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Immature platelets and reticulocytes fractions as a predictor of hematopoietic stem cell implantation

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ABSTRACT

Introduction: Classic biomarkers for hematopoietic stem cell implantation are absolute neutrophil count and platelet count. However, it is necessary to evaluate immature biomarkers in order to improve decision-making in the recovery of the transplant patient.

Objective: To determine the usefulness of immature platelet fractions and immature reticulocyte fractions as early predictors of hematopoietic progenitor implantation in oncology patients.

Methods: 67 clinical laboratory reports of transplant patients with hematopoietic progenitors were studied from day +1 to day +14. A panel analysis was applied for associations of variables.

Results: The absolute neutrophil count increased by 0.17 x109/L for each 1% increase in immature reticulocyte fraction (p<0.001); by 0.22 x109/L for each 1% increase in immature platelet fraction (p<0.001); and decreased by 0.13 x109/L for each day of follow-up (p<0.001). Platelet count increased by 1.71 x109/L for each 1% increase in immature reticulocyte fraction (p<0.001); and decreased by 6.99 x109/L for each day of follow-up (p<0.001). Immature platelet fraction was not associated with platelet count.

Conclusions: The immature reticulocytes fraction was associated with absolute neutrophil count and platelet count, so it could be considered as a predictor of hematopoietic stem cells implantation. The immature platelet fraction was only associated with absolute neutrophil count.

Key words: immature platelet fraction; immature reticulocyte fraction; hematopoietic stem cell transplantation.

INTRODUCTION

Hematopoietic stem cell transplant (HSCT) is a cellular immunotherapy that replaces hematopoietic cells in benign or malignant diseases with curative potential, such as some hematologic malignancies. The steps involved are the collection of hematopoietic stem cells (HSC) from an autologous or allogeneic donor, cell depletion by myeloablative, reduced intensity or non-myeloablative conditioning regimen, and subsequent hematopoietic recovery and immune reconstitution[(1)].

Recognition of the success of HSCT is due to the conditioning regimen (CR) used and the graft-versus-tumor effect that occurs after transplantation. This recognition is important since the stage of neutropenia and thrombocytopenia, originated by the CR, has more risk for the patient due to the danger of contracting infections. This risk decreases after neutrophil engraftment. For this reason and for patient safety, the classic criteria used as predictors of HSC implantation are defined as the first of three consecutive days of absolute neutrophil count (ANC) > 0. 5 x 10^9/L and the first of seven consecutive days of platelet count (PC) > 20 x 10^9/L, values associated with event-free survival before fifteen days post HSCT, and depend among other factors on the source of HSC, the number of cells administered and the prescription of granulocyte colony stimulating factor[(2-4)].

Some automated hematology analyzers have developed innovative parameters such as the immature platelets fraction (IPF) reflecting the number of immature or reticulated platelets by their residual RNA content. They remain in peripheral blood for 24 to 36 hours and the number circulating in the bloodstream is 2 to 3 times lower than in bone marrow. Moreover, due to their numerical relationship with platelet production, they may act as a marker of megakaryopoiesis in idiopathic thrombocytopenia

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after chemotherapy and act as a predictor of platelet recovery after HSCT[5-7]. Additionally, the development of automated reticulocyte counts has allowed an objective measure of reticulocyte maturity based on their residual RNA content, so that it is possible to accurately quantify the percentage of immature reticulocytes expressed as immature reticulocyte fraction (IRF). This parameter provides a very early and sensitive index of erythropoietic activity and has been related to the prognosis of treatment of hemolytic and non-hemolytic anemias, an early and reliable indicator of response to erythropoietin (EPO) therapy and successful implantation after bone marrow transplant[8-10]. At present, only cohort studies of hematopoietic recovery in transplant patients have been published, and IPF and IRF have been evaluated as predictors of HSC transplant on a single day (day of transplant)[11-14]. In the present research, the values of IPF and IRF were evaluated during the entire follow-up time, in such a way as to evidence real associations in relation to ANC and PC. The results will contribute to expand the scientific evidence on these immature biomarkers and will promote their clinical use.

