

# Immature platelet fraction: A useful marker for identifying the cause of thrombocytopenia and predicting platelet recovery

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## Abstract

**Introduction:** The assessment of bone marrow thrombopoietic activity in patients with thrombocytopenia is necessary to achieve an accurate diagnosis and administer effective treatment. We evaluated the discriminatory power of the immature platelet fraction (IPF) in differentiating hyperdestructive/consumptive thrombocytopenia from hypoproductive thrombocytopenia and its potential use as a predictive marker for platelet recovery.

**Methods:** In this observational study, platelet indices, including IPF, were measured in 105 healthy individuals, 27 patients with hyperdestructive/consumptive thrombocytopenia (all with immune thrombocytopenic purpura [ITP]), and 35 patients with hypoproductive thrombocytopenia (5 with aplastic anemia and 30 with cancer who were undergoing chemotherapy) using a Sysmex XN-3000 hematology analyzer.

**Results:** The platelet distribution width, mean platelet volume, platelet large cell ratio, IPF, and absolute immature platelet count (AIPC) were significantly higher in the hyperdestructive/consumptive thrombocytopenia group than in the hypoproductive thrombocytopenia group ( $P < .001$ ). The IPF showed the highest difference between the two patient groups (200%). Receiver operating characteristics analysis that showed the IPF had the largest area under the curve among all the platelet indices analyzed; its cut-off value was 2.3%. The IPF decreased 3 to 4 days in advance of platelet count elevation in patients with ITP, whereas the delta AIPC increased 3 days in advance. Furthermore, the IPF and delta AIPC increased 5.5 days and 8.5 days, respectively, before platelet counts increased up to  $130.0 \times 10^9/L$  in cancer patients receiving chemotherapy.

**Conclusion:** These data demonstrated that the IPF and delta AIPC are both excellent indicators of the etiology of thrombocytopenia and predictive markers for platelet recovery.

**Abbreviations:** AA = aplastic anemia, AIPC = absolute immature platelet count, AUC = area under the curve, BM = bone marrow, CI = confidence interval, FSC = forward scattered light, IPF = immature platelet fraction, IQR = interquartile range, ITP = immune thrombocytopenic purpura, MPV = mean platelet volume, PCT = plateletcrit, PDW = platelet distribution width, P-LCR = platelet-large cell ratio, ROC = receiver operating characteristic, SFL = side fluorescent light, TTP = thrombotic thrombocytopenic purpura.

**Keywords:** bone marrow, chemotherapy, immature platelet fraction, immune thrombocytopenic purpura, thrombocytopenia

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## 1. Introduction

The main causes of thrombocytopenia are increased destruction/consumption of circulating platelets and decreased platelet production in the bone marrow (BM).<sup>[1-7]</sup> Hyperdestructive and consumptive thrombocytopenia encompass conditions such as immune thrombocytopenic purpura (ITP), disseminated intravascular coagulation, and thrombotic thrombocytopenic purpura (TTP). In contrast, hypoproductive thrombocytopenia includes conditions of BM failure, such as aplastic anemia (AA) or myelodysplastic syndromes, as well as BM infiltration due to solidcancer, fibrosis, or leukemia. BM toxicity caused by chemotherapy, HIV, or cytomegalovirus infection can also cause hypoproductive thrombocytopenia. In clinical practice, it is crucial to distinguish a decrease in the platelet production rate from an increase in the rate of platelet destruction; therefore, the assessment of thrombopoietic activity can be useful for correctly diagnosing the etiology of thrombocytopenia.<sup>[1-7]</sup> Furthermore, the assessment of thrombopoietic activity can help avoid unnecessary platelet transfusion in thrombocytopenic patients. Reliably predicting the natural recovery of platelets within a few days can help inform the decision of whether to perform

prophylactic platelet transfusion.<sup>[8,9]</sup> However, platelet counts alone do not reveal the underlying pathomechanism of thrombocytopenia, nor do they predict platelet recovery.

Immature platelets can be a useful marker of thrombopoietic activity.<sup>[1–7,10–14]</sup> They represent the most recently produced platelets released into the circulation by regenerated BM megakaryocytes. These types of platelets are the analogs of reticulocytes and are similarly large; moreover, they contain elevated amounts of cytoplasmic RNA and decrease in size and RNA content as they age. The number and proportion of immature platelets reflect the rate of thrombopoiesis; the values of these parameters rise and fall concomitantly with the platelet production rate.<sup>[5,15,16]</sup> Automated hematology analyzers can calculate the absolute numbers of immature platelets and their proportions relative to mature platelets.<sup>[17]</sup> The residual ribonucleic acid transferred from megakaryocytic progenitor cells during platelet biogenesis can readily be stained with dyes such as thiazole orange and measured using flow cytometry.<sup>[16–18]</sup> Flow cytometric analysis of immature platelets is more useful than other staining protocols for evaluating thrombopoietic activity in patients with thrombocytopenia.<sup>[1,2,4,16,18–22]</sup>

In this study, we evaluated the immature platelet fraction (IPF) and absolute immature platelet count (AIPC), along with other platelet-related parameters, to investigate their clinical utility in identifying the cause of thrombocytopenia and predicting platelet recovery in thrombocytopenic patients. Patients with hyperdestructive/consumptive thrombocytopenia caused by ITP or liver cirrhosis, as well as those with hypoproducer thrombocytopenia caused by AA or chemotherapy, were included in the study, as were healthy individuals who served as control subjects.

