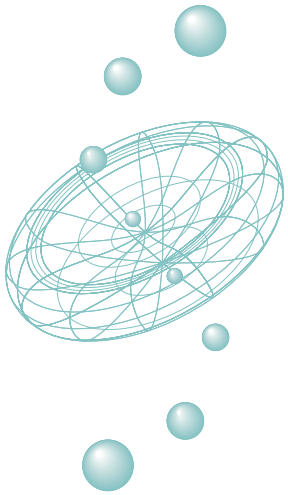
REVIEW ARTICLE

Clinical Utility of New Parameters Provided by XE-2100 RET Channel



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Key Words

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INTRODUCTION

Multiple parameter automated hematology analyzer XE-2100 (Sysmex Corporation, hereinafter called "XE-2100") has a channel intended to determine the count of reticulocytes automatically (RET Channel). In recent years, new clinical parameters were developed using this channel, and several studies have reported the clinical utility of them. In this article, we introduce the fragmented red cell quantification system (RET master) for which we involved in the development, as well as the experience to use and the usefulness of the immature platelet fraction counting system (IPF master) that was released recently.

THE FUNCTIONS OF XE-2100 AND THE PARAMETERS OF RET CHANNEL

XE-2100 possesses the following four channels: DIFF Channel to differentiate four types of white blood cells; WBC/BASO Channel for determining white blood cell (WBC) and basophil count; NRBC Channel for determining nucleated red blood cell count; IMI Channel for detecting immature WBCs and hemopoietic progenitor cells; and RET Channel for reticulocytes counting or determining platelet count by optical fluorescent method (PLT-O). [1)]

An example of RET scattergram is shown in ***Fig. 1***. The horizontal axis indicates the fluorescence intensity, which reflects the RNA level; and the vertical axis indicates the forward scatter, which depends on the cell size. It means that the system can quantify red blood cells (RBCs) with high fluorescence intensity and large RNA content as the reticulocytes. The system can also differentiate reticulocytes into those of low, middle, and high fluorescence ratio.

Below the RBCs data, this scattergram also plotted platelets, which can be applied in the determination of platelet count by optical fluorescent method (PLT-O). Platelet count has been determined conventionally based on the impedance method. The XE-2100 also uses impedance, however, PLT-O is useful to determine the platelet count accurately when large platelets are identi-

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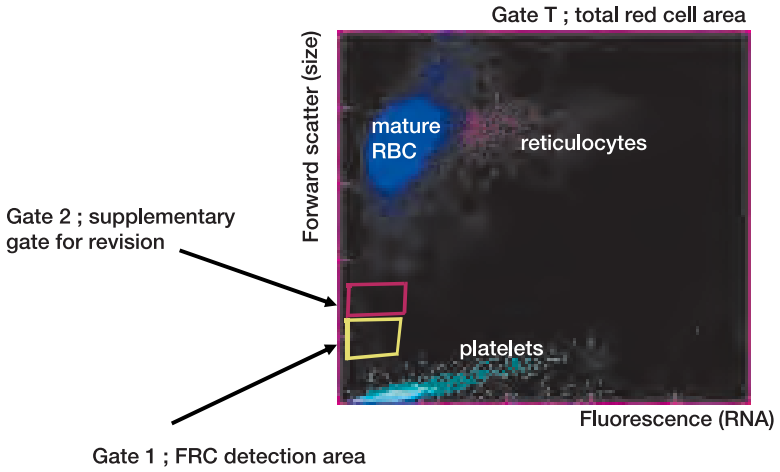

Fig. 1 *Scattergram of XE-2100 RET Channel*
The horizontal axis indicates the fluorescence intensity, which suggests the nucleic acid level in cell; and the vertical axis indicates the forward scatter, which suggests the cell size.

fied or when there are fragments close in size to platelets (fragmented red cells (FRCs) or fragmentations of leukemic cells). Differentiation of platelet maturity is done on the basis of size and RNA content. In fact, it was recently reported that PLT-O detected a patient with thrombotic thrombocytopenic purpura (TTP) after hematopoietic stem cell transplantation (SCT) whose platelet count by impedance had been determined to be higher falsely due to the presence of FRCs. The report suggests the usefulness of PLT-O. [2)]

