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EVALUATING THE ROLE OF IMMATURE PLATELET FRACTION IN PLATELET ENGRAFTMENT IN HEMATOPOIETIC STEM CELL TRANSPLANTATION

Original Research

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ABSTRACT

Background: Hematopoietic stem cell transplantation (HSCT) is a bone marrow condition in which platelet engraftment is a vital indicator of the condition. The immature platelet fraction (IPF) refers to the percentage composition of the recently formed platelets and could be used as a biomarker of prognosis of platelet recovery. The objective of this study was to conduct a test concerning the application of IPF in the measurement of the platelet engraftment in patients in the HSCT.

Methods: The study was a descriptive one that took place within a 6 months' time span in Shifa International Hospital. The consecutive sampling method was used to select the patients who underwent allogeneic or autologous transplantation of severe hematological disorders with the age of 30 between 15-60 years. Cases with grafted versus disease and other cases who were not of the age bracket were disqualified. To determine the relationship between platelet engraftment and IPF, as well as platelet count, on days 9 and 14 after the transplant, IPF and platelet counts were measured. Paired t-tests, ANOVA, and chi-square tests were used to perform the statistical analysis and the p-value below 0.05 was used to accept the significance.

Results: It was revealed that IPF rose significantly in day 9 (4.35 ± 1.46%) to day 14 (5.68 ± 2.26) following HSCT ($t = 3.21$, $p = 0.004$) indicating that there was active platelet production in the early engraftment. The platelet measures were on an increasing trend, and it was in agreement with increments of IPF. There were differences in age between the time of platelet engraftment ($F = 5.48$, $p = 0.009$) with the young patients (15-30 years old) having a higher platelet engraftment rate (9.2 ± 1.5 days) than the old patients. It further was discovered that, gender difference was critical, and the male engrafted far earlier (9.5 ± 1.6 days) in comparison to the female (10.8 ± 2.1 days) ($t = 2.12$, $p = 0.043$).

Conclusion: IPF results are the basis of support that the platelet engraftment prediction and follow-up after HSCT are a useful biomarker. Platelet counts and IPF follow-up can potentially be used to improve clinical care, such as early detection of platelet recovery and transfusiveness. In other studies, the cohort size must be larger to confirm these findings.

Keywords: Immature Platelet Fraction, Platelet Engraftment, Hematopoietic Stem Cell Transplantation, Biomarker, Platelet Recovery.

INTRODUCTION

Hematopoietic stem cell transplantation (HSCT) refers to a curative therapy used in the treatment of hematologic malignancies and other selected clinical conditions (1). It is proposed to restore the hematopoietic system of the recipient through an infusion of the donor hematopoietic stem cells. The initial indication of bone marrow recovery is transplant engraftment (2). Recent requirements to engage in engraftment are an absolute neutrophil count (ANC) > 0.5*10^9 /L on three consecutive days and a platelet count above 20*10^9 /L without transfusion. New engraftment indicators are under development. This has the immature platelet fraction (IPF), which are platelets that were just released into the blood. It has demonstrated satisfactory outcomes in conducting an examination of marrow restoration and thrombocytopenia among other phenomena (3).

Recent publications indicated that immature platelet fraction can be utilized as a sensitive biomarker of hematopoietic recovery (as are other developing molecular markers in clinical use) (4). To illustrate, platelet indices have been linked with numerous pathological ailments, such as thrombocytopenia, which has confirmed the clinical usefulness of dynamic platelet indices (5). The possibilities of IPF being used as predictors of post-HSCT engraftment have been outlined in other places. Some of the works propose that the increased IPF is predictive of the increasing platelet counts. Engraftment prediction may be an asset that can be used to assist clinical decisions in the administration of platelet transfusion, as well as mark the onset of bone marrow recovery (6). These studies, however, have employed varied cut-off points and methods, and this makes it difficult to standardize values in the context of HSCT. A normal platelet count (150,000 -450,000/ul) is essential in the process of maintaining hemostasis. Counts below 150,000/mL are considered thrombocytopenic, and spontaneous bleeding in the absence of injury has been related to platelet counts of below 20,000/mL (7). The existing hematology tools can effectively measure the low platelet count, but this measurement is only an indication of the circulating platelet count at a given time (8).