## 2. Materials and methods

### 2.1. Patients

This study included 105 healthy individuals who comprised the control group, 27 patients with ITP who comprised the hyperdestructive/consumptive thrombocytopenia group, and 35 patients (5 with AA and 30 undergoing chemotherapy for cancer) who formed the hypoproducer thrombocytopenia group. The recruited patients were treated at the Hallym University Sacred Hospital, Anyang, Republic of Korea between January 2016 and July 2016 and again between January 2018 and July 2018. The 30 patients with cancer included those with breast cancer (11), colon cancer (5), lung cancer (4), stomach cancer (3), melanoma (2), bladder cancer (1), brain cancer (1), osteosarcoma (1), ovarian cancer (1), and ureteral cancer (1). All patients with ITP and AA were newly diagnosed with no history of prior treatment. To investigate the usefulness of the IPF as a predictive marker of platelet recovery, follow-up samples from 3 hyperdestructive/consumptive thrombocytopenia patients (3 with ITP) and nine hypoproducer thrombocytopenia patients (2 with AA and seven undergoing chemotherapy) were also included. The study was approved by the institutional research board (no. 2016-I071), and the requirement for written informed consent was waived owing to the observational and anonymized nature of the study. The study was performed according to guidelines of the declaration of Helsinki.

### 2.2. Measurement of complete blood cell counts, including platelet indices

K<sub>2</sub>-EDTA (BD, Franklin Lakes, NJ) anticoagulated venous whole blood samples were analyzed using an XN-3000 automated

hematology analyzer (Sysmex, Kobe, Japan). When analysis was not performed immediately, the samples were stored at room temperature up to 30 minutes before testing. The internal structures of nucleic acid-containing platelets, such as mitochondria and endoplasmic reticulum, were stained using reagents containing oxazine fluorescent dyes. Two-dimensional scattergrams were plotted based on data obtained via flow cytometry using a semiconductor laser, with the X-axis representing the intensity of the side fluorescent light (SFL) and the Y-axis indicating the intensity of the forward scattered light (FSC). The platelets were measured using a platelet-specific channel after staining. Red blood cells typically show weak-to-medium SFL and strong FSC, while normal platelets also show weak-to-medium SFL but weak FSC. Immature platelets are distinguished by their strong SFL and medium FSC (Supplementary Figure 1, <http://links.lww.com/MD/D732>). The IPF (%) was calculated as using the following formula: (particle count in IPF zone/the particle count in the platelet zone) × 100. The AIPC was calculated by multiplying the IPF (%) by the platelet count. The delta absolute immature platelet count (delta AIPC) on day X was calculated by dividing (AIPC<sub>day X</sub> – AIPC<sub>day 0</sub>) by AIPC<sub>day 0</sub>.

### 2.3. Treatment for thrombocytopenia

Treatment options for thrombocytopenia included corticosteroids, intravenous immunoglobulins, and combined therapy. Platelet transfusion was also performed according to the national transfusion guideline. Prophylactic transfusions were performed when the patient's platelet count was under  $10.0 \times 10^9/L$ ; the threshold was higher in cases of clinical complications, surgical intervention, or the use of anticoagulants.

### 2.4. Treatment response and prediction of platelet recovery

The baseline platelet counts in patients with hyperdestructive/consumptive thrombocytopenia were below  $10.0 \times 10^9/L$ , which was consistent with severe thrombocytopenia according to the general classification criteria for thrombocytopenia used in the Republic of Korea (mild thrombocytopenia:  $90.0–130.0 \times 10^9/L$ , moderate thrombocytopenia:  $40.0–90.0 \times 10^9/L$ , and severe thrombocytopenia:  $<40.0 \times 10^9/L$ ). Therefore, the primary platelet recovery goal was the lower limit of moderate thrombocytopenia, that is,  $40.0 \times 10^9/L$ , which was considered the threshold of platelet recovery in these patients. Cancer patients in the hypoproducer thrombocytopenia group showed normal baseline platelet counts, so the threshold for platelet recovery in these patients was the lower limit of the normal range (ie,  $130.0 \times 10^9/L$ ).

### 2.5. Statistical analyses

Statistical analyses were performed using the SPSS Statistics version 24 (IBM Corporation, New York, NY) and MedCalc version 18 (MedCalc Software, Mariakerke, Belgium).

Quantitative variables are presented as the mean and standard deviation or median and interquartile range (IQR: 25th–75th percentiles). Student *t* test was applied to compare parametric quantitative variables between two groups, and comparisons between 3 groups were performed using analysis of variance with a post-hoc test. The Mann-Whitney test was applied to compare nonparametric quantitative variables between 2 groups, whereas

the Kruskal–Wallis test was used to compare more than 2 groups. Statistical data are represented in box-and-whisker plots, with the box depicting the upper and lower quartile and the line representing the median; whiskers represent the lowest and highest values.

