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

Saigo, Katsuyasu ; Sakota, Yasuyuki ; Masuda, Yukako ; Matsunaga, Kyoko ; Takenokuchi, Mariko ; Nishimura, Kunihiro ; Sugimoto, Takeshi ;…

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

Katsuyasu Saigo[a], Yasuyuki Sakota[b], Yukako Masuda[b], Kyoko Matsunaga[b], Mariko Takenokuchi[b], Kunihiro Nishimura[b], Takeshi Sugimoto[a], Kosuke Sakurai[a], Makoto Hashimoto[a], Tomoko Yanai[c], Akira Hayakawa[c], Yasuhiro Takeshima[c], Tsutomu Nomura[d], Yoshitsugu Kubota[d], Shunichi Kumagai[a,b]

a;   Blood Transfusion Division, Kobe University Hospital, Chuo-ku, Kobe 650-0017, Japan

b;   Clinical Laboratory, Kobe University Hospital, Kobe 650-0017, Japan

c;   Department of Pediatrics, Kobe University School of Medicine. Kobe 650-0017, Japan

d;   Department of Transfusion Medicine, Kagawa University Hospital, Kagawa 761-0793, Japan

Correspondence to;   Katsuyasu Saigo, MD

Blood Transfusion Division, Kobe University Hospital,

Kusunoki-cho, Chuo-ku, Kobe, 650-0017 Japan

Tel +81 78 382 6460   Fax +81 78 382 6469   E mail   saigo@med.kobe-u.ac.jp

Running title

Reticulated platelets and platelet transfusion

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.

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

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 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 [3,4,5,6,7,8].   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 y.o.) 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_2EDTA was evaluated at least 3 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 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 20×10^9/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%, Group3 from 6 to 10%, and Group4 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 30×10^9/l of platelets or an increase 10×10^9/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

3.2.1   Clinical course of Case #2 (13y.o., 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 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 $40x10^9$/l, the third platelet transfusion

turned out to be unnecessary.

3.2.2   Fig.4 shows the clinical course of Case #11 classified as Group 2, 15y.o.,

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 $40x10^9$/l on day 17, and was subsequently maintained for

several days.   Platelet recovery of over $30x10^9$/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 3 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 was therefore classified as Group 2.   In this case,

platelet recovery occurred 3 days after the day of the IPF% peak.

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 [3,4,6,7,8].   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 hrs after venipuncture, the PC supply must be reserved 24 hrs 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.

6.   Acknowledgement

References

[1] Kurata Y, Hayashi S, Kiyoi T, Kosugi S, Kashiwagi H, Honda S, et al.　Diagnostic value of tests for reticulated platelets, plasma glycocalicin, and thrombopoietin levels for discriminating between hyperdestructive and hypoplastic thrombocytopenia.　Am J Clin Pathol 2001; 115: 656-64.

[2] Wang C, Smith BR, Ault KA, Rinder HM.　Reticulated platelets predict platelet count recovery following chemotherapy.　Transfusion 2002; 42: 368-74.

[3] Briggs C, Kunka S, Hart D, Oguni S, machin SJ.　Assessment of an immature platelet fraction (IPF) in peripheral thrombocytopenia.　Br J Haematol 2004; 126: 93-99.

[4] Abe Y, Wada H, Tomatsu H, Sakaguchi A, Nishioka J, Yabu Y, et al.　A simple technique to determine thrombopoiesis level using immature platelet fraction (IPF). Thromb Res 2006; 118: 463-9.

[5] BriggsC, Hart D, Kunka S, Oguni S, Machin SJ.　Immature platelet fraction measurement; a future guide to platelet transfusion requirement after haematopoietic stem cell transplantation.　Transfus Med 2006; 16: 101-9.

[6] Nishiyama M, Hayashi S, Futsukaichi Y, Suehisa E, Kurata Y.　Measurement of reticulated platelets (immature platelet fraction) using automated hematology analyzer (XE-2100) in patients with thrombocytopenic disorders. Jpn J Lab Hematol 2006; 7: 313-8 [in Japanese, abstract in English].

[7] Tomatsu H, Sakaguchi A, Wada H, Abe Y, Nishioka J, Morishita Y, et al.　Clinical usefulness of fully automated measurement of immature platelet fraction (IPF) in

patients with thrombocytopenia. Jpn J Lab Hematol 2005; 6: 238-46 [in Japanese, abstract in English].

