Sistema Socio Sanitario

Fondazione IRCCS Ca' Granda
Ospedale Maggiore Policlinico

Regione Lombardia

Dipartimento Area dei Servizi
SC Medicina Trasfusionale

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Title:

Biological variables affecting CD34+ peripheral cells collection efficiency using the Spectra Optia continuous mononuclear cell collection system: hematocrit as determinant for CD34+ Collection Efficency.

Acronym: CD34+CE_2023

Promotor: Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Via Sforza 28, 20122 Milano, Italia

Coordinating Centre: S.C. Medicina Trasfusionale
Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico,
Via Sforza, 35, 20122 Milano, Italia

Principal Investigator: Maria Cristina Mocellin

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Type of study: non interventional study

Version Number: v.1.0

Date: 10/07/2023

DECLARATION OF CONFIDENTIALITY

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1. Index

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2. Abbreviations

HSCT: Hematopoietic stem cell transplantation

PBCS: Peripheral blood stem cells

LP: Leukapheresis

G-CSF: Granulocyte-colony stimulating factor

MNC: Mono-nuclear cells collection

cMNC: Continuos Mono-nuclear cells collection

RBC: Red blood cells

CE: Collection Efficiency

PBV: Processed blood volume

Ht: Hematocrit

WBC: White blood cells

PLTs: Platelets

Hb: Hemoglobin

3. Responsibilities

The Study Promoter is the Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico.

The Coordinating center is the S.C. Medicina Trasfusionale (Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico).

The role of **Principal Investigator (PI)** will be held by Dott.ssa Maria Cristina Mocellin, M.D at S.C. Medicina Trasfusionale.

The PI will be responsible for ideation and realization of the study design, adequacy and consistency of collected data, supervision of data analysis.

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Internal Collaborations

Unit	***Participant Name***	***Role and functions in the study***
SC Medicina Trasfusionale	Giacomo Aquilino	Data collection, bibliography research, article writing, data analysis.
SC Medicina Trasfusionale	Sara Alberti	Data collection, bibliography research, article writing, data analysis.
SC Medicina Trasfusionale	Cristiana Bianco	Statistical analysis
SC Medicina Trasfusionale	Luca Valenti	Supervising study design, Statistical analysis
SC Medicina Trasfusionale	Maddalena Loredana Zighetti	Bibliography supervising, writing supervising, data analysis
SC Medicina Trasfusionale	Daniele Prati	Data analysis

4. Amendments

NA

5. Timelines

Start of data collection	01/09/2023
End of data collection	01/11/2023
Final Report of the study	01/12/2023

6. Background and Rationale

Hematopoietic stem cell transplantation (HSCT), either autologous or allogenic, is a treatment strategy widely used in the onco-hematology field and less often for other diseases[1]. The most employed source of stem cell is peripheral blood stem cell (PBSC) identified as circulating CD34+ cells and collected by leukapheresis (LP) following mobilization process using granulocyte-colony stimulating factor (G-CSF) alone or in association with chemotherapy and/or plerixafor, the latter in case of poor-mobilizer patients[2].

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The PBSC harvest needs dedicated platform for being properly performed: in our institution the Spectra Optia apheresis system (Terumo BCT) is routinely employed with a continuous mononuclear cells collection (cMNC) system. Spectra Optia (Terumo BCT) is designed to optimize PBSC harvest through automated buffy coat interface identification and management, low volume tubing set, low extracorporeal volumes and support of cMNC processing system[3]. In the dual-step mononuclear cells collection (MNC) system an intermediate chamber is used to collect white blood cells while returning the majority of platelets to the subject. Then optical sensors detect red blood cells (RBC) overflow and leucocytes are intermittently flushed in the collection bag. The cMNC system differs from the former dual-step system by eliminating the intermediate collection chamber used and thus allowing continuous collection of leucocytes from the buffy coat. This approach appears to be less time consuming, more manageable, and more efficient in returning platelet and RBC to the subject[4].