Receiver operating characteristic (ROC) curves were used to compare the IPF and other platelet parameters between the hyperdestructive/consumptive thrombocytopenia and hypoproductive thrombocytopenia patient groups. The diagnostic cut-off value of the IPF with the best combined sensitivity and specificity was also determined. The area under the curve (AUC) and 95% confidence interval (CI) were calculated for each plot. *P*-values <.05 were considered significant in all analyses.

### 3. Results

#### 3.1. Patient characteristics

The distributions of age, sex, and other complete blood count parameters (other than platelets) of 105 healthy individuals, 27 hyperdestructive/consumptive thrombocytopenia patients, and 34 hypoproductive thrombocytopenia patients are summarized in Table 1. The data were collected either at the time of initial diagnosis (ITP and AA patients) or at the time of initial sample collection (cancer patients undergoing chemotherapy).

#### 3.2. The utility of platelet indices in identifying the cause of thrombocytopenia

**3.2.1. Platelet number and plateletcrit (PCT).** The mean platelet numbers in the control, hyperdestructive/consumptive thrombocytopenia, and hypoproductive thrombocytopenia groups were  $250.1 \pm 43.3 \times 10^9/L$ ,  $45.8 \pm 26.1 \times 10^9/L$ , and  $82.4 \pm 26.7 \times 10^9/L$ , respectively. The platelet number in the hyperdestructive/consumptive thrombocytopenia group was significantly lower than that in the hypoproductive thrombocytopenia group (*P* <.001).

The PCT was significantly different between the 3 groups: it was lowest in the hyperdestructive/consumptive thrombocytopenia group (median: 0.05%; IQR 0.04–0.09), higher in the hypoproductive thrombocytopenia group (median: 0.06%; IQR 0.06–0.11), and highest in the control group (median: 0.25%; IQR 0.22–0.27) (Fig. 1-A).

**3.2.2. IPF and AIPC.** The IPF was significantly higher in the hyperdestructive/consumptive thrombocytopenia group (median: 6.6%; IQR 4.0%–11.7%) than in both the control group (median: 1.8%; IQR 1.3%–2.4%; *P* <.001) and the hypopro-

ductive thrombocytopenia group (median: 1.8%; IQR 0.9%–2.3%; *P* <.001). However, the difference between the hypoproductive thrombocytopenia and control groups was not statistically significant (*P* = .31, Fig. 1-B). Compared to the control group, the hyperdestructive/consumptive thrombocytopenia group showed a 266.7% higher median IPF value.

The AIPC was also significantly different between the three groups; it was highest in the control group (median:  $4.3 \times 10^9/L$ ; IQR  $3.5\text{--}5.8 \times 10^9/L$ ), lower in the hyperdestructive/consumptive thrombocytopenia group (median:  $3.0 \times 10^9/L$ ; IQR  $1.2\text{--}4.2 \times 10^9/L$ ), and lowest in the hypoproductive thrombocytopenia group (median:  $1.3 \times 10^9/L$ ; IQR  $0.7\text{--}2.0 \times 10^9/L$ ) (all *P*-values <.001, Fig. 1-C). The hyperdestructive/consumptive thrombocytopenia group showed a median AIPC that was 25.6% lower than that of the control group, whereas the hypoproductive thrombocytopenia group showed a median AIPC that was 69.8% lower.

**3.2.3. Platelet distribution width (PDW), mean platelet volume (MPV), and platelet-large cell ratio (P-LCR).** Among the three groups investigated, the PDW, MPV, and P-LCR were highest in the hyperdestructive/consumptive thrombocytopenia group (14.0 fL [IQR: 12.4–15.5],  $11.7\% \pm 1.1\%$ , and  $38.5\% \pm 8.0\%$ , respectively); the differences were significant. The corresponding values were, respectively, 20.7%, 14.7%, and 44.2% higher than in the control group (11.6 fL [IQR: 10.7–12.5],  $10.2\% \pm 0.6\%$ , and  $26.7\% \pm 5.5\%$ , respectively; all *P* <.001). The PDW in the hypoproductive thrombocytopenia group was 6.1% lower than in the control group ( $11.0 \pm 1.9$  fL, *P* = .04), but the MPV and P-LCR showed no statistically significant difference.

**3.2.4. Correlation between platelet number and IPF.** The IPF in the hyperdestructive/consumptive group increased as the number of platelets decreased, especially in patients with severe thrombocytopenia with platelet counts under  $40.0 \times 10^9/L$  (Fig. 2). The IPFs were 3.4%, 5.7% (4.0%–8.9%), and 10.2% (4.4%–16.4%) in patients with platelet counts  $>90.0 \times 10^9/L$ ,  $40.0$  to  $90.0 \times 10^9/L$  and  $<40.0 \times 10^9/L$ , respectively (*P* = .33). In the hypoproductive thrombocytopenia group, the IPFs were 1.5% (0.9%–2.1%), 1.8% (0.9%–2.8%), and 1.7% (0.9%–2.2%) in patients with platelet counts  $<40.0 \times 10^9/L$ ,  $40.0\text{--}90.0 \times 10^9/L$ , and  $>90.0 \times 10^9/L$ , respectively (*P* = .91).