PRINCIPLE AND CLINICAL APPLICATION OF RET MASTER

Fragmented red cells quantification system

Purpose of FRCs quantification

There are various underlying diseases known to develop symptoms with increased FRCs in peripheral blood (red cell fragmentation syndrome) (***Table 1***). [3)] Among these, those that are caused by microangiopathies often need immediate differential diagnosis and treatment.
Post SCT, stem cell transplant-associated thrombotic microangiopathy (TMA), a disorder similar to TTP can occur. Because the disorder requires immediate action such as dose reduction or suspension of immunosuppressive therapy, it is suggested that FRCs should be monitored over time. In the Zeigler's grading system that has been conventionally used for clinical diagnosis (***Table 2***) [1)] or in a more recent report of consensus meeting (***Table 3***), [5)] presence of certain level of FRCs is indicated as an important marker for diagnosis.
However, quantification of FRCs by manual counting is time consuming for busy laboratory technicians. Additionally, the differentiation of FRCs differs among observers. Therefore, the establishment of an automated counting method of FRCs has been awaited.
Transplant-associated TMA is also called "stem cell transplant-associated microangiopathy (TAM)" [5)], because it is not always complicated with thrombosis. [6)]

Automatic counting method of FRCs

In the situations described above, a study was started to utilize small-sized RBCs detected on the XE-2100 RET Channel as a parameter. A detection system was devised incorporating a supplementary gate (Gate 2) for minimization of the effects of small-sized normal RBCs that can be observed in iron deficiency anemia or other disorders (***Fig. 1***). [7)] In order to obtain FRC count, if there are a large number of small-sized RBCs and the proportion of RBCs detected by the Gate 2 reaches a certain level, the system does not automatically use the count detected by the Gate 1 — the gate for FRCs detection, but corrects the number using the count detected by the Gate 2 based on the relation between the two counts that was obtained experimentally.
Upon clinical application of the detection system, the users should bear in mind that the method is, as obvious from the principle of detection, entirely dependent on the size of cells, and thus tends to indicate false positive as other automatic counting methods often do. [8)] As one of the future improvements, we suggested a possible automatic counting method that uses fixated blood cells to reflect the characteristic morphologies of FRCs and consider the shapes of the fragments. [9)] However, the application to daily practice is not currently feasible.
The reference range of the FRC rate was calculated to be

Table 1 *Red cell fragmentation syndrome (modified from Reference 3)*

Cardiovascular disorders or macroangiopathies related:
• Prosthetic valve • Valvuloplasty • Valvular disorder • Chordal rupture • Infectious endocarditis • Aortic aneurysm • Coarctation of the aorta
Microangiopathies related:
• Thrombotic thrombocytopenic purpura (TTP) • Hemolytic uremic syndrome (HUS) • Disseminated carcinomatosis (e.g. gastric cancer, breast cancer, lung cancer, pancreatic cancer) • Post chemotherapy (e.g. mitomycin, cisplatin, bleomycin) • During pregnancy or puerperal period (e.g. eclampsia or preeclampsia) • Disseminated intravascular coagulation syndrome (DIC) • Infectious diseases • Autoimmune diseases • Hemangioma • Malignant hypertension • Thermal burn • Hematopoietic stem cell transplantation • March haemoglobinuria

Table 2 *Zeigler's grading system (from Reference 4)*

Grade	LD	% of FRC	Clinical TMA
0	Normal or elevated	< 1.2%	Ruled out
1	Normal	> 1.3%	Pre clinical
2	Elevated	1.3 - 4.8%	Mild
3	Elevated	4.9 - 9.6%	Moderate
4	Elevated	> 9.7%	Severe

Table 3 *Draft of a new criteria for TMA diagnosis (from Reference 5)*

1	The proportion of fragmented RBCs in peripheral blood exceeds 4%.
2	Persistent or progressive thrombocytopenia (platelets count < 50,000/μL or decreased > 50% compared to the previous test)
3	Sudden or persistent elevation of LD (LDH)
4	Progress of anemia or increased need of blood transfusion
5	Decreased haptoglobin

0.03 - 0.56%, using the values obtained from 762 samples of healthy individuals, which was comparable to that of textbooks.

