for assays evaluating *in vitro* proliferative capacity. Stem cell culture assays are time consuming and results are investigator-dependent. Furthermore, flow cytometry-based evaluation of viability or apoptosis markers of progenitor cells after freezing–thawing are not standardized [19]. Reduced numbers of viable CD34+ cells have been reported to be associated with a risk of delayed platelet engraftment or graft failure. Further it has to be mentioned that interlaboratory discrepancies in the results of the assays exists, due to the fact that standardization is difficult and that the performance is variable. These problems can only be overcome by participating in external proficiency testing and by individual validation studies to establish specifications for release in each center [20].

The UK NEQAS Program

UK NEQAS for Leukocyte Immunophenotyping is an international External Quality Assessment (EQA)/Proficiency Testing (PT) provider hosted by, and is legally accountable to, Sheffield Teaching Hospitals NHS Foundation Trust. UK NEQAS LI was established as a regional program in 1986. At that time a total of 20 UK laboratories participated in the single program available at that time. Currently, there are over 1700 active registrations worldwide within the 20 programs now operated by the center [21]. In haematopoietic stem cell transplantations the use of CD34+ stem cell enumeration is an essential part of the treatment process, allowing for monitoring of donor mobilization pre harvest and to ensure sufficient cells are collected to ensure engraftment will occur. CD34+ Stem Cell Program is currently the largest world-wide for CD34+ haematopoietic progenitor cell enumeration [22]. The program uses stabilized peripheral blood obtained from consenting patients following stem cell mobilization and is suitable for use with whole blood lysis techniques and sequential gating strategies. Laboratories are requested to report both percentage and absolute values (in cells per microlitre), although performance is only monitored using the absolute values. Two samples are issued per trial and this program issues trials a minimum of 4 times per annum and a maximum of 6 [23].

The enumeration of CD34+ cells

The CD34 antigen is present on immature hematopoietic precursor cells and hematopoietic colony-forming cells in bone marrow and blood, including unipotent and pluripotent progenitor cells. An accurate measure of CD34+ cell count is necessary for dose requirement protocols on stem cell transplantation. CD34+ cell count is the most widely used biologic parameter for monitoring progenitor cell mobilization and apheresis, as well as assay the quality of most, if not all, types of hematopoietic cell grafts for autologous and allogeneic transplantation. International guidelines for flow cytometric enumeration of CD34+ hematopoietic stem cells (HSC) recommend the use of a single-platform assay [24]. Currently, the two most frequently used single platform kits are the Stem-Kit™ enumeration kit (manufactured by Beckman-Coulter, Villepintes, France) [25] and ProCount™ kit (manufactured by BD Biosciences, Meylan, France) [26]. Stem-Kit allows CD34+ cell enumeration in all types of HSC; it includes software designed for Beckman Coulter cytometers, which uses the ISHAGE gating strategy and allows automated or semi-automated production of results. The ISHAGE protocol is the most reliable method currently available to quantitate accurately this important subset of cells. ProCount is validated for fresh (non-cryopreserved) apheresis and peripheral blood samples only; it does not include a viability reagent. This kit comes with specific software designed for BD Biosciences cytometers that uses a Boolean strategy for data analyses, and allows automated production of results. The two kits produce well-correlated results. The major limitations of ProCount are its restrictive use for fresh apheresis and blood samples, and a short stability (few weeks) of the anti-CD45–PerCP antibody. BD Biosciences recently commercialized the SCE™ (stem cell enumeration) kit that addresses these limitations by integrating an antibody combination (CD45–FITC, CD34–PE), a viability dye 7-amino-actinomycin-D (7-AAD) and an NH_4–Cl lysis reagent. The kit also includes Trucount™ tubes, containing known numbers of microbeads; this avoids bead pipetting, which decreases test precision. Because of these potential improvements, the SCE kit could be used to enumerate CD34+ cells in all

HSC [peripheral blood, apheresis, bone marrow (BM) and cord blood (CB)], fresh and thawed [27].