MATERIALS AND METHODS

A retrospective cohort study was conducted at the National Institute of Neoplastic Diseases (INEN, by its Spanish acronym) in Lima, Peru, from September 2018 to May 2019 in which clinical laboratory reports performed on 67 oncology patients transplanted with autologous HSC and myeloablative AR were studied from day +1 to day +14 of transplantation. Whole population was evaluated to obtain maximum representativeness. Subsequently, selection criteria were applied and the final sample was 56 clinical laboratory reports.

Inclusion criteria: Oncology patients undergoing autologous HSCT and myeloablative regimen (57 patients).

Exclusion criteria: Patients who manifested fungal infection the day after HSCT (1 patient), Patients who presented signs and/or symptoms of cardiovascular damage after HSCT (according to the literature review, but this characteristic was not found in any patient).

Procedures

Hematological Biomarkers: IPF, IRF, ANC, and PC of HSCT oncological patients were considered for data analysis.

These biomarkers were collected from automated hematology equipment Sysmex XN-2000™, its methodology is based on the flow cytometry method with cell lysing and fluorescent staining.

Clinical variables: Variables such as age, sex, type of neoplasm, and follow-up time were collected from clinical histories for data analysis.

Follow-up time: It was considered the first day of follow-up the day after transplantation (+1) and was considered the last day of follow-up on the average neutrophil and platelet recovery time (+14).

Statistical analysis

For the characteristics and purpose of this research, a panel analysis was applied (evaluations for each patient and between patients over time) and the identification of each patient was considered as the cluster or panel variable.

For the description of categorical variables, absolute and relative frequencies were used. For the description of numerical variables, mean ± standard deviation, minimum and maximum were used. For the decision between fixed or random effects, the Hausman test was applied. The p value of this test was less than 0.01 and the fixed effects analysis was chosen. Only variables that change over time were considered (age, sex and type of neoplasm did not participate in the regression analysis).

To determine which variables (IPF, IRF, follow-up time) were associated with ANC and PC, a “forward selection” nesting process was applied. The variables to be evaluated were entered one by one, starting from an empty model (regression model containing only the outcome variable). This was done in order to create parsimonious models explaining the maximum variability of each outcome (ANC and PC).

The selection of the variables that entered the models was determined based on the highest log likelihood (LL) difference that showed statistical significance and the lowest number of degrees of freedom of the variable.

Finally, the heteroscedasticity test was applied and its p-value was less than 0.001 as a consequence robust standard errors were used in the final regression models. For the evaluation of possible confounding variables, multivariate models were created, and for the observation of possible changes in the direction of associations, bivariate models were also displayed. STATA version 16.0 statistical software was used, and p-values less than 0.05 were considered statistically significant.

Ethical aspects

This research did not use informed consent because the participation of humans subjects was not required. In addition, the present study was approved by the Ethics Committee of the National Institute of Neoplastic Diseases of Peru.

RESULTS

Data description

Table 1 shows male sex and non-Hodgkin's lymphoma were the most representative qualitative variables in this population (57.1% and 35.7%, respectively). On the other hand, table 2 demonstrates that for most of the quantitative variables (except age, because it did not change over the follow-up period), there was greater variation per patient over time in comparison with between patients. For age, the mean ± standard deviation was 42.6 ± 17.9 years old and the range was 3 to 65 years old. For follow-up time, the average number of days per patient ranged from 5-7 days. The average ANC and PC, for each patient ranged from 0.1 to 3.4 x10^9/L and 25.3 to 95.2 x10^9/L, respectively. Finally, for IPF and IRF, the average for each patient ranged from 1.1% to 5.6% and 0.16% to 26.7%, respectively.

TABLE 1 - Qualitative characteristics of HSCT oncological patients (n=56).