Application of the FRC rate (%) to microangiopathies

(1) Thrombotic thrombocytopenic purpura (TTP) / hemolytic uremic syndrome (HUS)

We evaluated the feasibility of the clinical application of this new parameter in 14 patients with child HUS, a typical microangiopathic hemolytic anemia, or with TTP. [10] The changes of parameters in a typical patient (aged 25 years, female, TTP) determined by automatic counting were shown in ***Fig. 2***. Decreases of the FRC rate, creatinine and thrombomodulin levels as well as recovery of platelet count were observed.

When we plotted the FRC rates of these TTP/HUS patients obtained by manual counting and by automatic counting, the two numbers correlated well and thus it suggested clinical applicability of the automatically counted FRCs (***Fig. 3***).

However, there were cases for whom FRCs were detected by visual examination but automatic counting could not detect them, although such cases are considered to be rare. ***Fig. 4*** shows the parameters of a patient with systemic lupus erythematosus (SLE) who exhibited decreased platelet count due to unknown causes during observation period. Although the disorder took a relatively slow progress initially, during certain period, FRCs increased rapidly. The patient must be the one who needs to be monitored over time.

However, for this patient, it should be noted that the measurements of automatic counting were extremely low, ranging from 0.1% to 1.5%, which suggests a possibility that automatic counting can indicate false negative. In the case of this patient, ADAMTS13 activity had been low consistently from the beginning to the time when the patient had a good response to plasma exchange and rituximab therapy and the platelet count improved. The association of the activity with the disease status was unclear. Right now, there is no marker to always reflect the disease status. We recognized again that comprehensive diagnosis is indispensable. It has been shown that ADAMTS13 activity tends to be decreased in some collagen diseases, [11] and that extremely low activities were observed in some cases with disseminated intravascular coagulation syndrome (DIC). [12]

(2) Transplant-associated TMA

In order to evaluate the usefulness of the FRC rate as a parameter for diagnosing TMA after SCT, we followed up the courses of 33 patients after stem cell transplantation (27 patients, allogeneic; 6 patients, autologous). [13] In patients who received autologous peripheral blood stem cell transplantation (auto-PBSCT), no increased FRC rate was observed. Of 27 patients who received allogeneic SCT, 2 exhibited increase in the FRC rate before transplantation both by automatic counting and by manual counting, and disappearance of FRCs after engraftment. [14] In hematologic diseases, the underlying disease needs to be considered as a cause of an emergence of FRCs. Thus, quantification of FRCs at blood counts before treatment or transplantation is necessary for accurate diagnosis.