[8] Sakota Y, Saigo K, Masuda Y, Yano M, Matsunaga K, Mukai M, et al. Clinical significance of measurement of reticulated platelets for the patients with collagen diseases or hematologic disorders. Sysmex J 2005; 28: 26-33 [in Japanese, abstract in English].

[9] Jiang M, Saigo K, Kumagai S, Imoto S, Kosaka Y, Matsumoto H, et al. Quantification of red cell fragmentation by automatic hematology analyzer XE-2100. Clin Lab Haematol 2001; 23: 167-172.

[10] Saigo K, Sakota Y, Masuda Y, Matsunaga K, Narita H, Hashimoto M, et al. Clinical utility of new parameters provided by XE-2100 RET channel. Sysmex Journal Int 2007; 17:81-94.

[11] Takami A, Shibayama M, Orito M, Okumura H, Yamashita T, Shimadoi S, et al. Immature platelet fraction for prediction of platelet engraftment after allogeniei stem cell transplantation. Bone Marrow Transplant 2007; 39: 501-7.

[12] Slichter SJ. Relationship between platelet count and bleeding risk in thrombocytopenic patients. Transfus Med Rev 2004; 3: 153-67.

Fig Legends

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

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

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.
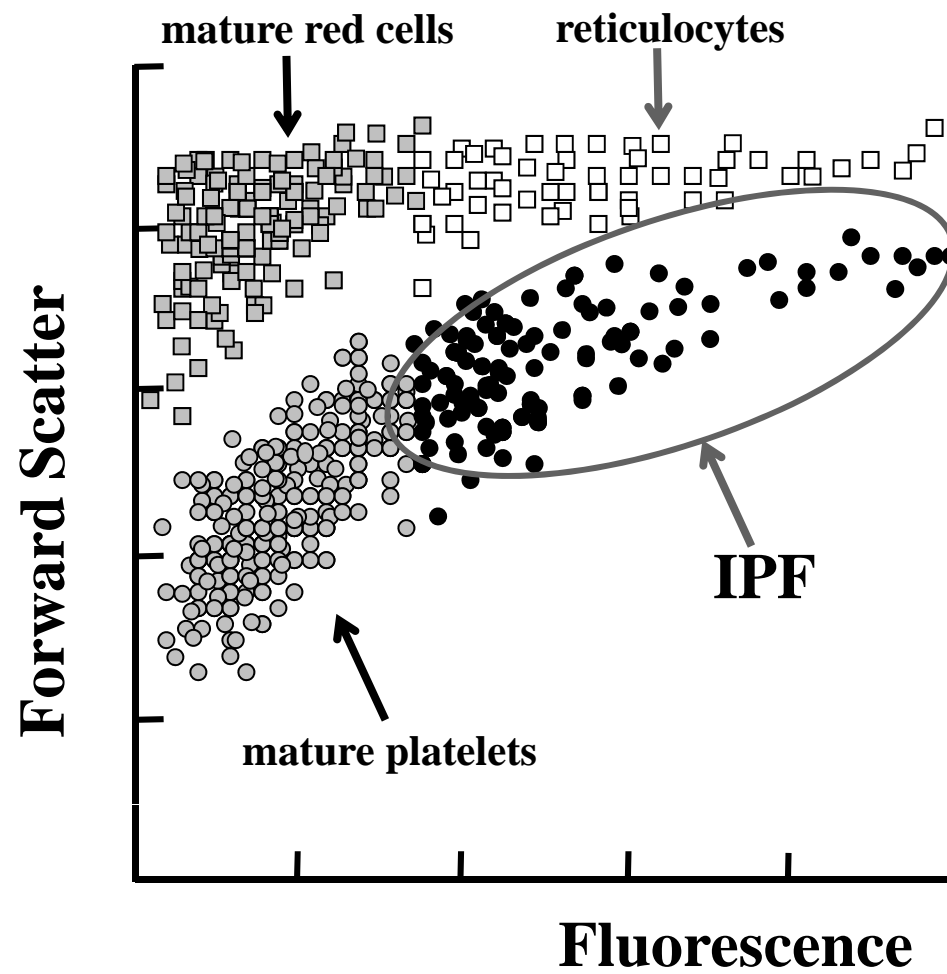
Fig.4.　Clinical course of Case # 11 of Group 2.　Horizontal arrow in the lower part indicates the period between platelet nadir and recovery.　PLT, platelet; WBC, white blood cells; PC, platelet concentrate.
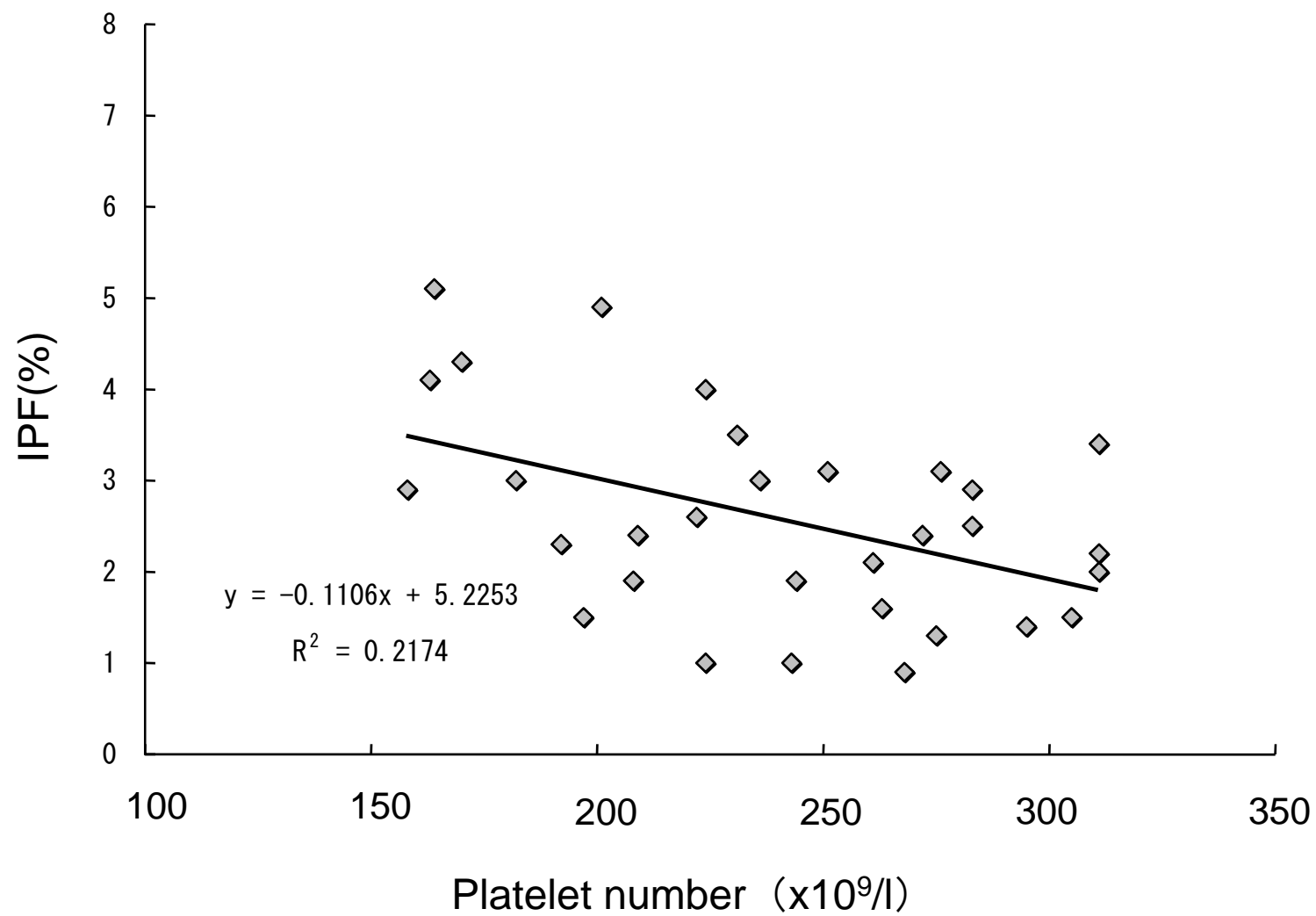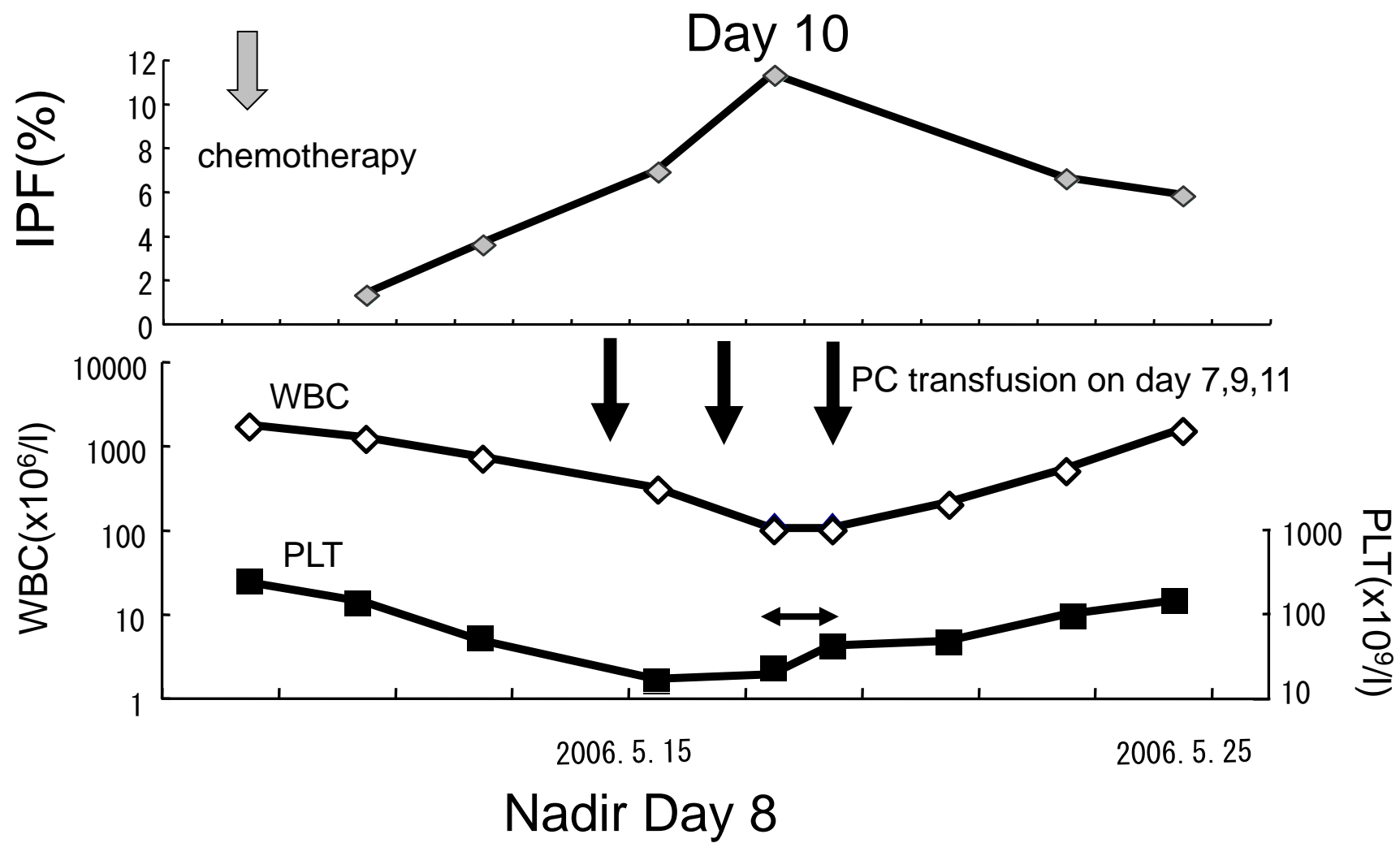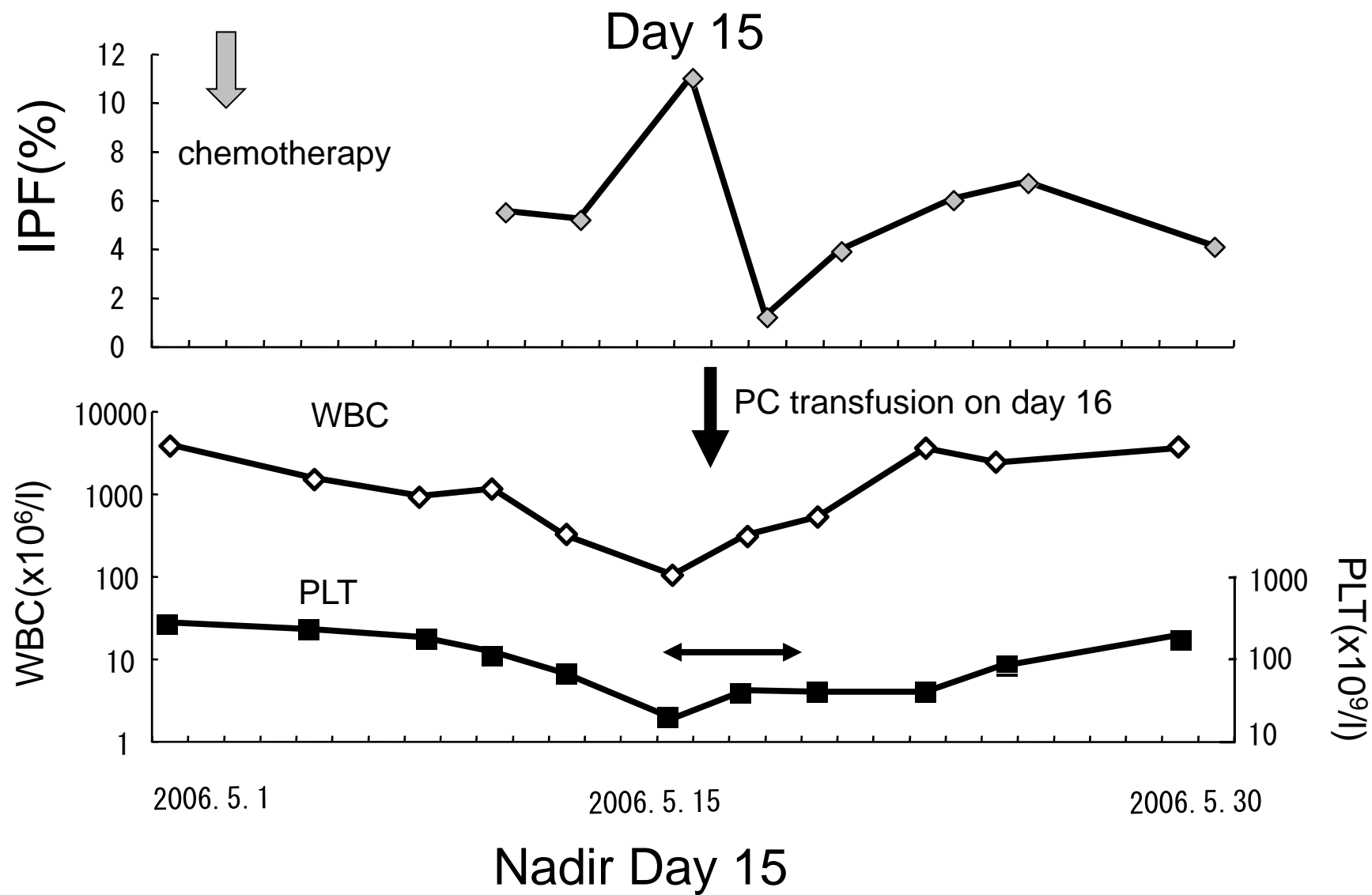
Fig.1

