Novel hematological parameters for the evaluation of patients with myeloproliferative neoplasms: the immature platelet and reticulocyte fractions

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Abstract New automated hematology analyzers have led to the availability of novel hematological parameters, including the immature platelet fraction (IPF) and the immature reticulocyte fraction (IRF), both of potential interest in patients with myeloproliferative neoplasms (MPNs). We performed a prospective analysis of 217 patients with MPN, including 32 (15%) with essential thrombocythemia (ET), 43 (20%) with polycythemia vera (PV), and 142 (65%) with myelofibrosis (MF); the IPF and IRF were measured by the Sysmex XN analyzer. As compared to patients with ET, both a higher IPF and IRF were observed among patients with PV and MF. Factors associated with high IPF among patients with PV/ET were male sex, thrombocytopenia, and diagnosis of PV; among patients with MF, they were elevated peripheral blasts, low platelet count, *JAK2* V617F mutation, and previous therapy. Factors associated with high IRF among patients with PV/ET were low hemoglobin, high reticulocyte count, and PV diagnosis; among patients with MF, they were peripheral blasts and elevated reticulocytes. The IPF and IRF represent novel parameters in patients with MPN with potential relevant clinical implications. Comparison with healthy subjects and those with secondary polycythemia is needed to confirm our preliminary findings.

Keywords Immature platelet fraction · Immature reticulocyte fraction · Myeloproliferative neoplasm

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Introduction

Recently, new automated hematology analyzers have led to the development of novel and more precise hematological parameters, including the immature platelet fraction (IPF) and the immature reticulocyte fraction (IRF) [1].

Immature platelets are reticulated platelets, still containing residual RNA, newly released from the bone marrow [2]. Their fractions reflect the rate of thrombopoiesis [3] and they exhibit a greater mass and higher prothrombotic potential compared to smaller and older platelets [4]. As a consequence, the IPF has shown clinical significance in idiopathic thrombocytopenia (differentiating decreased production from accelerated destruction) [5], in myelosuppression after chemotherapy or stem cell transplantation (predicting platelet recovery) [6, 7], and in thrombosis risk assessment in patients with acute coronary syndrome or sickle cell disease [8, 9].

Immature reticulocytes are distinguished from conventional reticulocytes based on their RNA content and represent an early and sensitive index of erythropoiesis [10]. The IRF may be of only limited utility in anemias secondary to hemolysis, bleeding, or bone marrow suppression, as in these situations it parallels the conventional reticulocyte count; however, it acquires significant utility in anemia secondary to acute infections or myelodysplastic syndromes (MDS), in which the total reticulocyte count is reduced or normal, whereas the IRF is increased [11–14].

The fractions of immature platelets and reticulocytes are of obvious potential interest in clonal disorders of hematopoietic stem cells, such as the myeloproliferative neoplasms (MPNs). The elevated IPF has been shown to associate with *JAK2* V617F mutation, hydroxyurea treatment, and a higher thrombotic risk in patients with polycythemia vera (PV) and essential thrombocythemia (ET) [15, 16]. However, its role in

myelofibrosis (MF) has never been explored. In addition, the clinical significance of IRF has not yet been investigated in any of the MPNs.

We present here a large prospective analysis of the clinical significance of the IPF and IRF in patients with MPN, including PV/ET and MF.

Methods

Patients and samples

We performed a prospective study of 217 unselected patients with a confirmed diagnosis of PV/ET or MF (including both primary MF and post-ET/post-PV MF) in whom IPF/IRF were measured between March 2014 and February 2015, during their routine visit to our center. Samples were collected once per patient, randomly during the course of their disease. PV/ET and MF were diagnosed according to the World Health Organization criteria [17]. For patients with MF, the Dynamic International Prognostic Scoring System (DIPSS) score was assigned to each patient as previously described [18]. Demographic and clinical information, including medical history, results of physical examination, complete blood count and blood chemistries, and assessment of bone marrow aspiration/biopsy, was collected at the time of IPF and IRF measurement. In particular, blast count was measured on the same sample used for IPF and IRF assessment. *JAK2* mutation was assessed as previously described [19]. Patients were subsequently followed per physician's preference.

This study was approved by the Institutional Review Board of MDACC and conducted in accordance with our institutional guidelines and the principles of the Declaration of Helsinki.