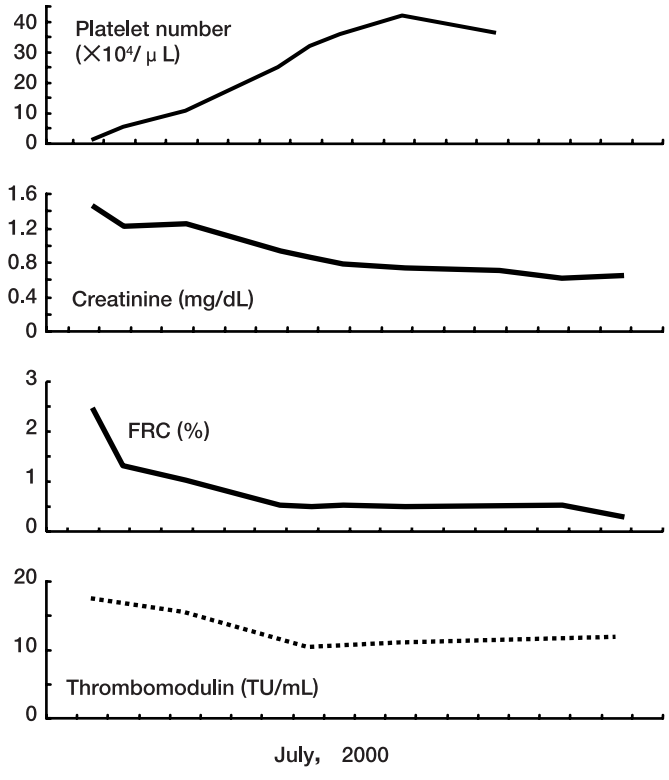

Fig. 2 *Changes of parameters in a TTP patient (aged 25 years, female)revision*

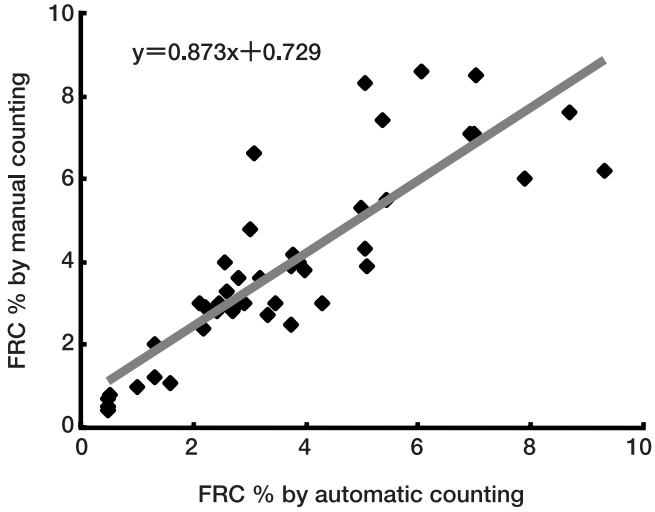

Fig. 3 *Correlation between the FRC rates detected by manual counting and automatic counting (from Reference 10)*

