

# Automatic detection of immature platelets for decision making regarding platelet transfusion indications for pediatric patients

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## Abstract

Immature or reticulated platelets are known as a clinical marker of thrombopoiesis. Recently, an automatic method was established to detect reticulated platelets as immature platelet fraction (IPF) by means of hematology analyzer XE-2100. We assessed the effects of IPF detection after chemotherapy for various pediatric malignant disorders of 16 patients. Our results indicate that IPF should be considered a useful marker of imminent platelet recovery so that unnecessary platelet transfusion can be avoided.

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**Keywords:** Immature platelet fraction (IPF); Reticulated platelets; XE-2100; Platelet transfusion

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## 1. Introduction

Newly released platelets from bone marrow containing more RNA than mature platelets are known as ‘reticulated platelets’ and their number in peripheral blood reflects the rate of thrombopoiesis [1,2].

However, clinical determination of reticulated platelets has not been realized for two reasons. First, it requires expensive procedures such as flowcytometry, and second, detection methods have not been standardized. Recently, however, automatic detection of reticulated platelets using the reticulocyte detection channel (RET channel) equipped to the XE-2100 hematology analyzer (Sysmex, Kobe, Japan) has been developed [3,4]. For this method, platelets containing more RNA than normal are

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considered immature, and are expressed as immature platelet fraction (IPF). Theoretically, IPF is proportional to reticulated platelets, and IPF% has previously been reported by several authors as a clinically useful marker of thrombopoiesis [3–8]. We report here the utility of IPF% to detect imminent platelet recovery after chemotherapy for the pediatric patients so that unnecessary platelet transfusion can be avoided.

## 2. Materials and methods

### 2.1. Patients/samples

Twenty-seven chemotherapeutic courses and seven courses of stem cell transplantation (SCT) for 16 pediatric patients (0–15 yo) at Kobe University Hospital were serially observed between Feb 2006 and May 2007. The SCT comprised four autologous peripheral blood stem cell transplantations (PBSCT) and three allogeneic SCTs (two with matched related bone marrow and one with cord blood). The cases consisted of five acute lymphoblastic leukemias, two rhabdomyosarcomas, one each of acute promyelocytic leukemia, acute megakaryoblastic leukemia, anaplastic large cell lymphoma, osteosarcoma, Ewing's sarcoma, primitive neuroectodermal tumor, neuroblastoma, hepatoma, and aplastic anemia. The parents of all the patients provided written informed consent for their children's participation before their entry in this study.

### 2.2. Methods

IPF% of anticoagulated blood with K<sub>2</sub>EDTA was evaluated at least three times a week by means of the IPF master program equipped to the XE-2100. XE-2100 is a multi-parameter automatic hematology analyzer employing flowcytometry and a semi-conductor diode laser system to analyze leukocytes, nucleated red cells, and reticulocytes (RET channel) [9,10]. In the RET channel, two fluorescent dyes consisting of polymethine and oxazine dye in the RET-SEARCH (II) reagent penetrate into the cells and stain DNA/RNA. Reticulocytes are then separated from mature red cells according to the differences in RNA content and from nucleated cells according to the differences in DNA/RNA content. With a similar procedure, platelets are divided into two parts, mature and immature platelets according to their fluorescence intensity derived from RNA. A schematic representation of IPF detection is shown

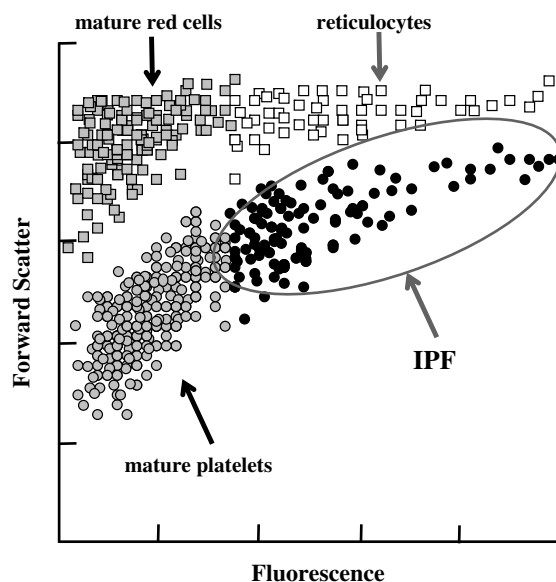


Fig. 1. Scatter gram of RET channel. Horizontal axis shows fluorescence and RNA content, and the vertical axis the size of the particles.

in Fig. 1. IPF was defined as platelets containing more RNA (horizontal axis), and at the same time large platelets are included (vertical axis). For this study, the results of IPF% were not reported to the pediatricians in charge.