**3.2.5. ROC curve analysis.** ROC analyses were performed to evaluate the differences in the sensitivities and specificities of the platelet indices in the hyperdestructive/consumptive thrombocytopenia and hypoproductive thrombocytopenia groups (Fig. 3). The AUC was highest for the IPF (0.931), indicating that this parameter showed the best discriminatory ability between the 2 groups, followed by the PDW (0.878), P-LCR (0.821), AIPC (0.761), and MPV (0.783) (all *P* <.001). The AUC of the PCT was under 0.5 (0.306, *P* = .02), showing no discriminatory power. The IPF cut-off value with the highest sensitivity and specificity was 2.3%; this value had a sensitivity of 95.5% (95% CI: 83.3–99.9), specificity of 73.5% (95% CI: 58.8–89.3), positive predictive value of 78.9% (95% CI: 61.1–87.3), and negative predictive value of 96.3% (95% CI: 78.9–99.4).

#### 3.3. The IPF and delta AIPC are predictive markers for platelet recovery

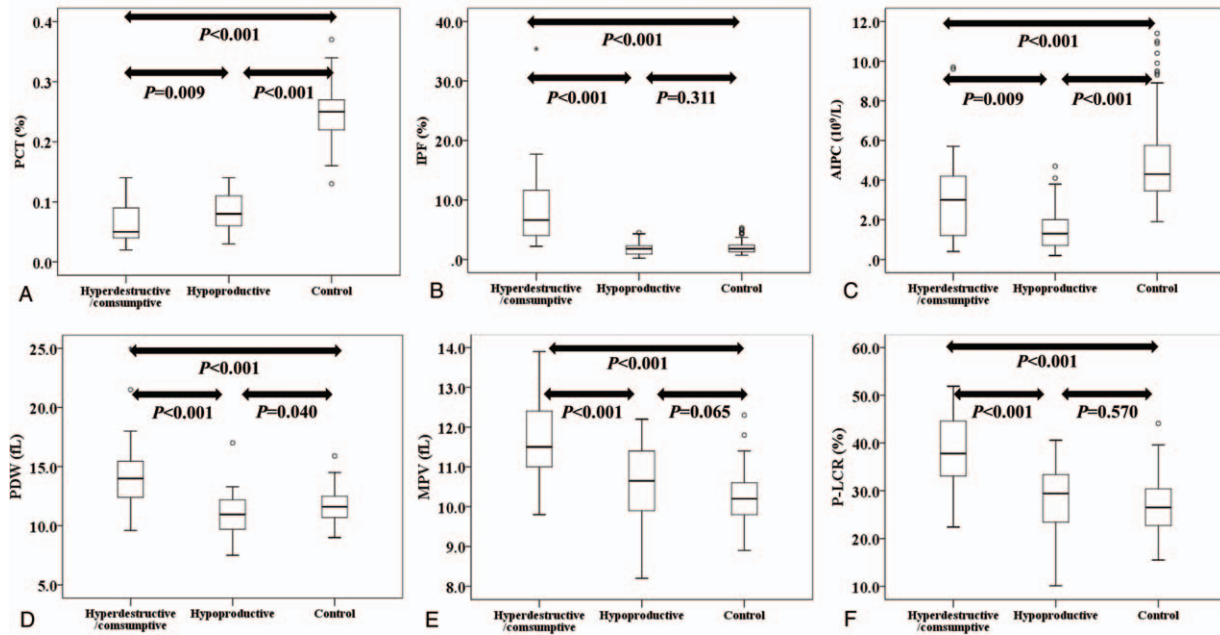
Although the IPF was found to be predictive of platelet recovery in the hyperdestructive/consumptive thrombocytopenia group,

**Table 1**  
Demographic data of control subjects and patient groups.

	Healthy control group (N=105)	Hyperdestructive/consumptive thrombocytopenia group (N=27)	Hypoproductive thrombocytopenia group (N=34)
Age, yr	46.5 ± 9.2	48.0 ± 17.6	61.1 ± 15.1
Male:female ratio	71:34	7:20	13:21
WBC ( $\times 10^9/L$ )	5.51 (4.66–6.20)	5.78 (3.16–9.4)	2.89 (1.18–4.78)
Hemoglobin (g/dL)	14.3 ± 1.3	11.4 ± 2.2	9.5 ± 1.9
Platelet ( $\times 10^9/L$ )	250.0 ± 43.3	57.4 ± 32.6	82.4 ± 26.7

WBC = white blood cells.