Variable	N (%)
Sex	
Female	24 (42.9)
Male	32 (57.1)
Neoplasm	
Multiple myeloma	18 (32.2)
Chronic myeloid leukemia	1 (1.8)
Hodgkin's lymphoma	4 (7.1)
Non-Hodgkin's lymphoma	20 (35.7)
Acute myeloid leukemia	5 (8.9)
Acute lymphocytic leukemia	5 (8.9)
Neuroblastoma	2 (3.6)
Mixed-phenotype acute leukemia	1 (1.8)

Factors associated with ANC: Table 3 indicates all variables entered the parsimonious regression model after being evaluated with the requirements mentioned in the methods section. There was no change in the direction of the association of ANC with IPF and IRF. On the other hand, the association between ANC and follow-up time did change.

Multiple regression models showed that mean of ANC increased by 0.17 x10^9/L for each 1% increase in IRF ($p<0.001$), adjusted by IPF and follow-up time; mean of ANC increased by 0. 22 x10^9/L for each 1% increase in IPF ($p<0.001$), adjusted for IRF and follow-up time; and mean of ANC decreases by 0.13 x10^9/L for each day of follow-up ($p<0.001$), adjusted by IRF and IPF.

Factors asociated with PC: Table 4 shows only the variables IRF and follow-up time entered the parsimonious regression model after being evaluated with the requirements mentioned in the methods section. Although there was no change in the direction of the association between PC and follow-up time, the association between PC and IFR did change.

Multiple regression models showed that mean of PC decreases by 6.99 x10^9/L ($p<0.001$) for each day of follow-up, adjusted by IFR. Finally, mean of PC increases by 1.71 x10^9/L ($p<0.001$), for each 1% increase in IRF, adjusted by follow-up time.

TABLE 2 - Quantitative characteristics of HSCT oncological patients (n=56).

Variable		Mean	Standard deviation	Minimum	Maximum
Age (years of age)	General	42.6	17.9	3	65
	Between patients		18.0	3	65
	Per patient		0	42.6	42.6
Follow-up time (days)	General	6.7	4.2	0	14
	Between patients		0.5	5	7
	Per patient		4.1	-0.3	13.7
Absolute neutrophil count (x10^9/L)	General	1.6	2.5	0	16.4
	Between patients		0.8	0.1	3.4
	Per patient		2.3	-1.3	15.3
Platelet count (x10^9/L)	General	55.7	42.48	0	264
	Between patients		19.88	25.3	95.2
	Per patient		37.69	-31.60	235.5
Immature platelet fraction (%)	General	2.6	1.9	0	15.6
	Between patients		1.1	1.1	5.6
	Per patient		1.6	-1.2	12.6
Immature reticulocyte fraction (%)	General	7.3	10.4	0	42.9
	Between patients		4.7	0.16	26.7
	Per patient		9.4	-7.9	43.7

TABLE 3 - Factors associated with ANC in HSCT oncological patients. Bivariate and multiple regression analysis, parsimonious model (n=56).

Variable	Bivariate analysis			Multiple regression (parsimonious model) *		
	M	CI 95 %	p	M	CI 95 %	p
Immature reticulocyte fraction (%)	0.15	0.11 - 0.19	<0.001	0.17	0.12 - 0.22	<0.001
Follow-up time (days)	0.14	0.08 - 0.20	<0.001	-0.13	-0.18 - 0.07	<0.001
Immature platelets fraction (%)	0.60	0.48 - 0.71	<0.001	0.22	0.10 - 0.34	<0.001

*Adjusted by variables: Immature reticulocytes fraction, follow-up time and immature platelets fraction. M: Mean. 95% CI: 95% confidence interval.

TABLE 4 - Factors associated with PC in HSCT oncological patients. Bivariate and multiple regression analysis, parsimonious model (n=56).

Variable	Bivariate analysis			Multiple regression (parsimonious model) *		
	M	CI 95 %	p	M	CI 95 %	p
Immature reticulocyte fraction (%)	-0.30	-0.59 - 0.01	0.041	1.71	1.33 – 2.09	<0.001
Follow-up time (days)	-4.60	-5.85 - -3.34	<0.001	-6.99	-8.56 - -5.41	<0.001

* Adjusted by variables: Immature reticulocytes fraction and follow-up time.
M: Mean. CI 95 %: 95% confidence interval.