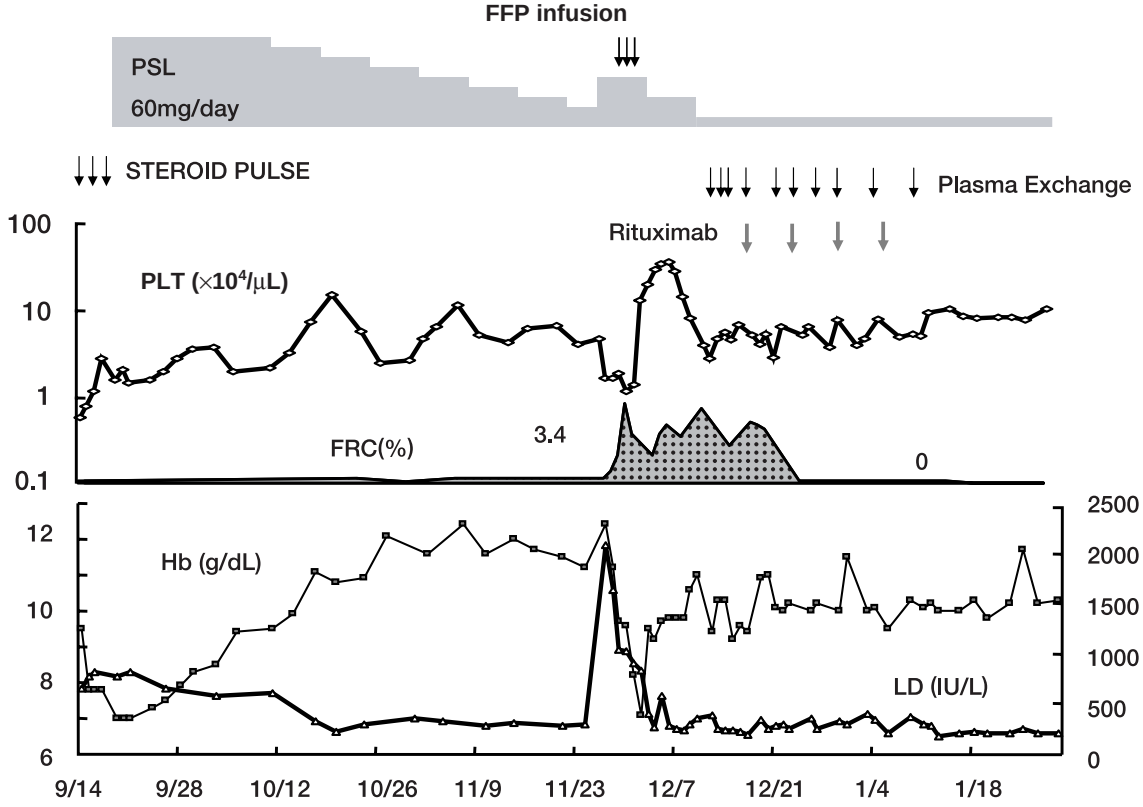

Fig. 4 *A patient with recurrent SLE, with robust thrombocytopenia*
FFP; fresh frozen plasma, PSL; prednisone, STEROID PULSE; high-dose methylprednisolone therapy, Rituximab; Rituxan (anti-CD20 monoclonal antibody)

Of 25 remaining patients, 23 exhibited elevation of FRC rate that exceeded the reference range during follow-up, and the FRC rate exceeded the level of the Zeigler's criterion (1.3%) in 11 patients (44%). An elevation of LD was also observed in 5 out of 11 patients, which suggested a possibility of TMA. Two of them were diagnosed to be TMA based on the change of platelet count or others. Therefore, the complication rate of TMA in these patients was 8%.

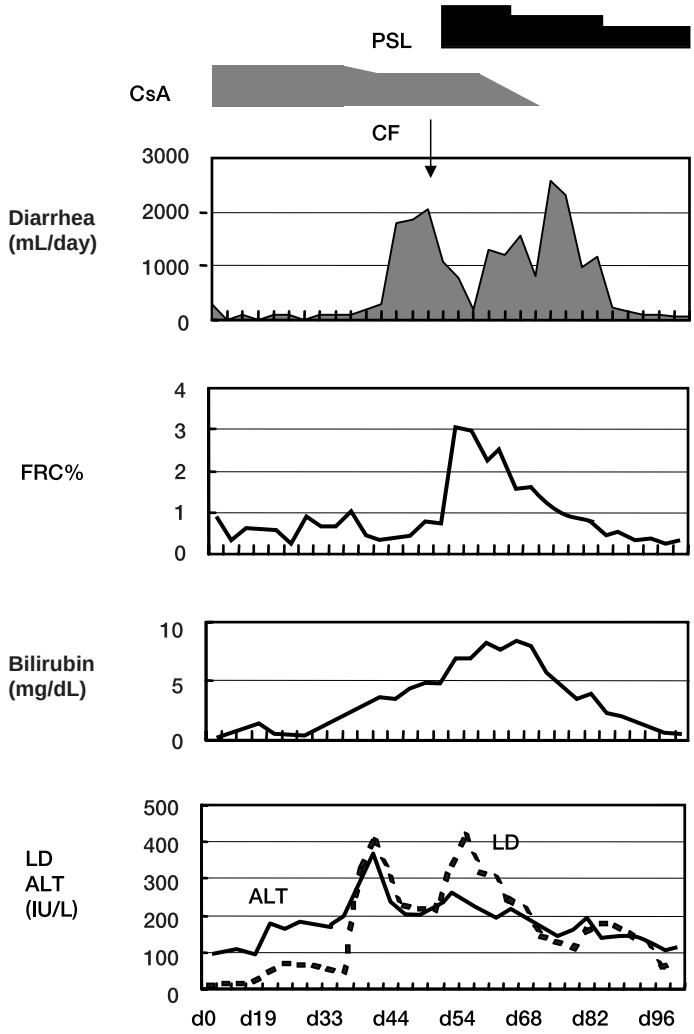

Fig. 5 *Parameters with SCT-associated THA (from Reference 13)*
CyA; cyclosporine, PSL; prednisone, CF; colon fiberscopy