We classified the clinical courses into four groups according to the thrombocytopenia of less than  $20 \times 10^9/l$  and the peak value of IPF% before platelet recovery. Patients without prominent thrombocytopenia were classified as Group 1, and patients suffering from thrombocytopenia were included in Groups 2, 3, and 4, which were defined based on the IPF% peak value before recovery of bone marrow function, Group 2 with an IPF% peak value of more than 10%, Group 3 from 6 to 10%, and Group 4 of less than 6%. The periods between the day the IPF% peak value was observed and the day of platelet recovery were recorded. Platelet recovery was defined as either more than  $30 \times 10^9/l$  of platelets or an increase  $10 \times 10^9/l$  over the platelet nadir without platelet transfusion.

## 3. Results

### 3.1. Reference value

As it is difficult to obtain blood samples from healthy children, IPF% values of the patients, when bone marrow function, especially leukocyte and

platelet numbers, recovered to the normal range without the use of cytokines such as G-CSF, were employed as reference values for this study ( $n = 33$ ). At this time point, all the patients were free from fever. As shown in Fig. 2, the average IPF% value was 2.6% with an SD of 1.1%, and IPF% and platelet number showed significantly negative correlation ( $p < 0.01$ ).

### 3.2. Case presentation

Clinical course of Case #2 (13 yo, Rhabdomyosarcoma) of Group 2 is shown in Fig. 3. The upper half shows the change in IPF%, and the peak value of 11.3% on day 10. The lower half shows leukocyte and platelet numbers, while platelet transfusion is indicated by arrows. The platelet nadir was

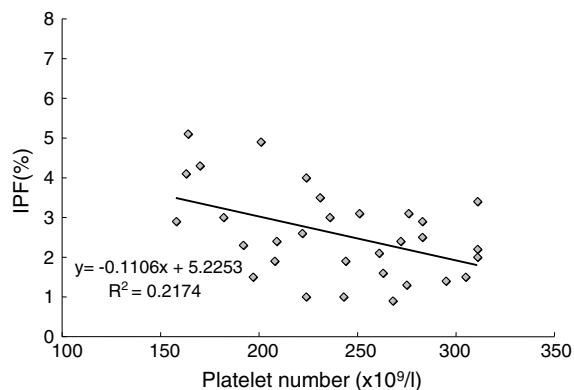


Fig. 2. Reference values of IPF% and the relationship between platelet number and IPF%.

recorded on day 8, and platelet transfusion was carried out on days 7, 9, and 11. As the platelet number on day 11, the day after the IPF% peak, was already  $40 \times 10^9/l$ , the third platelet transfusion turned out to be unnecessary.

Fig. 4 shows the clinical course of Case #11 classified as Group 2, 15 yo, Ewing's sarcoma. Platelet nadir was observed on day 15, and IPF simultaneously displayed its peak value of 11%. Platelet transfusion was carried out on day 16. Platelet count exceeded  $40 \times 10^9/l$  on day 17, and was subsequently maintained for several days. Platelet recovery of over  $30 \times 10^9/l$  was probably achieved on or around day 18.

### 3.3. Classification of patients

As shown in Table 1, 10 therapeutic courses were administered to Group 1, six each to Group 2 and 3, and five to Group 4. Average peak IPF% values for Groups 2, 3, and 4 were 12.1%, 7.2%, and 4.2%, respectively.

While platelet recovery for all the patients in Group 2 was observed within three days after the day of peak IPF%, it took 6.7 days (range; 1–15) and 7.4 days (6–9), respectively, for the patients in Groups 3 and 4.