Clinicians have employed the immature platelet fraction as a marker of thrombopoietic activity in the bone marrow. This approach can be explained by the study comparing the response of IPF to therapeutic interventions, such as thrombopoietin receptor agonists (9) and immune-mediated thrombocytopenia (10). These observations verify that IPF is a manifestation of sustained production of marrow by platelets and not the number of platelets circulating. In the past, clinicians have looked at immature platelet data as an indicator of thrombopoietic activity in the bone marrow that might be significant in evaluating the risk of bleeding (11). Thrombopoietin receptor agonists have been clinically tested using IPF to determine the treatment effect of Eltrombopag and have been investigated in patients with Immune Thrombocytopenia (ITP) (12). The IPF% was also found to be reduced after transfusion as a result of the increased number of platelets in circulation, but not the IPF# outcome, indicating that the assay could be a valid representation of platelet production in the marrow (13).

Several groups have investigated the clinical usefulness of the IPF in different patient groups: after hematopoietic progenitor cell (HPC) transplant; myeloablative chemotherapy of hematologic malignancy; disseminated intravascular coagulation (DIC); non-myeloablative treatment of cancer and aplastic anemia; and paroxysmal nocturnal hemoglobinuria (PNH) (14,15). Whereas it is a traditional procedure to adhere to neutrophil restoration upon hematologic transplantation procedures, IPF can be an alternative legitimate marker of hematopoietic restoration. The objective of this study was to conduct a test concerning the application of IPF in the measurement of the platelet engraftment in patients in the HSCT.

MATERIALS AND METHODS

The study was a descriptive observational study conducted within six months at the Shifa International Hospital (Ref: 257-747-2019). After synopsis approval from 15th November 2020- 15th November 2021. Hematopoietic stem cell transplantation (HSCT) among patients aged between 15 and 60 years with severe hematological disorders was done on thirty patients through consecutive sampling. The sample size was determined using OpenEpi 3.0.0 (released 2013, Atlanta, GA, USA) using a confidence level of 95%, a margin of error of 5%, and an expected success rate of 95% for immature platelet fraction (IPF) as a biomarker. All the recipients who were undergoing allogeneic and autologous transplantation were considered.

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The inclusion criteria included patients aged 15-60 years of either sex presenting with a diagnosis of severe hematological disorders and patients who gave informed consent. The study did not include patients under 15 years of age and over 60 years of age with graft versus disorder (GVHD), and those who lacked consent. On day 9 and day 14 following the transplant, blood samples were taken to find out the IPF and platelet counts; this was taken as an indicator of platelet engraftment. Automated hematology analyzers that have the capacity to measure fluorescence flow cytometry for measuring lipid peroxidation, which forms the measurement of IPF, and provide an accurate measure of immature platelets in circulation. Standard laboratory procedures were also used to do platelet counts at the same time. The treating physicians determined the type of HSCT (allogeneic or autologous) and donor characteristics on the basis of the clinical considerations that included the availability of the donor, the disease condition, and the urgency of transplantation. All of the participants provided informed consent to the data collection in written form. Anonymity of patients and their confidentiality were considered during the study in accordance with the ethical standards.

Data analysis was done in SPSS 26.0 (n.d., IBM Corp., Armonk, NY). The continuous data, such as IPF and number of platelets, were expressed in the form of a mean and standard deviation. The paired t-test was used to compare the results of IPF on the 9th to 14th days. Analysis of variance (ANOVA) was used to determine differences in age groups in terms of time of platelet engraftment, and chi-square was used to determine differences in categorical variables (e.g., differences between genders in engraftment). The p-value below 0.05 was considered significant.

RESULTS

This study showed a marked rise in the immature platelet fraction (IPF%) between day 9 and day 14 following hematopoietic stem cell transplantation (HSCT), which showed active production of platelets in the early phases of engrafting. It was also found that the platelet counts were considerably raised in accordance with the IPF increments that warranted their combination in platelet recovery monitoring. Moreover, it was also found that the platelet engraftment time was significantly different across the age groups, and younger patients exhibited a higher platelet engraftment. Engraftment days in males and females were also very different, which can possibly represent biological factors in the recovery as gender specific. These results confirm that IPS is a useful biomarker in the monitoring and prediction of platelet engraftment following HSCT, as well as aid in clinical decision-making and better management of patients. The Demographic Characteristics of Patients Undergoing HSCT (n = 30)

