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Interferon-gamma in mobilized stem cells: A possible prognostic marker in early post-transplant management in multiple myeloma

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ABSTRACT

Introduction: A complex network of cytokines in the bone marrow microenvironment has been implicated as an important factor in the pathogenesis of multiple myeloma (MM). Different cytokines have been studied in MM, both in peripheral blood and/or bone marrow, but there are few data correlating cytokines in leukapheresis product with post-transplant response depth to treatment.

Materials and Methods: In a retrospective cross-sectional study, levels of tumor necrosis factor alpha (TNF-α), transforming growth factor beta-1 (TGF-β1) and interferon gamma (IFN-γ) in peripheral hematopoietic stem cells/leukapheresis product (PHSC) of patients with MM eligible for transplantation were evaluated. Association of these cytokines with certain factors such as mobilized CD34 + cells/kg, staging, response to treatment and outcome were analyzed.

Results: The median baseline IFN-γ level was 826.4 pg/mL. IFN-γ levels in the leukapheresis product were significantly lower in patients who achieved complete response (CR) three months post-transplant when compared to patients with very good partial response (VGPR) (674.75 ± 80.32 pg/mL versus 939.6 ± 106.8 pg/mL, $p = 0.02$), respectively. Patients who lost depth of response at the third-month post-transplant had a median level of IFN-γ 1133, being considered "high-expressors" of IFN-γ, while those reaching improved response were called "low-expressors" (median level IFN-γ 485 pg/mL). Overall and progression–free survival did not have a statistically significant correlation with TNF-α, TGF-β1 or IFN-γ, as well as TNF-α and TGF-β1 levels in post-transplant response assessment.

Conclusion: IFN-γ in PHSC seems to be an important biomarker of loss of response in MM, suggesting a role in early post-transplant therapeutic management.

1. Introduction

Multiple myeloma (MM) is a malignant hematologic malignancy that is widely studied, but it is still an incurable disease. The heterogeneity of multiple myeloma is reflected not only in the outcome, ranging from a few months to more than 10 years, but also in the understanding of its pathogenesis [1]. In the last two decades, several studies have been done to elucidate the biology of MM and the standardization of diagnostic criteria and response to treatment, as well as the identification of biomarkers and prognostic factors. Thus, there has been a substantial impact on the treatment of MM with an improvement of duration and quality of response with current therapeutic strategies [2]. MM treatment for patients eligible for transplantation consists of induction chemotherapy followed by mobilization, stem cell collection (if stable or responsive disease), high-dose chemotherapy with autologous transplantation, and finally consolidation or post-transplant maintenance.

Several biomarkers and tools are used to determine the risk stratification of patients with multiple myeloma. Among them are the International Staging System (ISS) algorithm based on β2-microglobulin and albumin levels, and the Revised International Staging System (R-ISS), which recommends the combination of ISS with serum lactate dehydrogenase (LDH) and fluorescence *in situ* hybridization at the interphase (iFISH) [3–5]. High levels of β2-microglobulin reflect the

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presence of a large tumor mass and alteration in renal function, low levels of albumin result from the secretion of inflammatory cytokines in the bone marrow microenvironment, and the increase in LDH denotes disease aggressiveness, suggesting high proliferation index and/or the presence of tumor mass (extrabone and/or extramedullary) [6,7]. FISH detects the presence of chromosomal abnormalities which are also elements that define biological characteristics in multiple myeloma, stratifying into low (standard) or high-risk patients [8–10].

The bone marrow microenvironment has been a focus of research in understanding the pathogenesis of multiple myeloma: malignant clonal evolution, progression and survival of the disease, as well as treatment failure. The medullary niche is a primary modulator, capable of allowing infiltration, growth, proliferation, adhesion and cell migration, mediated by inflammatory factors (cytokines, chemokines, adipokines and growth factors) and endocrine signaling pathways. Therefore, the complex interaction in the bone marrow microenvironment has great importance in the growth of malignant cells (plasmocytes) and the cytotoxicity of healthy cells, as well as the resistance to drugs used in the treatment of myeloma [2,11–15].

Several studies have been described involving cytokines in both peripheral blood and bone marrow of MM patients. Among the cytokines studied, tumor necrosis factor-alpha (TNF-α), transforming-beta1 (TGF-β1) and interferon-gamma (IFN-γ) are cytokines highly relevant in tumors, closely involved in the interaction between plasma cells and the bone marrow microenvironment and related to the clinicopathological characteristics and outcome of MM [16–20].

Therefore, our study aimed to characterize the cytokine profile of TNF-α, TGF-β1 and IFN-γ in the leukapheresis product (peripheral stem cells) of patients with transplant-eligible MM. Thus, these cytokines could be identified as possible prognostic factors and associated with clinical impact after TACTH, not only in survival outcomes, but also in response depth to treatment, allowing early post-transplantation management as consolidation and/or maintenance.