### 3.4. SCT cases

Results of SCT cases are shown in Table 2. Six cases were classified into Group 3 or 4 except for Case #16, which was diagnosed as aplastic anemia and showed a high IPF% peak value of 23.6% and

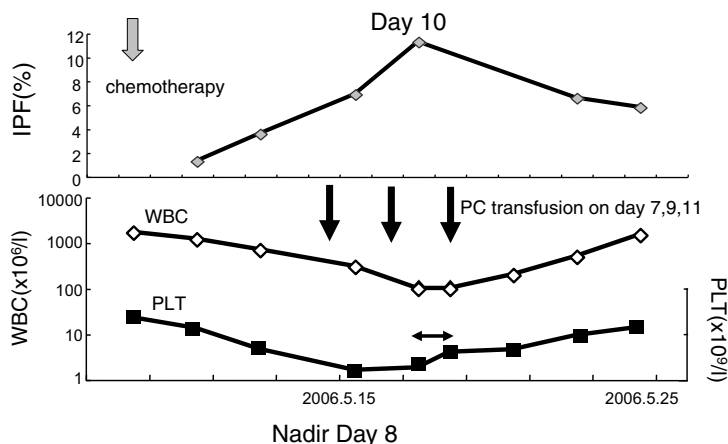


Fig. 3. Clinical course of Case #2, classified as Group 2. Horizontal arrow in the lower part indicates the period between platelet nadir and recovery. PLT, platelet; WBC, white blood cells; PC, platelet concentrate.

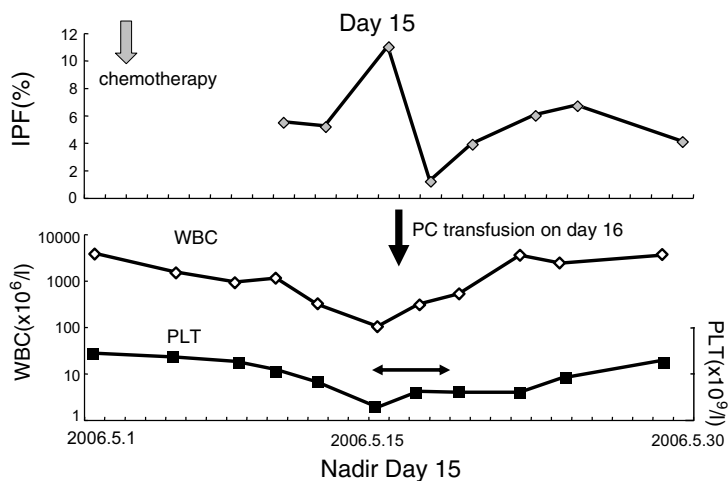


Fig. 4. Clinical course of Case #11 of Group 2. Horizontal arrow in the lower part indicates the period between platelet nadir and recovery. PLT, platelet; WBC, white blood cells; PC, platelet concentrate.

Table 1  
Classification of clinical courses and platelet recovery

	Group 1	Group 2	Group 3	Group 4
Thrombocytopenia <sup>a</sup>	Absent	+	+	+
Mean value of peak IPF% ( $M \pm SD$ )	nd <sup>c</sup>	$12.1 \pm 1.9$	$7.2 \pm 1.2$	$4.2 \pm 1.0$
Period until platelet recovery <sup>b</sup>	nd	2.2 days [1.4–3.0]	6.7 days [1.6–11.8]	7.4 days [6.0–8.8]
Numbers of courses included	10	6	6	5

<sup>a</sup> Less than  $2 \times 10^9/l$ .

<sup>b</sup> Days between platelet nadir and recovery (mean [95% CI]).

<sup>c</sup> Not determined.

was therefore classified as Group 2. In this case, platelet recovery occurred three days after the day of the IPF% peak.