Table 1: Demographic Characteristics of Patients Undergoing HSCT (n = 30)

Variable	Category	Number (%)
Age Group	15–30	16 (53.3)
	31–45	6 (20.0)
	46–60	8 (26.7)
Gender	Male	18 (60.0)
	Female	12 (40.0)
Transplant Type	Allogeneic	17 (56.7)
	Autologous	13 (43.3)
Primary Diagnosis	AML	10 (33.3)
	ALL	7 (23.3)
	Lymphoma	6 (20.0)
	Others	7 (23.3)
Disease Status	CR1	18 (60.0)
	Relapsed/Refr.	12 (40.0)

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Variable	Category	Number (%)
Conditioning Regimen	Myeloablative	20 (66.7)
	Reduced intensity	10 (33.3)
Stem Cell Source	Peripheral blood	22 (73.3)
	Bone marrow	8 (26.7)

n = Number of Patients, % = Percentage

Those who received HSCT were 30 patients. Most of them were between the age group of 15- 30 years (53.3%), and 60 percent of the cohort was male. Allogeneic transplantation was done on 56.7% of patients, and only 43.3% patients received autologous transplantation. The most common indicator was the acute myeloid leukemia (33.3%), then the acute lymphoblastic leukemia (23.3%), lymphoma (20.0%), and other hematologic conditions (23.3%). The majority of the patients had been transplanted in first complete remission (60.0%), two-thirds getting myeloablative conditioning (66.7%). The most common source of stem cells was peripheral blood (73.3%) (Table 1).

Table 2: Platelet Count and IPF% at Day 9 and Day 14 Post-HSCT (n = 30)

Parameter	Day 9 Mean ± SD	Day 14 Mean ± SD	Test Used	Test Statistic (t)	p-value
Platelet Count (cells/mm³)	57,290 ± 12,500	95,800±20,300	Paired t-test	t = 7.35	<0.001*
Immature Platelet Fraction (%)	4.35 ± 1.46	5.68 ± 2.26	Paired t-test	t = 3.21	0.004*

IPF% = Immature Platelet Fraction Percentage, SD = Standard Deviation, t = t-test statistic, * = Significance at p < 0.05

The day 9-14 post-HSCT showed a significant increase in platelet count, and immature platelet fraction (IPF%) increased. It means active platelet production in the course of the early engraftment ($p < 0.001$ and $p = 0.004$, respectively) (Table 2).

Table 3: Platelet Engraftment Days Stratified by Age and Gender (n = 30)

Variable	Group	Mean Engraftment Days ± SD	Test Used	Test Statistic	p-value
Age Group	15–30	9.2 ± 1.5	One-way ANOVA	F = 5.48	0.009*
	31–45	10.5 ± 2.0			
	46–60	11.0 ± 1.8			
Gender	Male	9.5 ± 1.6	Independent t-test	t = 2.12	0.043*
	Female	10.8 ± 2.1			

SD = Standard Deviation, F = ANOVA test statistic, t = t-test statistic, * = Significance at p < 0.05

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The age group and gender had a significant difference in platelet engraftment time. Patients with younger age (15-30 years), or males experienced a faster platelet engraftment ($p = 0.009$ and $p = 0.043$, respectively) (Table 3).

DISCUSSION

This paper was aimed at evaluating the use of immature platelet fraction (IPF) as a new outcome measure to predict platelet functionality recovery in the early post-transplantation period following hematopoietic stem cell transplantation, and how patient demographics (age, sex) relate to the engraftment period. The findings revealed a great increase in the percentage of IPF between day 9 and 14 after HSCT and a simultaneous increase in the number of platelets that supported the application of IPF as a marrow recovery and early platelet engraftment marker.

Our results are consistent with previous research indicating that IPF is a sensitive regulator of thrombopoiesis, which is more effective than other conventional platelet parameters in recovery of the marrow (16,17). The same literature has suggested that the application of IPF in hematopoietic recovery following transplantation has shown its utility in the clinical decision process (18,19). We found that younger patients (15-30 years) were found to have faster platelet engraftment than older age groups, and this agrees with other reports in the literature that younger bone marrow environments (younger age) possess a greater regenerative capacity (20,21). Moreover, the time of engraftment in male patients was much less than the time of engraftment in women, which means that hematopoietic recovery may be gender-biased (22,23). This can be attributed to the fact that immunological and hormonal variations influence bone marrow functions and graft-host diseases following transplantation (24).