IPF and IRF measurement

The IPF and IRF were measured by a fully automated hematology analyzer, Sysmex XN (Sysmex Corporation, Kobe, Japan). At our institution, internal quality controls on Sysmex XN are run three times daily, through the commercial control XN Check (Sysmex America, Inc., Lincolnshire, IL, USA). The storage temperature of the sample was room temperature and the time elapsed between collection and analysis was 30 min. The measured value was expressed as the percentage of the total concentration of platelets and erythrocytes, respectively. Using this method, the reference range for IPF in healthy individuals was 1–7.3% [20] and the reference range for IRF in healthy individuals was 2.3–18% in males and 2.6–16.7% in females [10]; values were considered low, normal, or high if below, within, or above these reference ranges, respectively.

Statistical analysis

Categorical variables were compared using the $\chi2$ or Fisher exact tests. Logistic regression was used to determine factors associated with high IPF and IRF. Only factors that were significant on univariate analysis were included in multivariable analyses. Time-to-event outcomes were analyzed using the method of Kaplan and Meier, and comparisons were made using the log-rank test. A p value of <0.05 (two-tailed) was considered statistically significant. Statistical analyses were carried out using IBM SPSS Statistics 22 software for Windows (SPSS Inc., Chicago, IL).

Results

Baseline characteristics

Two-hundred and seventeen patients were included in the study: 32 (15%) with ET, 43 (20%) with PV, and 142 (65%) with MF. Baseline characteristics are shown in Table 1.

Among all patients, the median IPF was 5% (range, 0.9–28.9%) and was high in 79 (36%) patients. As compared to patients with ET (median 2.3%, range 1–5.3%), a higher IPF was observed among patients with PV (median 3.4%, range 0.9–17.3%; $p = 0.02$) and MF (median 6.2%, range 1.3–28.6%; $p < 0.001$) (Fig. 1a). Specifically, all patients with ET had a low or normal IPF, whereas 9 (21%) patients with PV and 70 (49%) with MF had a high IPF.

Among all patients, the median IRF was 20.7% (range 1.2–47%) and was high in 132 (61%) patients. As compared to patients with ET (median 9.8%, range 3.8–33.7%), a higher IRF was observed among patients with PV (median 17.1%, range 1.2–34.4%; $p = 0.01$) and MF (median 24.9%, range 7.2–47%; $p < 0.001$) (Fig. 1b). Specifically, a high IRF was observed in 8 (25%) patients with ET, 23 (53%) with PV, and 111 (78%) with MF.

Factors associated with high IPF

Among patients with PV/ET, factors associated with a high IPF on univariate analysis were male sex (78 vs 35%, $p = 0.03$), low platelet count (median 180 vs 499 × 10^9/uL, $p = 0.005$), and a diagnosis of PV as compared to ET (100 vs 52%, $p = 0.008$). No associations were found for the following factors: age, race, constitutional symptoms, hepatomegaly, splenomegaly, white blood cell count, peripheral blasts, hemoglobin, hematocrit, reticulocyte percentage, IRF, peripheral CD34+ cells, bone marrow blasts, fibrosis grade, *JAK2* V617F positivity, previous therapy, previous use of JAK inhibitor, and history of thrombosis. Because of the small number of events (only nine patients had high IPF), no multivariable analysis could be performed.