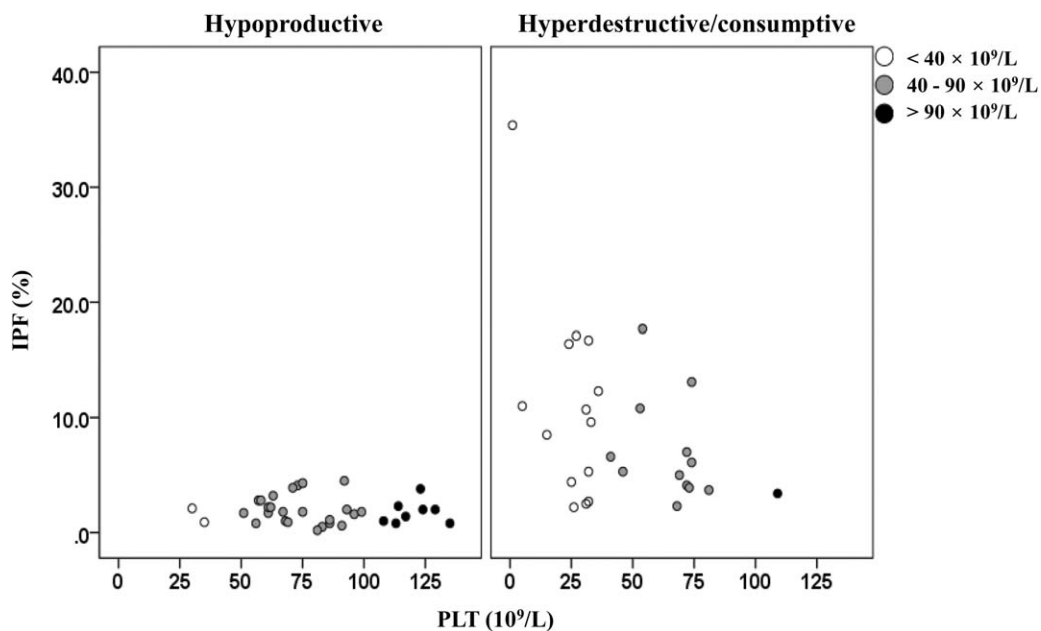
Values are ± standard deviations or medians (25th–75th interquartile ranges).



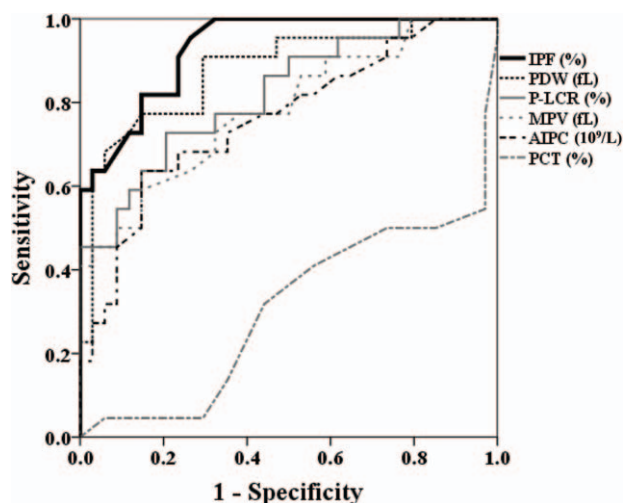
**Figure 1.** Platelet indices in patients in the hyperdestructive thrombocytopenia group, the hypoproduative thrombocytopenia group, and healthy individuals (control group) measured using an XN-3000 hematology analyzer. (A) Plateletcrit (PCT); (B) immature platelet fraction percentage (IPF); (C) absolute immature platelet fraction count (AIPC); (D) platelet distribution width (PDW); (E) mean platelet volume (MPV); (F) platelet-large cell ratio (P-LCR).

an inverse relationship was observed between the platelet count and the IPF. The platelet number increased to over  $40.0 \times 10^9/L$  3 to 4 days after the IPF decreased from its highest value, and 3 days after the delta AIPC (in which the AIPC was compared to its baseline [day 0] value as the denominator) increased in three ITP

patients (patients 1, 2, and 3). A representative case (patient 1) is shown in Figure 4. We were unable to examine the predictive values of the IPF or delta AIPC for platelet recovery since none of these patients showed obvious platelet recovery during the study period.



**Figure 2.** Distribution of immature platelet fractions (IPFs) measured using an XN-3000 as related to the total platelet count in the hyperdestructive thrombocytopenia and hypoproduative thrombocytopenia groups. The thrombocytopenia groups were divided into 3 subgroups based on the platelet (PLT) count (<40 × 10<sup>9</sup>/L; 40–90 × 10<sup>9</sup>/L; and >90 × 10<sup>9</sup>/L).



