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Assessment of immature platelet fraction and immature reticulocyte fraction as predictors of engraftment after hematopoietic stem cell transplantation

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SUMMARY

Introduction: Engraftment is a critical milestone of the hematopoietic stem cell transplantation (HSCT) process. The immature platelet fraction (IPF) and immature reticulocyte fraction (IRF) are considered early indicators of bone marrow recovery. The objective of this study was to assess these parameters as predictors of HSCT engraftment.

Methods: Neutrophil and platelet engraftment were defined as the first of three consecutive days with an absolute neutrophil count $>0.5 \times 10^9$/L or platelet count $>20 \times 10^9$/L, respectively. The IRF cutoff was 12%. Two IPF cutoffs were used: >6.2% and >10%.

Results: The study sample comprised 44 patients, of whom 24 had undergone autologous HSCT and 20 had undergone allogeneic HSCT. Absolute neutrophil counts $>0.5 \times 10^9$/L were preceded by IRF >12% in 86% of patients (38 of 44). Platelet counts $>20 \times 10^9$/L were preceded by an IPF >6.2% in 90% of patients (37 of 41) and by an IPF >10% in 63% of patients (26 of 41).

Conclusion: The results show that IRF and IPF are engraftment predictors. Peak in IPF was observed before rise in platelet count, while IRF rises before absolute neutrophil count (ANC) and persists increased. This indicates that IRF and IPF can be considered as new tools for hematopoietic assessment after HSCT.

INTRODUCTION

Hematopoietic stem cell transplantation (HSCT) is a curative treatment employed in hematologic malignancies and other select clinical conditions [1–5]. Its expected purpose is to reconstitute the hematopoietic system of the recipient by administering an infusion of donor hematopoietic stem cells. Transplant engraftment is the first sign of bone marrow recovery [1, 2]. Current criteria for engraftment are an absolute neutrophil count (ANC) $>0.5 \times 10^9$/L for three consecutive days and a platelet count $>20 \times 10^9$/L without the need for transfusion support.

New indicators of engraftment are being studied. These include the immature reticulocyte fraction (IRF) and the immature platelet fraction (IPF), which represent reticulocytes and platelets newly released into the bloodstream, with higher RNA content [6–9]. These fractions are calculated during automated reticulocyte counting by blood analyzers and thus constitute a rapid, simple test that carries no additional cost as compared to a standard reticulocyte count. They have shown good results in the assessment of marrow recovery, thrombocytopenias, and erythropoiesis among other phenomena [10–15].

The potential of IPF and IRF as predictors of post-HSCT engraftment has been described elsewhere. Several studies suggest that a rise in IPF predicts an increase in platelet counts and that increases in the ANC are mirrored by IRF [6–9]. Prediction of engraftment can be a valuable asset to support clinical decisions such as administration of granulocyte colony-stimulating factor or platelet transfusions, and can signal the start of bone marrow recovery [6–8].

However, these studies have used different cutoff points and methods, which hinder standardization of values within the context of HSCT [6, 7, 12]. The purpose of this study was to assess IPF and IRF as early indicators of post-HSCT engraftment in patients at a university hospital in southern Brazil.

MATERIALS AND METHODS

The study sample comprised patients who underwent HSCT between March and September 2013 at a pediatric oncology unit and isolation unit (protected environment unit). Peripheral blood specimens collected into K_3EDTA-containing tubes were used to obtain full blood counts and IPF and IRF levels, which were assessed in a Sysmex XE 5000® blood analyzer (Sysmex Corporation, Kobe, Japan). Neutrophil and platelet counts were obtained from a review of electronic medical records, as were relevant clinical data. All tests were performed within 6 h of peripheral blood collection.

Platelet levels were analyzed by hydrodynamic focusing direct current detection. The WBC differential was generated by flow cytometry. IPF and IRF were determined by fluorescence and light scatter-based methods, using fluorescent RNA markers that enable distinction between immature and mature cell fractions.