Case report (Fig. 5)

A 34-year old male patient relapsed acute myeloid leukemia and received peripheral blood stem cell transplantation from his HLA- and ABO-matched sister. He had diarrhea after SCT, and because the cause was considered to be GVHD at first, steroid therapy was initiated. However, based on rapid increase of the FRC rate and findings of colon biopsy using fiberscopy, he was diagnosed with transplant-associated TMA. After suspension or dose reduction of cyclosporine and steroid administration, his symptoms resolved. As shown by this case, to grasp the fluctuation of the FRC rate in a timely manner with frequent monitoring is expected to be highly useful for making accurate diagnosis, even if it may not be early diagnosis.

According to Kanamori et al.[15] who reported the findings of visual examinations of FRCs after allogeneic transplantation, the FRCs increased in many patients and were higher among the patients complicated with TMA, which indicated a similar result to ours. However, they performed visual examinations once per two weeks. The fact is considered to reflect that it is difficult to monitor real-time fluctuation only by manual approach in practice.

Future progress

In recent years, various organ transplant methods made progresses in Japan, and the patients who received high-dose immunosuppressive therapy are increasing. Ito et al. [16] reported a patient with TMA post liver transplantation from a living donor for whom automatic counting of FRCs was used effectively. Thus, automatic counting of FRCs is expected to contribute to improved safety of transplantation therapy as a whole.

Reticulocyte hemoglobin equivalent (RET-He)

It was found that the hemoglobin level in reticulocytes can be estimated from the data of the reticulocyte sizes (forward scatter) and can be used for the diagnosis of functional iron deficiency. [17] This is considered to be a parameter equivalent to reticulocyte hemoglobin content (CHr) of Bayer's ADVIA 120 Hematology System. [18] The parameter has been shown to be a marker for appropriate administration of iron in dialysis patients during the use of erythropoietin. In future, further studies are needed to demonstrate a wider range of its usefulness.

CLINICAL RELEVANCE AND APPLICATION OF IPF MASTER

Reticulated platelets

In 1969, Ingram et al. [19] reported two types of reticular cells in new methylene blue stained peripheral blood specimens obtained from dogs. One of them was "reticulocyte," immature red cell, which is a highly useful marker of erythropoietic process in laboratory tests at diagnosis and follow-up of anemia. The parameter is now widely used because of the recent prevalence of automatic counting systems, in addition to manual counting methods using supravital staining.

Another type of reticular cells is "reticulated platelet," that is, immature platelet. Although its clinical usefulness in thrombocytopenia was recognized, it has been rarely applied to clinical practices because the quantification is not easy. Expensive monitoring equipment such as flow cytometer (FCM) are necessary, and standardization of the detection methods is difficult due to varying methods in individual institutions.

Quantification of immature platelet fraction

As mentioned above, platelet count indicated by RET Channel, PLT-O, is a clinically useful parameter. In addition, a method was developed to quantify relatively large platelet fractions with high RNA content (***Fig. 6***) as the immature platelet fraction (IPF) using the XE-2100. The parameter is considered to be usable as an alternative of reticulated platelet that has conventionally been determined by FCM. There is heterogeneity in platelet size, and immature platelets are differentiated to have larger fractions and are known to have higher functions. [20] Thus, reticulated platelets and IPFs are considered to consist of larger number of large platelets.

Although it is not clear to which fraction detected by conventional methods by FCM, IPF corresponds, development of automatic counting method must be highly useful in standardizing reticulated platelet counting as a global common marker, aiming for its clinical application. Currently, many institutions are examining feasibility of the clinical application and going to report the results.

A study reported that the normal range of the IPF rate (%) was 3.29% (1 - 10.3%) for male and 3.28% (1.1 - 9.5%) for female and the IPF rate correlated negatively with the platelet count. [21] Another study reported that there was no difference in the IPF rate between male and female and it is 2.7 ± 1.4% (MEAN ± SD), with the reference limit of 6.9% or less (MEAN + 3SD). [22] In this article, "IPF" mentioned below refers to the IPF rate (%) unless otherwise noted.