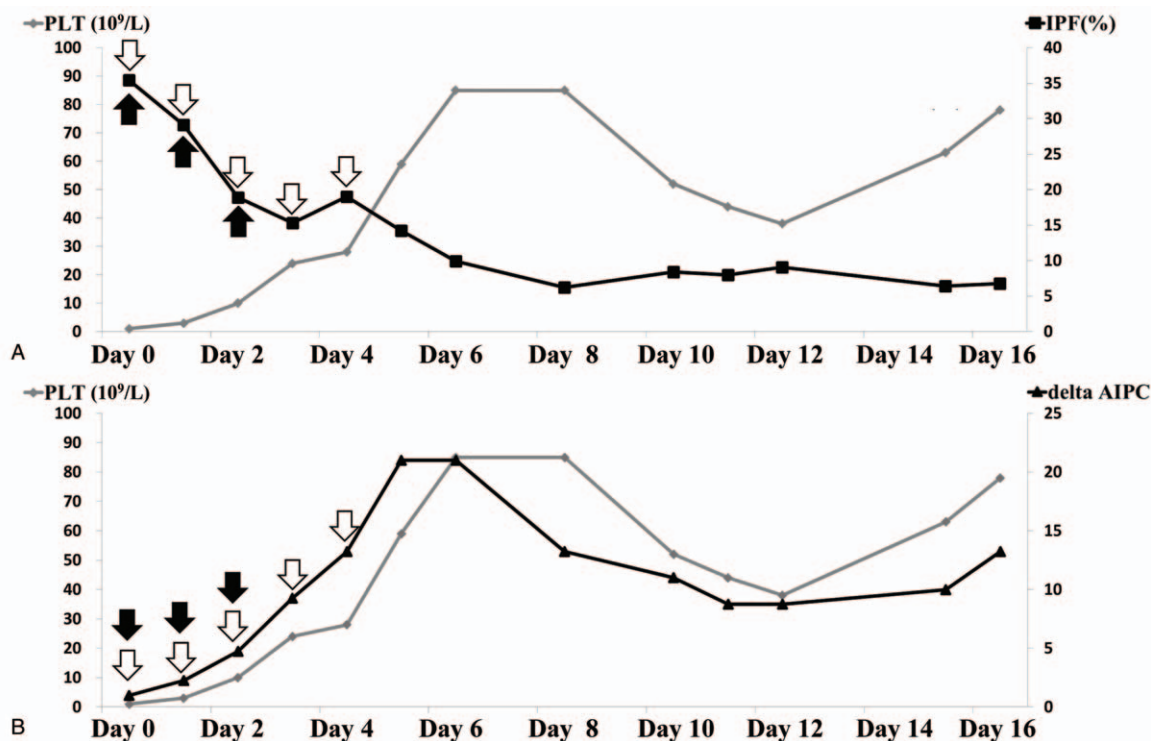
**Figure 3.** Receiver operating characteristics curves and areas under the curve of platelet indices measured using an XN-3000 hematology analyzer in the hyperdestructive thrombocytopenia and hypoproliferative thrombocytopenia groups. IPF (%): immature platelet fraction percentage; AIPC: absolute immature platelet fraction count; P-LCR: platelet-large cell ratio; PCT: plateletcrit; PDW: platelet distribution width.

We also evaluated the predictive ability of the IPF and AIPC in patients with hypoproliferative thrombocytopenia. As platelet recovery in patients undergoing cytotoxic chemotherapy follows very different kinetics than that in AA patients, the nine

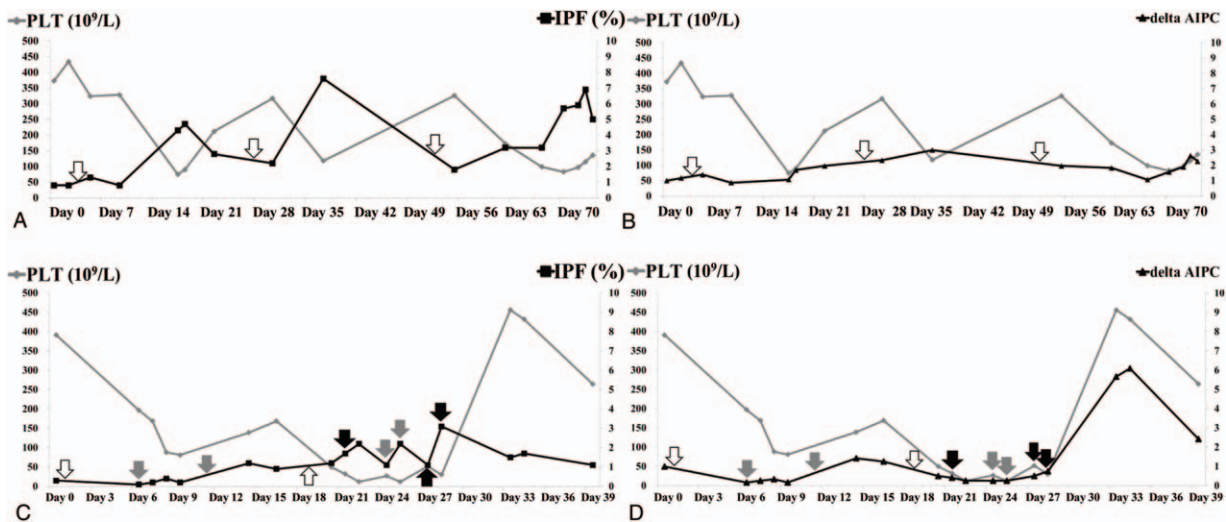
hypoproliferative thrombocytopenic patients who were followed for this purpose were divided into 2 groups: seven cancer patients receiving chemotherapy and 2 AA patients. The predictive values of the IPF and delta AIPC for platelet recovery were obvious in four of the 7 cancer patients receiving chemotherapy (patients 4, 5, 6, and 7). Whereas thrombocytopenia occurred during each cycle of chemotherapy, an increase in the IPF above its median value in the control group (2.1%) or an increase in the delta AIPC above the lowest point within the observation period coincided with a increase in the platelet count to  $>130.0 \times 10^9/L$  over a median of 5.5 days (IQR: 5.0–9.0 days) and a median of 8.5 days (7.0–12.3 days), respectively. Representative cases (patients 4 and 6) are shown in Figure 5. Three cancer patients (patients 8, 9, and 10) were excluded from the analysis because their platelet recovery appeared to be more dependent on platelet transfusion than on bone marrow thrombopoiesis during the study period. The abilities of the IPF and AIPC to predict platelet recovery were not evaluable in two AA patients (patients 11 and 12) because their platelet recoveries also appeared to be mainly dependent on platelet transfusion during the study period.