Neutrophil engraftment was defined as the first of three consecutive days with an ANC $>0.5 \times 10^9$/L, and platelet engraftment, as the first of three consecutive days with a platelet count $>20 \times 10^9$/L. The IRF cutoff point was set at >12%, on the basis of the reference range (RR) calculated at our center. Two cutoffs were established for the IPF: >6.2%, based on the RR calculated at our center, which was determined as 0.6–6.1%; and >10%, on the basis of the existing literature.

Statistical calculations were carried out in PASW Statistics for Windows 18.0 (SPSS Inc., Chicago, IL, USA). Sample size calculation was based on a significance level (α) of 0.05 and a statistical power of 80%. Variables were expressed as median and interquartile range (Shapiro–Wilk test). The Wilcoxon test was used to ascertain differences between the day of platelet engraftment and IPF and the day of neutrophil engraftment and IRF. The study was approved by the institutional Research Ethics Committee. Written informed consent was obtained from all patients or their legal guardians.

RESULTS

The sample comprised 44 patients who had undergone HSCT at the study center. The mean age was 37 years (range, 1–71 years), and 21 patients (48%) were female. One patient never achieved a platelet count $>20 \times 10^9$/L unsupported by transfusion (i.e., platelet engraftment did not occur) and two never had a platelet nadir (platelet count $<20 \times 10^9$/L). These patients were analyzed for IRF and neutrophil engraftment alone.

Of the patients included in analysis, 24 underwent autologous HSCT and 20 underwent allogeneic HSCT. Of the latter, 12 received related-donor and five received unrelated-donor grafts. There were two haploidentical transplants and one syngeneic transplant. Table 1 describes the characteristics of the study population.

Table 2 shows median ANCs, platelet counts, IPF, and IRF on the day indicative of engraftment in the autologous and allogeneic HSCT subgroups. Table 3 shows the day of engraftment (median) by each marker and the statistical differences between these indicators.

Figures 1 and 3 provide a timeline of median IRF and neutrophil counts after autologous and allogeneic HSCT, respectively, while Figures 2 and 4 present a timeline of median IPF and platelet counts after autologous and allogeneic HSCT, respectively.

Table 1. Patient characteristics

	Autologous	Allogeneic	Total
Sex			
Female	13	8	21
Male	11	12	23
Age (years)			
>18	20	14	34
<18	4	6	10
Disease			
ALL	0	7	7
AML	0	6	6
CLL	0	1	1
MDS	0	1	1
AA	0	1	1
PNH	0	1	1
NHL	1	1	2
HL	3	0	3
MM	13	0	13
Solid tumors	5	0	5
Amyloidosis	2	0	2
Other	0	2	2
Cell source			
Bone marrow	0	16	16
Peripheral blood	24	4	28
Conditioning			
Myeloablative	24	14	38
Nonablative	0	6	6

ALL, acute lymphoid leukemia; AML, acute myeloid leukemia; CLL, chronic lymphoid leukemia; MDS, myelodysplastic syndrome; AA, aplastic anemia; PNH, paroxysmal nocturnal hemoglobinuria; NHL, non-Hodgkin lymphoma; HL, Hodgkin's lymphoma; MM, multiple myeloma.

Table 2. Parameters on the day of engraftment

	Autologous	Allogeneic
Platelets (10^9/L)	27.0 (22.5–34.50)	34.0 (27.0–47.50)
Neutrophils (10^9/L)	0.97 (0.70–1.95)	0.67 (0.57–0.84)
IRF > 12%	19.4 (16.05–24.5)	19.3 (14.9–22.8)
IPF > 6%	9.7 (7.1–12.2)	7.8 (7.1–11.9)
IPF > 10%	12.5 (10.5–14.87)	11.9 (10.4–12.8)

IPF, immature platelet fraction; IRF, immature reticulocyte fraction.
Data expressed as median (interquartile range).

Immature reticulocyte fraction predicted neutrophil engraftment in 86% of patients (38 of 44). In five patients, an ANC >0.5 × 10^9/L occurred before the IRF exceeded 12%, and one patient never reached the IRF cutoff.

An IPF >6.2% predicted platelet engraftment in 92% of patients (38 of 41). In three patients, a platelet count >20 × 10^9/L preceded an IPF >6.2%. An IPF >10% predicted engraftment in 70% of patients (29 of 41). Seven patients never reached the 10% cutoff. In five patients, this parameter did not precede a platelet count >20 × 10^9/L.