Table 1 Baseline characteristics

N = 217	Median [range], number (%)	
	PV/ET (75)	MF (142)
Age (years)	61 [22–86]	68 [28–91]
Caucasian	64 (85)	123 (87)
Non-caucasian	11 (15)	19 (13)
Males	30 (40)	83 (58)
Females	45 (60)	59 (42)
CS	25 (33)	114 (80)
No CS	50 (67)	28 (20)
Liver (cm BCM)	0 [0–7]	0 [0–8]
Spleen (cm BCM)	0 [0–13]	4.5 [0–26]
WBC (10^9/uL)	7.5 [1.6–30.9]	8.5 [0.6–111]
PB blasts (%)	0 [0–0]	0 [0–39]
Hgb (g/dL)	13.5 [9.3–17.7]	10.3 [4.4–16.1]
Hct (%)	42 [13–56]	32 [15–50]
IRF (%)	14.8 [1.2–34.4]	24.9 [7.2–47]
IRF(%)		
High	31 (41)	111 (78)
Normal	44 (59)	31 (22)
Ret (%)		
High	37 (49)	115 (81)
Normal	38 (51)	29 (19)
PLT (10^9/uL)	464 [121–1975]	168 [14–1180]
IPF (%)	2.8 [0.9–17.3]	6.2 [1.3–28.6]
IPF (%)		
High	9 (12)	70 (49)
Normal	66 (88)	72 (51)
PB CD34+ (%)	0.03 [0–2]	1.01 [0.01–17]
BM blasts (%)	1 [0–3]	1 [0–14]
Fibrosis		
1	7 (20)	18 (19)
2	26 (74)	35 (37)
3	2 (6)	41 (44)
Post-ET-MF	–	35 (25)
Post-PV-MF	–	29 (20)
Primary MF	–	78 (55)
JAK2 V617F		
Positive	52 (73)	92 (65)
Negative	19 (27)	46 (35)
DIPSS		
Low	–	8 (6)
Int-1	–	53 (37)
Int-2	–	58 (41)
High	–	23 (16)
Treated	35 (47)	79 (56)
Non-treated	40 (53)	63 (44)
JAK-i	12 (16)	69 (87)
No JAK-i	63 (84)	10 (13)

PV polycythemia vera, *ET* essential thrombocythemia, *MF* myelofibrosis, *CS* constitutional symptoms (anorexia, weight loss, low grade fever, and night sweats), *BCM* below the costal margin, *WBC* white blood cells, *PB* peripheral blood, *Hgb* hemoglobin, *Hct* hematocrit, *IRF* immature reticulocyte fraction, *Ret* reticulocytes, *PLT* platelets, *IPF* immature platelet fraction, *BM* bone marrow, *JAK* Janus kinase, *DIPSS* dynamic international prognostic scoring systems, *JAK-i* JAK inhibitor

Among patients with MF, factors associated with a high IPF on univariate analysis were elevated white blood cell count (median 10 vs 7.7×10^9/uL, $p = 0.04$), elevated peripheral blasts (median 1 vs 0%, $p = 0.04$), low platelet count (median 109 vs 234×10^9/uL, $p < 0.001$), a diagnosis of post-PV MF (30 vs 11%, $p = 0.004$), positive *JAK2* V617F mutation (80 vs 54%, $p = 0.002$), and previous treatment (67 vs 44%, $p = 0.007$); no associations were found for the following variables: age, race, sex, constitutional symptoms, hepatomegaly, splenomegaly, white blood cell count, peripheral blasts, hemoglobin, hematocrit, reticulocyte percentage, IRF, platelet count, peripheral CD34+ cells, bone marrow blasts, fibrosis grade, DIPSS score, and previous use of JAK inhibitor.

On multivariable analysis, the factors that remained associated with high IPF were elevated peripheral blasts (odd ratio [OR] 3.3, 95% confidence interval [CI] 1.4–8.1; $p = 0.009$), low platelet count (OR 3.7, 95% CI 1.6–8.8; $p = 0.003$), *JAK2* V617F mutation (OR 4.3, 95% CI 1.7–10.8; $p = 0.002$), and previous therapy (OR 2.6, 95% CI 1.2–5.8; $p = 0.02$) (Table 2).

Factors associated with high IRF

Among patients with PV/ET, factors associated with a high IRF on univariate analysis were the following: presence of constitutional symptoms (50 vs 23%, $p = 0.02$), lower hemoglobin (13 vs 14.1 g/dL, $p = 0.04$), high reticulocyte percentage (65 vs 39%, $p = 0.04$), and PV diagnosis (74 vs 45%, $p = 0.02$); no associations were found for the following variables: age, race, sex, hepatomegaly, splenomegaly, white blood cell count, peripheral blasts, hematocrit, platelet count, IRP, peripheral CD34+ cells, bone marrow blasts, fibrosis grade, *JAK2* V617F positivity, previous therapy, previous use of JAK inhibitor, and history of thrombosis.

On multivariable analysis, the factors which remained associated with high IRF were lower hemoglobin levels (OR 4.9, 95% CI 1.4–16.8; $p = 0.01$), high reticulocyte count (OR 6, 95% CI 1.8–19.9; $p = 0.003$), and PV diagnosis (OR 4.6, 95% CI 1.4–15.3; $p = 0.01$) (Table 2).