One of the main benchmarks of LP quality is the collection efficiency (CE) defined as the ratio between total CD34+ cells yield in apheresis and total CD34+ cells in the processed blood volume (PBV)[5]. The following formula is currently use:

$$CE\ CD34{+}{=}\ \frac{\text{CollectedCD34} + (\text{cells}\text{x}10^4)}{\left\{\frac{[\text{preLP CD34} + (\text{cells/mcL}) + \text{postLP CD34} + (\text{cells/mcL})]}{2}\ \text{x PBV}\right\}}$$

Obtaining the highest possible CE for every LP procedure is critical as long as several studies have shown the importance of targeted CD34+ cells yield in order to assure a safe and rapid haematological recovery after the high dose chemotherapy regimens used in HSCT[6]. Therefore the objective is to reach the target PBSC yield in the safest and quickest way possible: an high CE allows to reduce the PBV and the procedure length.

The CE values are affected by different factors among which blood sample's physical properties. Such factors have been widely studied in MNC protocol[7], but less studies concern the cMNC protocol[8]. In 147 consecutive apheresis procedures performed both on patients and healthy donors with Spectra Optia (Terumo BCT) with cMNC system, Kondo et colleagues found a moderate positive correlation between CD34+ CE and hematocrit (Ht) and only a very week correlation with white blood cells (WBC) and platelet counts. We would like to confirm these data in a setting of subjects with a predominance of patients over healthy donors, thus evaluating the influence of clinical and biochemical factors on CE for autologous and allogenic hematopoietic stem cell production. Finally we would like to assess a possible pre-LP Ht threshold associated with a sufficient (> 60%) CD34+ CE[9].

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7. Objectives of the study

7.1 Primary objective

In this study we will evaluate the impact of clinical and biochemical factors on Collection Efficiency (CE) for autologous and allogenic hematopoietic stem cell production using the cMNC system.

7.2 Secondary objectives

- Identify clinical and/or biochemical factors associated with an optimal (≥60%) CD34+ CE

- Confirm data, from the published literature, regarding the correlation between Ht and CD34+ CE in a cohort of subjects largely composed by hematological patients.

- Develop a prediction model for optimal (≥60%) CD34+ CE using pre-LP clinical and/or biochemical factors.

8. Methods

8.1 Study Design

This is an observational, retrospective, non-pharmacological, single-center, no profit study conducted on cohort of patients and stem cell donors referred to the Transfusional Medicine Department of IRCCS Ca' Granda Ospedale Maggiore Policlinico in Milan for PBSC harvesting.

8.1.1 Primary Endpoint

Impact, measured by statistical correlation, of clinical (age, sex, type of donor, diagnosis, recent RBC transfusion) and biochemical (pre-LP values of Ht, Hb, WBC and PLT) variables on CD34+ CE.

8.1.2 Secondary Endpoints

- Correlation of clinical (age, sex, type of donor, diagnosis, recent RBC transfusion) and biochemical variables (pre-LP values of Ht, Hb, WBC and PLT) with an adequate (≥60%) CD34+ CE.
- Correlation of pre-LP Ht levels and recent (≤ 60 days) RBC transfusion with CD34+ CE, especially in the autologous setting.
- AUROC (Area Under the ROC Curve) of a pre-LP model based on clinical parameters aimed at predicting an adequate (≥60%) CD34+ CE.

8.2 Setting

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Data will be collected from hospital official documents record (Order Entry, Amb Web, Concerto, Emonet), considering patients referred to the Apheresis Unit (SC Medicina Trasfusionale) for two years' time (from 1/1/2021 to 31/12/2022). All patients are referred to Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico in Milan, Italy. Data will be pseudonymized: at enrollment each participant will be assigned a unique code so that only local investigators will be able to identify the subject.

8.2.1 Study Population

We will consider consecutive patients and donors referred to the Apheresis Unit within the Transfusional Medicine Department of Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico in Milan (from 1/1/2021 to 31/12/2022) for PBSC collection. Total study population: 113 subjects of which 74 hematological/non hematological patients and 39 stem cell donors.

Patients are defined as subjects affected by a hematological or non-hematological disease for which an autologous stem cells transplantation is recommended. These cases are referred as autologous use of stem cells.

Stem cells donors are volunteer healthy subjects who have decided to undergo the procedures needed to collect blood stem cells. These subjects can donate their blood stem cells in favor of a blood relative or, through subscription of a specific registry (IBMDR – International Bone Marrow Donor Registry), to an unknown subject. These cases are referred as allogenic use of stem cells.