DISCUSSION

Hematopoietic recovery following HSCT begins when neutrophil and platelet counts start to rise [1, 2]. Delays in engraftment may require therapeutic measures, such as administration of granulocyte colony-stimulating factor (G-CSF) and platelet transfusions. The IPF and IRF can facilitate this process by providing early evidence of bone marrow engraftment [6–9].

The results of the present study confirm the potential of IRF as a predictor of neutrophil recovery, as it preceded an ANC >0.5 × 10^9/L in both autologous and allogeneic HSCT recipients ($P < 0.001$ and $P = 0.006$). The 12.2% IRF cutoff was based on the RR of 1.6–12.1%, which was calculated from a sample of hematologically healthy patients at our center. Other cutoffs have been used in previous studies, such as IRF >10% [7, 8] and IRF-D (time to IRF doubling)

Table 3. Day of engraftment by each parameter

	Autologous	*P*	Allogeneic	*P*	Overall
Neutrophils	13.5 (10.25–15.0)	–	19.0 (14.25–22.5)	–	15
Platelets	11.0 (10.0–12.0)	–	19.0 (12.0–27.0)	–	12
IRF > 12%	9.5 (9.0–10.75)	<0.001*	13.0 (11.0–15.0)	0.006*	10
IPF > 10%	9.0 (8.0–10.75)	0.011**	15.0 (10.0–18.0)	0.004**	10
IPF > 6%	9.0 (8.0–9.75)	<0.001**	12.0 (7.5–15.0)	0.001**	9

IPF, immature platelet fraction; IRF, immature reticulocyte fraction.
Data expressed as median (interquartile range).
*Significant difference between neutrophil count and IRF (Wilcoxon test).
**Significant difference between platelet count and IPF (Wilcoxon test).
$P < 0.05$ was considered significant.

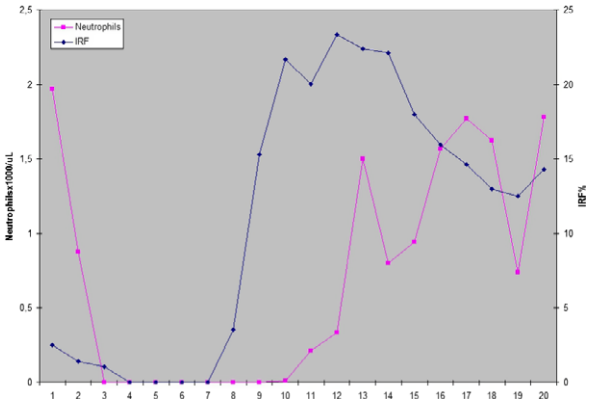

Figure 1. Median immature reticulocyte fraction *vs.* median neutrophil count of patients after autologous hematopoietic stem cell transplantation.

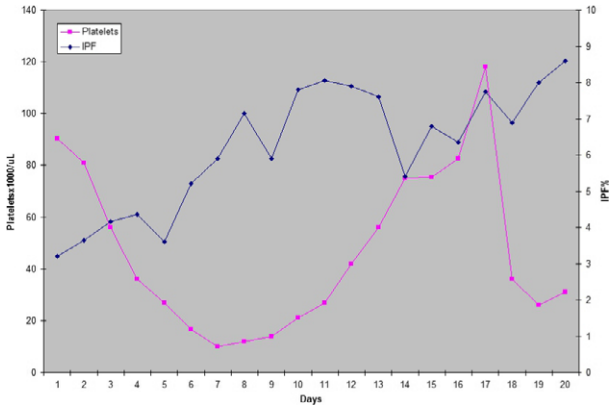

Figure 2. Median immature platelet fraction *vs.* median platelet count of patients after autologous hematopoietic stem cell transplantation.

[10]. The prediction of neutrophil engraftment by IRF was similar to that found in these studies, despite differences in equipment and methods [6, 7, 9, 12].