Fig.2

Fig.3

Fig.4

Table 1. Classification of clinical courses and platelet recovery

	Group 1	Group 2	Group 3	Group 4
Thrombocytopenia [1]	absent	+	+	+
Mean value of peak IPF%(M±SD)	nd [3]	12.1±1.9	7.2±1.2	4.2±1.0
Period until platelet recovery [2]	nd	2.2days[1.4-3.0]	6.7days[1.6-11.8]	7.4days[6.0-8.8]
Numbers of courses included	10	6	6	5

[1] Less than 2x10^9/l

[2] Days between platelet nadir and recovery(Mean [95%CI])

[3] not determined

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 transplatation
aPBSCT [1]	11 (15yo, Ewing's sarcoma)	Group 3	8.4	15	36
aPBSCT	6 (2yo, neyroblastoma)	Group 3	7.5	5	9
aPBSCT	4 (15yo, rhabdomyosarcoma)	Group 4	nd [4]	nd	10
aPBSCT	15 (15yo, osteosarcoma)	Group 4	nd	nd	12
allo-BMT [2]	9 (15yo, anaplastic large cell lymphoma)	Group 4	nd	nd	6
CBT [3]	10 (0yo, acute lymphblastic leukemia)	Group 4	nd	nd	12
allo-BMT	16 (13yo, aplastic anemia)	Group 2	23.6	3	23

[1] autologous peripheral blood stem cell transplantation

[2] allogeneic bone marrow transplantation

[3] cord blood transplantation

[4] not determined