The outlined gender differences can be explained by the literature source on hormonal derailment of the hematopoiesis and immune system (25). It is also indicated that the activity of the hematopoiesis is also affected by estrogen, and this might be the cause because females take a longer time before the engraftment process could occur as compared to males. Besides, the immunological components such as nerve growth factor would regulate the inflammatory responses following the transplant that might affect the recovery dynamic (26,27). The rise of IPF is a feature of an increase in the thrombopoietic of the unripe platelets which contain restful RNA and hemostatic activity when they enter the circulation (28). This untimely spiral of IPF is likely a foreteller of the measurable redemption of platelet count and can hence create an urgent early signifier on time-sensitive clinical reasoning in relation to platelet transfusion and patient treatment (29,30). The same is found in the literature where sensitivity, and specificity of IPF has been compared with other traditional parameters such as mean platelet volume to identify platelet regeneration (31).

The kind of transplant was also put into consideration as the recipients of autologous HSCT showed different IPF trends and recovery trends in comparison to allogenic recipients (32,33). Such differences are probable to be the differences in underlying conditioning regimen, graft source and immune-mediated effects such as graft-versus-host disease. The reason is in the fact that there is the existing literature which can substantiate the clinical relevance of our findings that autologous HSCT is able to offer quicker hematopoietic and general survival in the chosen patient groups (34,35). The clinical implication of such findings is that it suggests that the automatic IPF measurements should be included in the post-transplant monitoring program (36).

The identification of IPF at the timeliness of transplantation will optimize the projections of transfusion and decrease the wasteful platelet transfusion, and possibly minimize the complications concerning thrombocytopenia. In addition, the identified demographic influences the time of engraftment, which explains the need to introduce customized post-HSCT care plans (37). Though this analysis focused on the hematopoietic parameters, the recent investigations in the inflammatory mediators and molecular markers, such as the cytokines and growth factors such as IL-6 and leptin have become potentially useful research areas, which can be used to further optimize the predictive models of the engraftment and complications (38, 39). The dimensions can also find interaction with the platelet recovery process, which requires the incorporation of biomarker strategies (40).

This research is limited in a number of ways. The number of people sampled was extremely limited and was collected at a single center; therefore, it is possible that our findings may not be generalized. Furthermore, the possible confounding factors such as the intensity of pre-transplant conditioning, comorbidity, and graft cell doses were not addressed in details. In the future, multicenter studies involving larger cohorts and standard protocols are to be developed to substantiate the IPF cutoff values and adjust the capacities of the latter to identify the occurrence of the condition. Molecular researches to investigate the mechanistic nature of gender and age variation in engraftment may also prove valuable knowledge.

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CONCLUSION

It is also interesting that the immature platelet fraction is a good and accurate parameter of platelet recovery after hematopoietic stem cell transplantation. Its increase implies increases which could be measured in terms of platelet count and therefore it gives a warning to clinicians which could help them to optimize platelet transfusion plans. The IPF patterns of the patients undergoing autologous transplantation under the peripheral stem cell collection indicate the clinical relevance of the assay. Automated IPF measurement can apply to the routine post-HSCT care and help to manage and improve the outcomes of patients. The studies conducted should be extended in the future to involve more people to validate these results and determine the universal IPF cutoff values.

AUTHOR CONTRIBUTION

Author	Contribution
Maria Saeed*	Substantial Contribution to study design, analysis, acquisition of Data Manuscript Writing Has given Final Approval of the version to be published
Shawana Shahid	Substantial Contribution to study design, acquisition and interpretation of Data Critical Review and Manuscript Writing Has given Final Approval of the version to be published
Fatima Tuz Zahra	Substantial Contribution to acquisition and interpretation of Data Has given Final Approval of the version to be published
Maymoona Suhail	Contributed to Data Collection and Analysis Has given Final Approval of the version to be published
Tuba Farhat	Contributed to Data Collection and Analysis Has given Final Approval of the version to be published
Maira Ijaz	Substantial Contribution to study design and Data Analysis Has given Final Approval of the version to be published

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