#### 4. Discussion

In this study, we showed that, of all the platelet-related indices examined, the IPF demonstrated the best discriminatory power for identifying the cause of thrombocytopenia (hyperdestructive/consumptive vs hypoproliferative). The IPF was significantly higher in the hyperdestructive/consumptive thrombocytopenia group (266.7% of the control group value) than in the hypoproliferative group; this difference can be used as a guide to distinguish the cause of thrombocytopenia in real-world



**Figure 4.** A representative example of changes in platelet indexes in a patient with immune thrombocytopenic purpura (patient 1). (A) The course of platelet counts (PLT) and the immature platelet fraction (IPF) percentage, and (B) the course of PLT and absolute IPF count compared to baseline (delta absolute immature platelet count). Black arrows: transfusion of platelet concentrates; white arrow: intravenous steroid treatment.



**Figure 5.** Representative examples of changes in platelet indexes in cancer patients undergoing chemotherapy. (A) The course of platelet counts (PLT) and the immature platelet fraction (IPF) percentage in patient 3, and (B) the course of PLT and absolute IPF count compared to baseline (delta absolute immature platelet count [AIPC]) in patient 3. (C) The course of PLT and IPF percentage in patient 6, and (D) the course of PLT and absolute IPC count compared to baseline (delta AIPC) in patient 6. Black arrow: transfusion of platelet concentrates; white arrows: chemotherapy. AIPC = absolute immature platelet count, IPC = immature platelet count, PLT = platelet counts.

practice. The AIPC showed the second largest difference between the 2 groups, at 130.8% of the control group value. The PDW, MPV, and P-LCR were higher in the hyperdestructive/consumptive thrombocytopenia group than in the control group, but the differences in percentages were not as large as those for the IPF and AIPC. Moreover, the differences between the control and hypoproducer thrombocytopenia groups were minimal or not statistically significant. Furthermore, it has previously been shown that the PDW, MPV, and P-LCR values increase as the AIPC increases<sup>[2,3]</sup>; therefore, these indices should not be used as independent markers for identifying the etiology of thrombocytopenia. The excellent discriminatory power of the IPF was also demonstrated using ROC curve analysis.

Our results are consistent with those of previous studies. Adly et al showed that the median IPF was significantly higher in patients with thrombocytopenia owing to increased peripheral platelet destruction than in those with thrombocytopenia owing to decreased platelet production, and that the IPF could be a marker for the diagnosis of ITP with high sensitivity and specificity.<sup>[10]</sup> Strauss et al found the IPF to be a suitable marker of thrombocytopenia caused by defective platelet production, whereas the AIPC (representing the immature platelet count) may potentially be practical for distinguishing acute ITP from thrombocytopenia in children with newly diagnosed acute lymphocytic leukemia.<sup>[2]</sup> However, most of the previous studies only compared patients with thrombocytopenia who had diverse underlying etiologies, and did not compare all patients to healthy individuals. We confirmed the discriminatory power of platelet indices, including the IPF, in patients with ITP (ie, the hyperdestructive/consumptive thrombocytopenia group) vs those with AA and cancer patients undergoing chemotherapy (the hypoproducer group), as well as with a control group of healthy individuals.

The IPF increased as the platelet count decreased in the hyperdestructive/consumptive thrombocytopenia group but not in the hypoproducer thrombocytopenia group, even though the

difference was not statistically significant (likely owing to the small number of study subjects). This suggests that the IPF reflects the BM's thrombopoietic activity and that the BM mediates thrombopoietic activity in a compensatory manner in response to the peripheral destruction or consumption of platelets during hyperdestructive/consumptive thrombocytopenia, but not hypoproducer thrombocytopenia. The median IPF was highest in patients in the hyperdestructive/consumptive thrombocytopenia group with platelet counts  $<40.0 \times 10^9/L$  and lowest in those with platelet counts  $>90.0 \times 10^9/L$ .

The most important finding in this study was that the IPF and delta AIPC are valuable predictive markers for platelet recovery. In 3 ITP patients with severe thrombocytopenia, the platelet count recovered to  $>40.0 \times 10^9/L$  3 to 4 days after the IPF dropped from its highest value and 3 days after the delta AIPC commenced increasing from its lowest value. A decrease in the IPF and an increase in the delta AIPC suggest that the BM has already produced a sufficient amount of new platelets, which in turn indicates that anti-antibody treatment is effective, demonstrating a negative feedback loop correlating with a response to treatment. The negative feedback loop was also evident in 4 cancer patients undergoing chemotherapy with intact BM thrombopoietic activity. The platelet count decreased due to transient BM suppression caused by the chemotherapy but began increasing when the BM activity was restored. The negative feedback loop was also demonstrated by the platelet count climbing to  $>130.0 \times 10^9/L$  5.5 days after the IPF dropped from its highest value and 8.5 days after the delta AIPC began climbing from its lowest value.