Table 2  
Clinical data of the patients treated with stem cell transplantation

Case # (age, Dx)	Classification	IPF% peak value	Platelet recovery (days)	
			After IPF% peak	After transplantation
aPBSCT <sup>a</sup> 11 (15 yo, Ewing's sarcoma)	Group 3	8.4	15	36
aPBSCT 6 (2 yo, neuroblastoma)	Group 3	7.5	5	9
aPBSCT 4 (15 yo, rhabdomyosarcoma)	Group 4	nd <sup>d</sup>	nd	10
aPBSCT 15 (15 yo, osteosarcoma)	Group 4	nd	nd	12
Allo-BMT <sup>b</sup> 9 (15 yo, anaplastic large cell lymphoma)	Group 4	nd	nd	6
CBT <sup>c</sup> 10 (0 yo, acute lymphoblastic leukemia)	Group 4	nd	nd	12
Allo-BMT 16 (13 yo, aplastic anemia)	Group 2	23.6	3	23

<sup>a</sup> Autologous peripheral blood stem cell transplantation.

<sup>b</sup> Allogeneic bone marrow transplantation.

<sup>c</sup> Cord blood transplantation.

<sup>d</sup> Not determined.

#### 4. Discussion

Immature or reticulated platelets are reportedly a possible indicator of recovery from thrombocytopenia after chemotherapy [2]. A newly developed IPF detection method is regarded as simple and clinically applicable for the determination of reticulated platelets [3]. It has been reported by several authors, including us, that IPF is useful for diagnosis of the etiology of thrombocytopenia [3,4,6–8]. A high proportion of IPF is observed when platelets are destroyed in peripheral circulation due to such disorders as idiopathic thrombocytopenic purpura or thrombotic thrombocytopenic purpura. On the other hand, slight or no increase in IPF of patients with aplastic anemia or liver cirrhosis is also informative.

Briggs et al. [3] have shown that the ratio of IPF (IPF%) is better than absolute numbers for IPF to monitor recovery from thrombocytopenia, although absolute numbers of IPF are useful for differentiation of thrombocytopenia due to bone marrow dysfunction from thrombocytopenia due to peripheral destruction of platelets [4]. We have therefore used IPF% as an indicator.

No reports have been published on the reference values of IPF% for pediatric patients. Hematologic data after recovery of bone marrow function were therefore used as reference values as described in the results section. The results showed a negative correlation for IPF% values and platelet number ( $p < 0.01$ ), similar to that reported for adults [4]. Also, the average value of IPF% was similar to that reported for adults [4,6,11], so that IPF% was considered to be also applicable for pediatric patients.

It seems reasonable to omit platelet transfusion when the increase in IPF% after chemotherapy is classified as Group 2 provided there are no other complications of hemorrhagic factors such as sepsis or disseminated intravascular coagulation. However, IPF measurement cannot be used to decide whether to perform a transfusion after stem cell transplantation, although IPF is reported to be a good indicator of engraftment [5,11]. Since one aplastic anemia patient showed excellent IPF response in our experience, it seems to be warranted to conduct further studies into the effects of underlying disease, or the source and quality of transplanted stem cells on IPF response.

Since all of the platelet concentrates (PC) supplied from Red Cross blood centers in Japan are allowed to use for only 72 h after venipuncture, the PC supply must be reserved 24 h before transfusion. This means that ‘trigger platelets transfusion’, that is, a decision based on the complete blood count data in the morning of the day of transfusion [12], is difficult to perform. Under these circumstances, a surrogate marker such as IPF% for the prediction of platelet recovery can be expected to be useful to avoid unnecessary PC transfusion.

A decrease in IPF% after PC transfusion is observed and probably due to either a decrease in thrombopoietin levels, as pointed out by Briggs et al. [5], or to dilution of immature platelets by transfused mature cells. At the same time, these investigators detected a transient increase in IPF% during infection and speculated that an increase in cytokines such as IL-6 could be the cause [5]. Thus,

we have to pay attention to these spurious conditions for the clinical application of IPF%.

Although it is not clear whether our results are applicable to adult patients, several authors [3,4] have pointed that this may be the case. Control studies should therefore be performed to clarify the usefulness of IPF for both pediatric and adults patients.

## 5. Conclusion

IPF% is a clinically useful concept to estimate platelet recovery after chemotherapy and to avoid unnecessary PC transfusion for pediatric patients.

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