2. Materials and methods

2.1. Study design and sampling

Participant sample collection was authorized by signing of the Free and Informed Consent Terms for Collection and Transplantation of Peripheral Blood Stem Cells, a documentation required by the National Transplant System and conducted according to the Declaration of Helsinki (2008). Plataforma Brasil approved this project under the number 1.389.996, on January 18th of 2016, and it was also approved by the Institutional Ethics Committee on Human Research. It is available for consultation at Plataforma Brasil under the number CAAE 50070515.0.0000.0107.

This was a retrospective cross-sectional study with all multiple myeloma patients submitted to autologous hematopoietic stem cell transplantation (N = 63) from September 2010 to September 2014 and February 2015 to August 2017, at the Bone Marrow Transplantation Unit (BMT) of the University Hospital of Londrina/State University of Londrina. Patients were referred by oncohaematologists from Londrina and North Macroregion of Paraná State-Brazil for evaluation of eligibility for transplantation. Diagnosis, treatment and response evaluation (pre-HSCT) were performed according to the criteria proposed by the International Myeloma Working Group (IMWG). Of these 63 patients, 42 fulfilled the inclusion criteria, which were mobilization with granulocyte colony stimulating factor (G-CSF) exclusively and being submitted to high-volume leukapheresis (six processed total blood volumes). Among the excluded patients, two had transplant-related mortality (< 100 days of the procedure), due to infection in D + 32, D + 43 and D + 39, nine patients underwent leukapheresis of normal volume (≤3 volemias) and nine patients were mobilized with different regimens (granulocyte colony-stimulating factor – G-CSF with the addition of chemotherapy or Plerixafor™) (Fig. 1).

Although patients received different induction treatment regimens for multiple myeloma, all patients were submitted to mobilization and peripheral stem cell harvesting with the same clinical protocol. They received 10 μg/kg/day of Filgrastim (G-CSF) twice a day, subcutaneous, for five consecutive days for mobilization. On the fourth day (D4) of the mobilization process, peripheral $CD34^+$ cells were counted by flow cytometry (FacsCanto™II Cytometer, BD Biosciences) aiming at least $CD34^+ > 10$ μg/mL. Leukapheresis was performed from the fifth day of mobilization, harvesting at least 2×10^6 $CD34^+$ cells/kg for transplantation (1 or 2 procedures), with a CobeSpectra/TerumoBCT apheresis machine, processing six volemias per day, through an 11 French double lumen central venous catheter. Anticoagulants used during the procedure were anticoagulant citrate dextrose solution-(ACD-A) and heparin, according to the institutional protocol for large volume collection. At the end of apheresis, the stem cell product was sent to the Laboratory of Cryopreservation of the University Hospital of Londrina for processing and storage until the time of transplantation. CTHP processing for autologous transplant involved several steps. First, the leukoapheresis product was weighed and stored in a refrigerator (2–6 °C) until total nucleated cell count (TNC-total leukocytes) and $CD34^+$ cell count were determined. After cell results, calculation of the number of TNC/kg ($\times 10^8$) and of $CD34^+$ cells/kg ($\times 10^6$), cell concentration (approximately 2.5–3.0% TNC/kg per bag) and number of bags to be cryopreserved were determined. Leukapheresis product (50 mL cells + 40 mL autologous plasma + 10 mL dimethylsulfoxide 10% (DMSO cryoprotective solution) without buffer solution or human albumin) resulted in a final volume of 100 mL in each bag, which was stored at – 86 °C (Indrel freezer) until the transplant. Cell viability test by Trypan blue staining method was performed prior and post DMSO addition and immediately after thawing at the time of transplant. Although there was a difference in viability at the three times of evaluation, this difference was not reflected in post-transplant hematologic recovery time. Besides cell viability, microbiological analysis was also performed for leukapheresis product quality control before cryopreservation and on the day of transplantation.

2.2. Cytokine level assays

An aliquot containing 50 μL of cryopreserved stem cells plus plasma and DMSO, under the same conditions as stem cells for transplantation, was obtained from the last cryopreservation bag of the first day apheresis product and stored in a vial. TNF-α, TGF-β1 and IFN-γ levels were determined at two different times (December 2016, N = 27 and January 2018, N = 15), in a duplicate sample, immediately after sample thawing by enzyme-linked immunosorbent assay – ELISA according to the manufacturer's conditions (e-Biosciences, USA) and quantified in a Victor Multilabel Plate Reader (Perkin Elmer, Waltham, MA, USA). Patients were classified into groups, according to the median basal level of IFN-γ as “high or low-expressors”.

2.3. Study population

Relevant information on the diagnosis of MM was obtained according to IMWĠs consensus and ISS risk factors for disease. The heterogeneity of the treatment of MM induction was due to the fact that the patients were treated at different reference centers, with different numbers of induction cycles and lines of therapies, according to the best therapeutic response obtained. Response criteria assessment was classified as complete response, very good partial response and partial response, based on serum and/or urinary monoclonal component by protein electrophoresis and immunofixation, reduction of plasmocytomas and percentage of plasma cells in the bone marrow. Complete stringent response was not reported, since no free light chain or minimal residual disease by flow cytometry were determined. There was limited cytogenetic analysis, free light chain screening and minimal residual disease by flow cytometry due to socioeconomic unavailability.