8.2.2. Inclusion Criteria

Subjects must meet all the following inclusion criteria:

Patients and stem cell donors:

- Patients and stem cell donors who have been evaluated as suitable for PBSC apheresis and collection, either for autologous or allogenic use
- Patients and stem cell donors that have completed at least one CD34+ apheresis procedure at Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico in Milan.
- Age ≥16 years

8.2.3 Exclusion Criteria

- Patients affected by Acute Lymphoblastic Leukemia.

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8.2.4 Description of study subject

All procedures performed during the study will comply with current clinical practice, international and national guidelines. Main object of the study is the PBSC apheresis procedure performed by using the continuous mononuclear cells collection (cMNC) system with Spectra Optia (Terumo BCT), specifically the biological and clinical factors affecting the procedure efficiency.

8.2.5 Programmed visits and evaluations

Given the retrospective nature of the study, the visits and evaluations already took place between January 2021 and December 2022 during normal clinical activities and as required by practice for the management of patients in this clinical situation.

Patients and stem cell donors were evaluated as suitable for apheresis procedures with out-patient visits at Apheresis Unit within the Transfusional Medicine Department.
Specifically, to exclude any significant comorbidity, all stem cell donors have undergone the following evaluations:
- Record of complete familiar and personal anamnesis, patient's demographical parameters
- Medical examination
- Blood exams including: complete blood count and leukocyte formula, renal and liver function, coagulation tests, serology (HIV, HBV, HCV, syphilis et al), molecular biology (HIV, HBV and HCV), blood group, electrolytes, protein electrophoresis, iron status, vitamin B12, folic acid, lymphocyte subpopulations.
- Chest X-ray
- Full abdomen ultrasound
- Color-doppler echocardiography
- Specialist cardiologic evaluation comprehensive of ECG.
- In case of PBSC donors: specialistic anaestesiologic evaluation.

Patients, already been extensively evaluated by the reference specialists for all significant comorbidities, have undergone the following evaluations:
- Medical examination and concomitant evaluation of vascular access adequacy.
- Blood exams including: complete blood count and leukocyte formula, renal and liver function, coagulation tests, electrolytes.

8.3 Variables

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The following variables will be collected:
- Clinical parameters: age (years), gender (male or female), body weight (kg), type of PBSC use (autologous or allogenic), date of apheresis procedure, type of venous access (peripheral veins or central venous access), total hematic volume (liters), type of mobilization (chemotherapy, G-CSF, Plerixafor), diagnosis (only for autologous setting: multiple myeloma, non-hodgking lymphoma, non haematological disease), RBC transfusion within 60 days before apheresis (yes or not).
- Biochemical parameters: complete blood count pre-LP (cell/mcL), complete blood count on apheresis product (cell/mcL), CD34+ count pre-LP, post-LP and in apheresis product (cell/mcL; cellx106 and cellx106/kg).
- Procedures parameters: total blood volume processed (L), total apheresis product volume (mL)

8.4 Documents source

All data will be collected from official hospital records, particularly AmbWeb, and stored in an ad hoc file. Patient's identity will be managed through an alphanumerical code.

8.5 Sample Size

Total study population: 113 subjects of which 74 hematological/non hematological patients and 39 stem cell donors.

The number of subjects evaluated is determined by the clinical activity of the Apheresis Unit (SC Medicina Trasfusionale) and has been considered adequate considering the number of subjects included in other similar study present in the literature[7,8]. Due to descriptive nature of the analysis planned, no pre-analysis sample size was determined.

8.6 Data Management

Data will be collected in ad hoc Excel file. To guarantee data protection, patients will be indicated with a unique pseudonymized code. Each process to promote data quality will be guaranteed. Data insertion in the database will be performed in a correct and accurate way according to the source documentation. To ensure adequate control over the quality of the study, the investigator will allow, if required from the regulatory authorities, direct access to all relevant documentation and devote part of his time to discuss the study results.

All the information collected in our database will be treated in accordance with the provisions of the European Privacy Regulation (General Data Protection Regulation UE 2016/679) and Italian legislation.

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8.7 Statistical Analysis

For descriptive analyses, quantitative variables will be expressed as mean and standard deviation or as median, interquartile range (IQR) and minimum-maximum range, while categorical variables will be expressed as absolute and relative frequencies.

Aiming to evaluate the impact of clinical and biochemical variables on CD34+ CE, we will examine the association between CD34+ CE and age, sex, type of donor (patients vs healthy donors), hematocrit, hemoglobin level, WBC counts, platelets count.