Application of IPF to differential diagnosis of thrombocytopenia

Classification of thrombocytopenia

As shown in ***Table 4***, causes of true thrombocytopenia are conventionally classified into diminished platelet production in bone marrow, increased platelet destruction in peripheral blood, and abnormal distribution. For precise differential diagnosis of thrombocytopenia, check of myelopoiesis (e.g. bone marrow aspiration) is needed. However, in real clinical settings, it is desired to make accurate diagnosis only with peripheral blood tests or

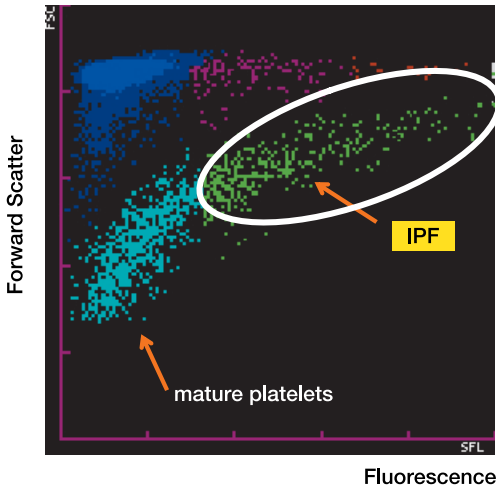

Fig. 6 *Scattergram of XE-2100 RET Channel for detecting IPFs*
IPF; immature platelet fraction

Table 4 *Diseases associated with decreased platelet count*

1. Diseases due to failed platelet production	2. Diseases involved with shortened platelet survival
1) With multilineage cytopenia Aplastic anemia, acute leukemia, myelodysplastic syndrome, megaloblastic anemia, metastasis to bone marrow, drug-induced thrombocytopenia, etc. 2) Decrease in platelet only Amegakaryocytic thrombocytopenia	1) Immunologic destruction Idiopathic thrombocytopenic purpura, drug-induced thrombocytopenia, etc. 2) Increased platelet use Thrombotic thrombocytopenic purpura, hemolytic uremic syndrome, disseminated intravascular coagulation syndrome, massive bleeding, etc. 3) Abnormal distribution Spleen, hemangioma, etc.

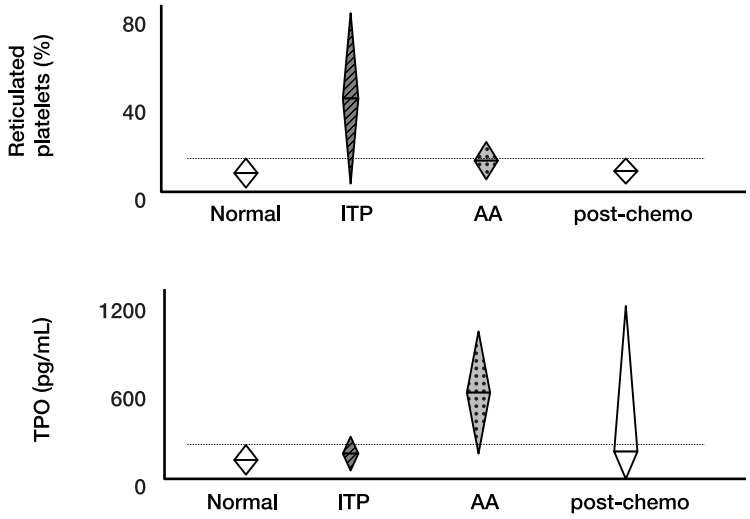

Fig. 7 *Differential diagnosis of thrombocytopenia based on the reticulated platelet rate and the blood throm bopoietin (TPO) level (modified from Reference 23)*
ITP; idiopathic thrombocytopenic purpura, AA; aplastic anemia, post-chemo; post chemotherapy patient
A horizontal line in each diagram indicates the upper limit of reference level.