The IPF preceded platelet engraftment in both autologous and allogeneic HSCT recipients with cutoff values of >6.2% ($P < 0.001$ and $P = 0.001$) and >10% ($P = 0.01$ and $P = 0.004$). An IPF >6.2% corresponds to the first measurement exceeding the within-laboratory RR calculated at our center (0.7–6.1%), and an IPF >10% has previously been reported as having predictive value. It bears stressing that previous studies employing the IPF as a potential predictor used different cutoff values [6, 8, 10, 11]. Divergent results regarding platelet engraftment have been reported with IPF >7% [6], IPF >3% [11], and IPF >10% or peak post-HSCT IPF [10].

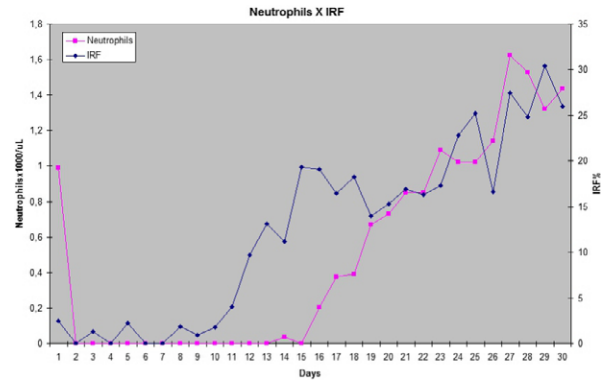

Figure 3. Median immature reticulocyte fraction *vs.* median neutrophil count of patients after allogeneic hematopoietic stem cell transplantation.

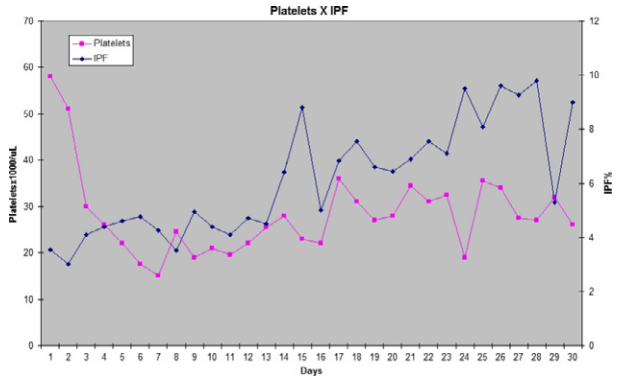

Figure 4. Median immature platelet fraction *vs.* median platelet count of patients after allogeneic hematopoietic stem cell transplantation.

Monitoring of the IRF in the post-HSCT period (Figures 1 and 3) showed that this parameter increased before neutrophil counts do and remains at high levels. The IPF, on the other hand, exhibited some variation, particularly in allogeneic HSCT recipients (Figures 2 and 4). This inconsistency makes the IPF difficult to interpret as a marker of platelet recovery. It has been reported that a rise in IPF precedes platelet recovery [6, 8, 10, 11]. In a previous assessment of the IPF as a predictor of engraftment, it was found that continuous platelet transfusions may decrease this fraction, whereas events such as sepsis can increase it [10]. Of all patients submitted to allogeneic HSCT, 70% (14/20) presented clinical symptoms of infection. This may explain the fluctuations seen in the allogeneic HSCT group.

Some aspects of our sample should be taken into account during data analysis. Only four peripheral blood transplants and six transplants employing non-ablative conditioning regimens were performed, which precluded separate analysis of these subgroups. Prior studies have reported a difference in the time to IPF and IRF prediction of engraftment depending on the type of transplant performed [6, 7, 11].

All participants received transfusion support during the thrombocytopenia period, as is part of routine post-transplant care. Although the influence of transfusions on IPF has been discussed elsewhere in the literature, it could not be assessed in the present study [8, 14]. Further studies would be useful to explain the interferences on IPF.

Our findings demonstrate the clinical applicability of the IPF and IRF as predictors of bone marrow engraftment after HSCT. Both parameters are available in a wide range of blood analyzers, but have yet to enter routine clinical practice. The IRF proved more stable for monitoring purposes, and both fractions rose before platelet and neutrophil counts did. Therefore, these parameters can be incorporated into routine care as a novel tool for assessment of post-HSCT hematopoiesis.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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