The apoptosis in HSCT

Before the hematopoietic stem cells reinfusion, the QCs consist of a total nucleated cell count, the viability assessment of both CD45+/7-AAD cell population and CD45+/CD34+/7-AAD subpopulation through flow cytometry, and the evaluation of proliferative capacity. The clonogenic assays consist of a 14-day incubation at 37 °C and CFU dose infused to the patient is one of the best markers of graft outcome. Therefore, it is necessary to perform the QCs at least 14 days before the transplantation to obtain a complete evaluation of the cryopreserved HPC unit. It should be important to have a rapid and efficient test in association with flow cytometry as a satisfactory QC of the HPC units before the reinfusion to the patient to have preliminary indications that allow clinicians to proceed with the transplantation before the clonogenic tests results [28]. For this purpose, in a paper Scerpa and colleagues described the use of the new instrument NucleoCounter NC-3000. The NucleoCounter NC-3000 enables automated cell counting and analyses of a wide number of samples to be performed at the same time; it is easy to use and also guarantees an excellent precision reducing the data variability due to the operator's work. Other tests used in the clinical routine, such as the determination of cell viability with trypan blue, are characterized by a huge variability of the results. In particular, two different NucleoCounter NC-3000 protocols, both related to the evaluation of cell functionality, have been used in the study of Scerpa et al. [28]. The quantification and detection of apoptotic cells was determined by the protocol "mitochondrial potential assay," which correlates the loss of the mitochondrial membrane potential and the early stage of apoptosis and chemical hypoxia-induced necrosis. Instead, the protocol "vitality assay: analysis of the level of cellular thiols" was applied to evaluate the detection of changes in the cellular level of reduced thiols directly related to apoptosis [29, 30].

Microbial contamination of hematopoietic stem cells products

Microbial safety of the hematopoietic progenitor cell (HPC) product is an important issue for successful HCT and is regarded as a quality marker of good medical practice. HSCT involve many different steps including harvesting of bone marrow or peripheral blood progenitor cells, processing for cryopreservation, freezing, thawing and finally infusion [31]. Despite using sterile precautions all of these steps are prone to contamination. The incidence of microbial contamination of stem cell products have been reported as 0.2–26.3% [32, 33]. Such a wide range of reported contamination rates may be related to different stem cell sources of studies, time dependent improvement of collection and processing systems, variations in harvesting and processing protocols of centers, and experience of apheresis teams. In addition, frequency of using central venous catheters (CVC) for HPC collection probably influence the rate of microbial contamination of products, as infections associated with CVCs are important factors for HPC contamination [34, 35].

The sterility testing process of hematopoietic stem cells

To decrease the risk of serious transplantation-transmitted infections, the current good tissue practices recommends avoiding the processing of stem cells from donors with positive cultures for pathogenic or enteric bacteria unless a final sterilization step is expected during product processing. In some hospitals, it is accepted that contaminated HSC products be transplanted if there are no other options for patient treatment. In these cases, an antibiotic therapy is applied based on the antimicrobial susceptibility of the contaminant microorganism [36, 37]. As required by cellular therapy accrediting organizations such as AABB and the Foundation for Accreditation of Cellular Therapy (FACT), cell therapy products must be tested for microbial contamination. In addition, as required by the Food and Drug Administration (FDA) Code of Federal Regulations, a validated testing method such as biologic sterility test immersion or membrane filtration must be used by the testing laboratory. Although automated culture systems are not FDA approved for sterility testing of human cellular therapy products or cellular-based products, both BacT/ALERT 3D (bioMérieux, Durham, NC) and Bactec 9240 (Becton Dickinson Franklin Lakes, NJ) systems are widely used [38, 39]. According to Khuu and coworkers, these two automated systems are more sensitive and specific and faster in detecting microbial contamination in cell therapy products than the Code of Federal Regulations methods [40]. It should be highlighted that PBPCPs should be processed in clean areas with air locks for personnel and equipment to minimize the risk of bacterial contamination during processing. This includes the particulate and microbiologic monitoring during various grades in operation as well as the control of surfaces and personnel after critical operations [41].

Conclusion

Hematopoietic stem cell transplantation is routinely used for the treatment of numerous oncohematologic malignancies. The stem cells collected by apheresis undergo minimal manipulation procedures such as volume reduction and cryopreservation [42, 43]. In particular, the cryopreservation and thawing procedures represent the crucial point of the whole process and may also affect the viability of hematopoietic stem cells contained in the HPC units. For this reason it is necessary to perform accurate QCs before the hematopoietic stem cells reinfusion [44, 45].

Authors' contributions/Wkład autorów

FZ – study design. ND – data collection and interpretation, statistical analysis, manuscript preparation, literature search.

Conflict of interest/Konflikt interesu

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None declared.

Ethics/Etyka

The work described in this article has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans; EU Directive 2010/63/EU for animal experiments; Uniform Requirements for manuscripts submitted to Biomedical journals.

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