Previous studies have shown that the IPF, AIPC, or delta AIPC reflect real-time thrombopoiesis in the BM.<sup>[1,4,24,25]</sup> Linden et al showed that IPF could be used as a predictor of platelet recovery within 2 days using a cut-off of 5.3% in patients receiving autologous stem cell transplantation.<sup>[1]</sup> Abe et al showed that the IPF was significantly higher in patients with ITP and in those who completed chemotherapy (ie, during the recovery phase),

significantly lower during the nadir phase post-chemotherapy, and within normal range in patients with incomplete ITP remission and in those with AA.<sup>[4]</sup> In contrast, Greene et al showed that the absolute immature platelet counts (AIPC in the present study), but not their fractions, are more suitable for differentiating thrombocytopenias such as ITP and TTP.<sup>[24]</sup> Hong et al showed that the AIPC ratio was a useful variable to confirm TTP diagnosis and to monitor clinical response using an arbitrary cut-off value of 3.<sup>[25]</sup> They also demonstrated that, ex-vivo, the IPF and AIPC actively increased even in stored platelet products, likely owing to biosynthetically active immature platelets that rely on nucleic acids for protein production, function, and longevity.<sup>[23]</sup> Our study demonstrated that the IPF and delta AIPC can reliably predict the natural recovery of platelet counts a few days in advance, thus facilitating decision-making concerning therapy and avoiding unnecessary platelet transfusions in patients with ITP or those undergoing chemotherapy. Our study also confirmed that the IPF and delta AIPC can predict platelet recovery to certain levels in advance in patients with ITP or those undergoing chemotherapy for cancer.

Nevertheless, there are a few aspects to consider when interpreting our findings:

- (1) The predictive ability of these platelet parameters may be affected by treatment regimens, including the administration of anti-autoantibodies or thrombopoietin receptor agonists (although these agents were not used in our study subjects). Therefore, any thorough studies should be powered to control for these differences, including with stratification.
- (2) The duration of 3 to 4 days can correspond to the latency period of steroids; therefore, the IPF or AIPC may be biased measures of platelet recovery. However, our data suggest that they can still serve as surrogate markers for platelet recovery, particularly in terms of achieving the threshold of  $40.0 \times 10^9/L$ .
- (3) The observed decrease in the IPF 3 to 4 days in advance of platelet count elevation may occur even though the absolute platelet count (the denominator) is actually rising.

However, the IPF is not only dependent on the total platelet count but also on the AIPC (the numerator), and the change in IPF reflects the degree of change in both the total platelet count and the AIPC. Moreover, the total platelet count may reflect bone marrow thrombopoiesis as well as platelet transfusion, whereas the IPF and AIPC (the latter likely more reliably) reflect bone marrow thrombopoiesis more directly and independently. Taken together, our results imply that the IPF along with the AIPC could serve as more reliable markers than PDW, MPV, and P-LCR when identifying the cause of thrombocytopenia (ie, hyperdestructive/consumptive vs hypoproduative). Furthermore, the IPF and AIPC could be useful for predicting platelet recovery in patients with ITP or in those undergoing chemotherapy. However, our data do not currently support using the IPF and AIPC as replacements for PDW, MPV, P-LCR, or other parameters in all situations, as validating this notion was beyond the scope of our study. Further studies performed under various conditions would provide additional evidence towards identifying the platelet parameters that are best suited to represent each particular circumstance.

A limitation of our study is that the composition of patients in the 2 disease groups and the control group were heterogeneous in terms of age and sex, which could have affected the distribution of platelet indices. Additionally, platelet counts, which affect the platelet indices, were different in the 2 disease groups, especially

when comparing PCT to AIPC. However, in a study of 306 individuals (101 male subjects and 205 female subjects), Giovanetti et al found that the MPV and PDW (determined using an Abbott Cell Dyn 3500 CS) show no significant sex- or age-dependent differences except when comparing patients <10 years to most other age groups (as categorized by decades).<sup>[26]</sup> Only PCT was affected by sex in that study. Our analysis also showed that neither the MPV nor the PDW correlates with age and sex in healthy individuals. It remains unknown if age and sex affect other platelet indices, such as the IPF. Further studies of the role of age, sex, and platelet count in matched study populations with different etiologies of thrombocytopenia are required to validate our findings.

In conclusion, we demonstrated that the IPF and AIPC are excellent markers for distinguishing hyperdestructive/consumptive thrombocytopenia from hypoproduative thrombocytopenia. Furthermore, by reflecting the BM's thrombopoietic activity, the IPF and AIPC are robust and reliable predictors of platelet recovery in patients with ITP and in those with cancer who are undergoing chemotherapy. This can assist in providing future therapeutic guidance and preventing unnecessary transfusions.

## Author contribution

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