Among patient with MF, factors associated with a high IRF on univariate analysis were the following: constitutional symptoms (84 vs 68%, $p = 0.04$), splenomegaly (median 5 vs 0 cm, $p = 0.04$), elevated peripheral blasts (median 1 vs 0%, $p < 0.001$), elevated reticulocyte counts (median 87 vs 58%, $p = 0.01$), and high DIPSS score (63 vs 35%, $p = 0.02$); no associations were found for the following variables: age, race, sex, hepatomegaly, white blood cell count, hemoglobin, hematocrit, platelet count, IPF, peripheral CD34+ cells, bone marrow blasts, fibrosis grade, type of MF (primary vs post-ET/PV), *JAK2* V617F positivity, previous treatment, and previous use of JAK inhibitor.
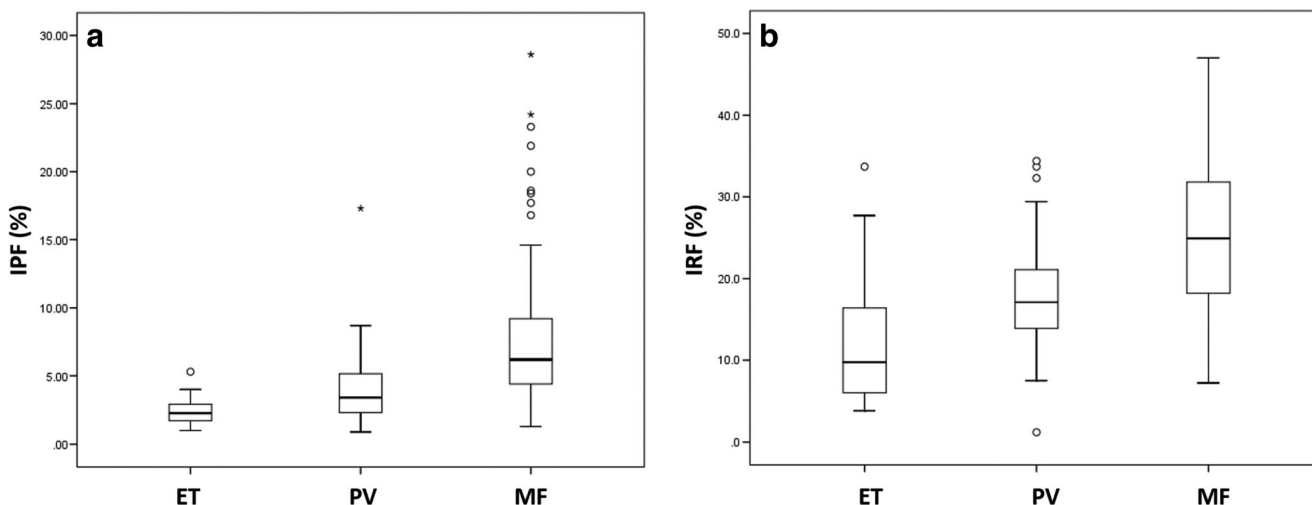
Fig. 1 IPF and IRF distribution by diagnosis. **a** Immature platelet fraction. **b** Immature reticulocyte fraction. *IPF* immature platelet fraction, *IRF* immature reticulocyte fraction, *MF* myelofibrosis, *PV* polycythemia vera, *ET* essential thrombocythemia

On multivariable analysis, the factors which remained associated with high IRF were elevated peripheral blasts (OR 7.8, 95% CI 2.1–29.5; $p = 0.002$) and elevated reticulocyte counts (OR 3.7, 95% CI 1.3–10.3; $p = 0.01$) (Table 2).

Association with thrombosis, transformation, and survival

After a median follow-up, 22 (1–26) months from IRP/IRF measurement, among patients with PV/ET, no thrombotic events or MF/AML transformations occurred, and only 1 patient died; among patients with MF, no thrombotic events were reported, 11 patients developed secondary AML, and 25 patients died.

No significant association between IPF or IRF and outcomes was observed.

Discussion

The clinical significance of the immature platelet and reticulocyte fraction is of potential interest in patients affected by myeloid malignancies, such as MPN. Here, we provide the first published analysis of the clinical and biological characteristics associated with IPF in patients with MF and of those associated with IRF in both MF and PV/ET.