Analysis will be performed by fitting data in generalized linear models for continuous variables and logistic regression for binary variables. In order to avoid overfitting, the selection of variables to be included in multivariables models will be performed by Backward stepwise regression. All models will be adjusted for significant clinical confounders.

Statistica analysis will be performed using the JMP Pro 16.0 Statistical Analysis Software (SAS Institute, Cary, NC). P values <0.05 (two tailed) were considered statistically significant.

8.7.1 Primary Endpoint: analysis

Influence of biological variables on CD34+ CE will be evaluated using a stepwise backward regression analysis; correlation intensity will be assessed using generalized linear model of regression and strength of correlation will be determined by estimate and standard error value.

8.7.2 Secondary Endopoint: Analysis

The association of biological variables with adequate CD34+ CE will be evaluated using linear logistic model of regression and strength of correlation was assessed evaluating Odd Ratio values. Accuracy of clinical prediction model for optimal CD34+ CE will be evaluated by estimating AUROC.

8.7.3 Ad interim analysis

NA.

8.7.4 Supplemental analysis

NA

8.8 Quality Control

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Each procedure proposed to the patients and performed will strictly adhere to current clinical practice and recommendations. All data attain current clinical practice.

As promoter, Fondazione IRCCS Ca' Granda, Ospedale Maggiore Policlinico could apply a quality control to the study. In this case, the PI must allow the monitor to verify directly to all the study documents and to spend a portion of his/her time and staff to discuss the monitoring results and other aspects of the study. Moreover, the Regulatory Authorities can perform inspections. In this case, the PI must allow the authorities to directly verify all the documents related to the study and spend a portion of his/her time and staff to allow the inspector to discuss the monitoring and eventually other aspects of the study

8.9 Study Limits

We will try to correct two possible bias within our cohorts:
- by excluding patients affected by Acute Lymphobastic Leukemia who underwent apheresis procedure in order to collect a salvage CD34+ autologous backup. Those patients are characterized by abnormally high pre-LP CD34+ counts due to disease immunophenotype (often CD34+) and low bone marrow disease burden. Moreover the practice of collecting an autologous CD34+ backup in case of allogenic engraftment failure or disease relapse, although not detrimental for patients, it's not supported by strong practicing evidence.
- By considering only the first apheresis procedure for each patients in order to avoid any possible selective bias.

The main limits of this study could be:
- the small cohort dimension which do not allow significant evaluations about influence of underlying disease, mobilization regimens and poor mobilizing condition.
- the retrospective nature of data which do not allow to clearly assess influence and utility of RBC transfusion pre-LP.

8.10 Other aspects

NA

9 Subjects protection

This study will be conducted by Good Clinical Practice (GCP) rules; in accordance with the ethical principles that have their origin in the Declaration of Helsinki and with the respect to the European clinical practice, in compliance with all international guidelines and national law regulation in Italy.

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The protocol must be submitted to the Independent Ethics Committee (IEC) for review and will receive IEC approval/favourable opinion before initiation of the study. During the study, any amendments to the protocol must also be approved by IEC.

9.1 Informative note to the subject and consent form for processing of personal data

Being the Policlinico Institute , promoter of the study, an IRCCS (Istituto di Ricovero e Cura a Carattere Scientifico- health and research institute), in compliance with current legislation on the protection and protection of personal data (EU Reg. 679/2016) and Legislative Decree 196/2003 (Code of Privacy) as amended by Legislative Decree 101/2018, for the data collected and processed by the Promoter and by the other participating centers having the same IRCCS nature, it will not be mandatory to ask the study participants for consent to the use of their personal data for the conduct of the study itself.

In compliance with the provisions of art. 110 bis paragraph IV of the amended Legislative Decree 196/2003, due to the instrumental nature that the health care activity assumes with respect to research at Scientific Hospitalization and Treatment Institutes, the treatment, for research purposes, of personal data already collected during standard clinical activity, including the anonymization of the data itself, does not in fact constitute further processing.

9.2 Insurance

Due to the observational nature of the study no additional insurance is due over those already used for normal clinical practice.

10. Publication

Communications, reports, and publication of the results of the study will be under the responsibility of the principal investigator of the study.

11 Financial funding

NA

12. Bibliography

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