DISCUSSION

To the best of our knowledge and after searches and reviews, this is the first research applying panel data analysis, association quantification and control of confounding variables to study the association of ANC and PC with IPF and IRF. Therefore, the presented results may provide more objective interpretations for choosing early biomarkers to determine the transplantation of autologous HSC with myeloablative regimen.

In this regard, the variables associated with ANC, the direction of the associations with IPF and IRF did not change between bivariate analysis and multiple regression; but the direction of the association with follow-up time, did change. In fact, the mean of ANC decreases for each additional day of follow-up (parsimonious model). Therefore, comparing the relationship between ANC and days of follow-up, where the same IPF and IRF values were obtained among patients after HSCT, would not be the best option to evaluate HSC implantation. Some authors such as Morkis, Rauf and Gonçalo reinforce the idea of the increase in mean of IFR days before the increase in mean of ANC, however, by not performing a multiple regression analysis, the change in direction of ANC with the follow-up time could not be contrasted, adjusting for the other variables[13,15,16]. Similarly, Park in a complete investigation on the different hematological parameters associated with ANC, concludes that IFR is a stable marker for the prediction of neutrophil recovery[12].

Relating to the variables associated with PC, the direction of the association with follow-up time did not change between the bivariate analysis and the multiple regression; but the direction of the association with IRF did change in this investigation. This would be due to adjusting and controlling for the influence of each covariate in the multiple regression model, which also considered all follow-up days. This information provided is new in the literature, therefore, it could not be contrasted by other previous studies.

Furthermore, IPF was not associated with PC; this is probably because all patients received platelet units to avoid post-transplant hemostasis complications, and the level of natural IPF production was influenced; this would be in agreement with the explanation provided by Briggs[17]. Additionally, Meintker in a similar study shows that mean of IPF varied considerably over follow-up time in patients who received platelet units at the end of HSCT[18]. In another study where platelet recovery without transfusion support was evaluated after liver transplantation, the predictive results of IPF were more evident than PC[19].

As a consequence, there may be measurement bias in the results of the evaluation of PC with the rest of the variables. However, transfusing platelet units to patients undergoing HSCT is a common process in several health institutions worldwide, so it could be uncontrollable for researchers in this field.

The fact that IPF and IRF were associated with these traditional biomarkers, with special mention to IRF for being associated with both (ANC and PC), coincides with the normal physiology of a person in a post-transplant process, in which the first markers to increase will be those coming from immature cells[20]. Therefore, the importance of measuring these early biomarkers to generate a better prediction of autologous HSC transplant with myeloablative regimen (inclusion criteria for the population of this study) is highlighted.

Potential limitations include a small sample size, which would make the estimates less accurate, and the fact that the samples came from a single hospital, which could affect the external validity of this study. The latter could be diminished because the mentioned location is the reference hospital for neoplastic diseases in Peru[21].

It is recommended to replicate studies with a larger sample size and evaluate these associations in different types of HSCT (other types of donors such as allogeneic, syngeneic, and haploidentical; and other types of conditioning such as non-myeloablative), in order to confirm these results. Apart, it is suspected that if IPF is reported by percentage, it is slightly affected by platelet transfusions, so Sakuragi proposed to report IPF by absolute number (A-IPF)[22]. On this basis, a study with a similar objective is recommended to perform, but with reporting in absolute values for IPF.

To conclude, IRF was associated with PC and ANC, whereas IPF was only associated with the latter. These early biomarkers should be considered for future evaluations in patients undergoing autologous HSCT with myeloablative regimen, as they could guide patient recovery with greater objectivity[23].

CONCLUSION

This research-based analysis of two new hematological

biomarkers as predictors of hematopoietic stem cell implantation into a regression model showed that the immature reticulocytes fraction was associated with ANC and PC, and demonstrated statistical reliability, so it could be considered a good predictor of hematopoietic stem cells implantation, whereas IPF was only associated with ANC.

Further studies in which only patients without previous platelet transfusion are considered are recommended to analyze the association between PC and IPF.

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