Our study included 217 patients with PV/ET or MF for whom these two parameters were tested and who were prospectively followed; of interest, the levels of both IPF and IRF were high in patients with MF, intermediate in patients with PV, and low-normal in patients with ET. Given the inverse relation of the IPF with platelet count [3] and of the IRF with hemoglobin levels [10], such findings may not be surprising for patients with MF, in whom anemia and thrombocytopenia

Table 2 Multivariable analysis of factors associated with high IPF and IRF. Because of low number of events, no multivariable analysis could be performed for factors associated with high IPF among patients with PV/ET

	High IPF		High IRF			
	MF		PV/ET		MF	
	OR [95% CI]	*p*	OR [95% CI]	*p*	OR [95% CI]	*p*
PB >1%	3.3 [1.4–8.1]	0.009			7.8 [2.1–29.5]	0.002
PLT <100 × 10⁹/uL	3.7 [1.6–8.8]	0.003				
JAK2 mutated	4.3 [1.7–10.8]	0.002				
Treated	2.6 [1.2–5.8]	0.02				
Hgb <14 g/dL			4.9 [1.4–16.8]	0.01		
High reticulocytes			6 [1.8–19.9]	0.003	3.7 [1.3–10.3]	0.01
PV			1.6 [1.4–15.3]			

IPF immature platelet fraction, *IRF* immature reticulocyte fraction, *MF* myelofibrosis, *PV* polycythemia vera, *ET* essential thrombocythemia, *PB* peripheral blasts, *PLT* platelets, *JAK* Janus kinase, *Hgb* hemoglobin

are common [21], or in patients with ET, typically presenting with normal to high hemoglobin and thrombocytosis [22]. A high-intermediate IPF and especially a high-intermediate IRF are, however, less intuitive in patients with PV, in whom lower levels would be expected; of particular interest, among patients with PV/ET, in addition to the expected parameters (such as anemia and high reticulocytes), a diagnosis of PV was the only factor associated with high IRF on multivariable analysis. Our data suggest that high IRF in individuals presenting with elevated hemoglobin may suggest a diagnosis of PV rather than secondary polycythemia; its analysis in patients with secondary polycythemia and its validation as a potential diagnostic criterion for PV deserve further investigation.

Among patients with PV/ET, factors associated with high IPF were male sex, thrombocytopenia, and a diagnosis of PV; higher IPF levels are more common in males [23], and an inverse relation with platelet count, as outlined above, is expected [3]. In addition, a higher frequency of high IPF in PV as compared with ET, where thrombocytosis predominates, is not surprising. A previous study had observed an association between high IPF, *JAK*2 V617F mutation, and absence of previous treatment with hydroxyurea [15], which was not confirmed in our analysis. While both cohorts had similar sizes (about 75 patients), the previous study was enriched with patients with ET (as opposed to PV in our cohort); in addition, the previous study compared untreated and *JAK2* V617F-positive patients to healthy individuals, as opposed to previously treated and *JAK2*-unmutated patients in our analysis.

Among patients with MF, factors associated with high IPF were elevated peripheral blasts, low platelets, previous therapy, and *JAK2* V617F mutation; all of these may represent markers of aggressive disease [18, 24–26], and the lack of association with DIPSS score may encourage its investigation as an independent prognostic marker in MF. By contrast, the association between high IRF and adverse prognostic factors in patients with MF was less evident.

The biological significance of IPF and IRF is supported by their increased levels in MF as compared to PV/ET and by their association with unfavorable prognostic factors in these conditions; in fact, the fraction of immature platelet and reticulocyte may indirectly identify pathological myeloid progenitors, responsible for disease progression and aggressive biological behavior; however, correlative studies are needed to confirm these speculations.

While a previous study had reported a higher incidence of thrombotic events in patients with MPN and elevated IPF [16], the limited follow-up of our analysis did not allow appreciation of any association between the IPF/IRF and outcomes.

In conclusion, the IPF and IRF represent novel parameters in patients with MPN; in particular, the IRF may support the diagnosis of PV in individuals presenting with elevated hemoglobin, and the IPF may have an independent prognostic role in patients with MF. Comparison with healthy subjects, those with secondary polycythemia and longer follow-up are needed to confirm our preliminary findings.

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