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Utility of routine evaluation of sterility of cellular therapy products with or without extensive manipulation: Best practices and clinical significance

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Abstract

Background. We analyzed the results of routine sterility testing performed in our center over the last 10 years, in the context both hematopoietic stem cell transplantation (HSCT) and Advanced Therapeutic Medicinal Products (ATMPs). *Methods.* For sterility tests 14-day cultures were performed in culture media detecting aerobic and anaerobic microorganisms. *Results.* In this study, 22/1643 (1.3%) of apheretic products for autologous or allogeneic HSCT were contaminated, whereas 14/73 bone marrow (BM) harvests (17.8%) were positive. In 22 cases, the contaminated HSCs were infused to patients, but there was no evidence of any adverse impact of contamination on the hematologic engraftment or on infections. Indeed none of the five positive hemocultures detected in patients following infusion could be linked to the contaminated stem cell product. Our Cell Factory also generated 286 ATMPs in good manufacturing practice (GMP) conditions since 2007 and all final products were sterile. In three cases of mesenchymal stromal cell expansions, the starting BM harvests were contaminated, but the cell products at the end of expansion were sterile, presumably thanks to the presence of an antibiotic in the culture medium. *Discussion.* The decreased rate of contamination of cell harvests observed with time suggests that routine sterility testing and communication of the results to the collecting centers may improve clinical practices. Furthermore, we recommend the use of antibiotics in the medium for ATMP expansion, to decrease the likelihood of expanding microorganisms within clean rooms. Finally we discuss the costs of sterility testing of ATMPs by GMP-approved external laboratories.

Key Words: *advanced therapeutic medicinal products, cost analysis, hematopoietic stem cell transplantation, microbial contamination*

Introduction

The current Joint Accreditation Committee (JACIE) standards require sterility tests to be routinely performed on unmanipulated or minimally manipulated cell products used in hematopoietic stem cell transplantation (HSCT). In the HSCT setting, contamination may result from inadequate skin antisepsis of donors, especially when the collection of hematopoietic stem cells (HSCs) is performed from the bone marrow (BM), or from contamination of patient's and normal donor's peripheral venous access in the case of apheretic procedures. In addition, possible contaminations can be derived from inadequate procedures during stem cell product harvesting or manipulation (bag transfers, purifications and red blood cell reduction procedures, etc.).

The Haematology and Bone Marrow Transplant (BMT) Unit at the Papa Giovanni XXIII Hospital in Bergamo performs more than 60 autologous and 50 allogeneic HSCT procedures each year. The clinical relevance of microbial contamination in the HSCT setting is still controversial because microbial contamination of stem cell products may potentially impact the quality of the graft, resulting in life-threatening infections in patients and/or poor engraftment. Several reports suggest that immediate adverse reactions or infectious complications in the host are rarely related to the contaminated graft [1–4], especially with optimized antibiotics prophylaxis. However, cases of

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infections in recipients as well as neutropenic fever possibly due to contaminated grafts have been reported [1,5–8]. The possible effects of contamination on engraftment have not always been investigated, but most reports suggest that infusion of contaminated HSCs does not affect engraftment [3,8,9].

The Haematology Unit also coordinates several mono- or multicentric clinical trials involving extensively manipulated Advanced Therapeutic Medicinal Products (ATMPs). These are produced according to European Good Manufacturing Practice (EU GMP) guidelines by the local Cell Factory (Center of Cellular Therapy "G. Lanzani"), which has been regularly approved by the national authorities (Agenzia Italiana del Farmaco [AIFA]) since 2008. Indeed the Centre of Cellular Therapy "G. Lanzani" acts as a processing laboratory for HSCs as well as a Cell Factory for ATMP production.

Sterility for both HSCs and ATMPs is performed in our center by an automated and validated 14-day microbial detection system that uses media specific for aerobic and anaerobic microorganisms (Bact/Alert), in compliance with European Pharmacopoeia (Ph.Eur.) guidelines.

We, therefore, analyse here the overall impact in our center of sterility testing on collection and processing practices for HSCT and define whether the infusion of a contaminated product has any potential impact on transplant outcome. Furthermore, we report our analytical results on the rate of microbial contamination of the ATMPs that were generated by the Cell Factory since 2007. Finally a cost analysis is presented.

Materials and methods

Microbiological cultures

All cellular products that were processed by the Centre of Cellular Therapy "G. Lanzani" in the context of the BMT program of the Haematology Unit or collected by the BM collection facility were tested for sterility. Briefly, ≥1% of the cell product (0.5–4 mL) was collected with a syringe under a sterile laminar flow cabinet, diluted in 10 mL final volume of the autologous or compatible plasma used for freezing and inoculated in a set of two bottles (BacT/Alert Aerobic FA Plus and Anaerobic FN Plus, which detect aerobic and anaerobic bacteria and, bioMérieux). The bottles were then sent at room temperature to the Microbiology & Virology Laboratory (M&V Laboratory) for a 14-day incubation in a BacT/Alert 3D instrument (bioMérieux) at 35 ± 2°C.

ATMPs were generated in flasks, as described previously [10–14], and were tested for sterility in the same manner as HSCs by M&V Laboratory up to 2014. The method was fully validated, as required by the Ph.Eur. Validation included testing, in duplicates, the growth of 10–100 colony forming units of control strains in presence or absence of the test material (both final cell products were resuspended in autologous plasma and final cell culture supernatant, which are the materials routinely used for the sterility test). Control strains used for validation were those requested in chapter 2.6.27 of Ph.Eur., i.e., *Staphylococcus aureus* (ATCC6538), *Bacillus subtilis* (ATCC6633), *Pseudomonas aeruginosa* (ATCC9027), *Candida albicans* (ATCC10231), *Aspergillus brasiliensis* (*Aspergillus niger* complex) (ATCC16404), *Streptococcus pyogenes* (UK NEQAS no. 3170), *Yersinia enterolitica* (UK NEQAS no. 3125) for aerobic medium and *Clostridium sporogenes* (ATCC19404), *Bacteroides fragilis* (ATCC25285) and *Propionibacterium acnes* (local isolate, sequenced) for anaerobic medium. All strains used in the validation grew within 7 days but *P acnes* usually required more than 7 and up to 14 days to be detected (data not shown). From 2014, the sterility testing for ATMPs has been carried out by a GMP-approved laboratory (Eurofins Biolab srl, Vimodrone, Italy). Inoculation of the same BacT/Alert culture bottles were performed by the Cell Factory operators and then sent to Eurofins Biolab for incubation, detection and reporting. The incubation conditions and time were the same as those that had been performed by M&V Laboratory up to that time. However, an additional control strain was added during the validation of the method transfer to this external laboratory, i.e. *Corynebacterium jeikeium* (local isolate, sequenced).

Strain identification and chemosensitivity

Before 2012, microbial identification was performed in the M&V Laboratory using Gram staining characteristics, and biochemical tests using a VITEK (bioMérieux) analyser (card ID GN, ID GP, ID ANC and ID YST). From 2012, the matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF) has been applied to identify bacteria, using the instrument (VITEK MS, bioMérieux). Procedures were performed according to the manufacturer's technical instructions.

Chemosensitivity testing was performed for all identified strains, except *P acnes,* because this strains is considered nonpathogenic. Tests were performed using an agar diffusion technique, performed according to Clinical Laboratory Standard International (CLSI) guidelines till 2010, then with the European Committee on Antimicrobial Susceptibility Testing (EUCAST) rules, if available. Disk diffusion lectures and data registrations were done using the SirSCAN 2000 automatic system (i2a Diagnostics) and confirmed using broth dilution technique using a VITEK (bioMérieux) analyser (card AST N201, AST P586, AST ST01 and AST P632).