noninvasive examinations. Kurata et al. [23,24] suggested that in differential diagnosis of aplastic anemia (AA) and idiopathic thrombocytopenic purpura (ITP), it was impossible to differentiate the diseases completely based on the size of platelets or platelet associated IgG (PAIgG), and that it was useful to combine the detection of reticulated platelet by FCM and the quantification of blood thrombopoietin (TPO). It is expected that, in ITP, the former is high and the latter is close to normal level, and in AA, the former is low and the latter is high (***Fig. 7***). In addition, it was demonstrated recently that detection of anti-glycoprotein IIb/IIIa antibody producing lymphocytes by using ELISPOT assay was effective for differential diagnosis of ITP, and a new diagnostic criteria was proposed. [25]

Application of IPF to differential diagnosis of thrombocytopenia

As shown above, it is obvious that counting of reticulated platelets is useful for differential diagnosis of thrombocytopenia. Currently, studies are underway to examine to what extent XE-2100's IPF is useful.
Briggs et al. [26] reported a clinical evaluation of IPF measurements and suggested that CV (%) was 2.04 - 14.17% for 10 measurements, that it was relatively stable for 48 hours when stored at room temperature, that IPF increased significantly during acute stage of ITP, and that IPF indicated high levels during decrease of platelets when ITP or TTP was followed up. In terms of the shelf life of the specimens, there was a report to suggest that it should be within one day, [22] and thus it must be better to use them for the measurement as soon as possible.

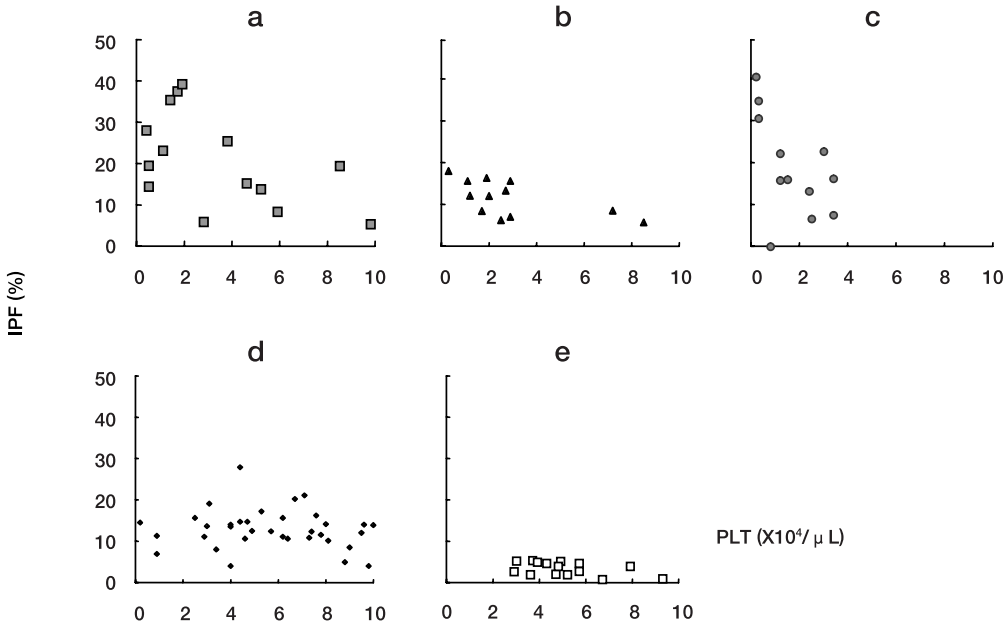

Fig. 8 *Relation of platelet count and IPF rate (%) in each disease (from Reference 27)*
a; idiopathic thrombocytopenic purpura (ITP), b; aplastic anemia (AA), c; myelodysplastic syndrome (MDS), d; thrombotic thrombocytopenic purpura / hemolytic uremic syndrome (TTP/HUS), e; hepatic cirrhosis

We also presented measurement results of IPF in thrombocytopenia due to various diseases (***Fig. 8***). [27] In ITP, IPF indicated extraordinarily high level when a decrease in platelet count was significant. In AA, the level was slightly high; in TTP/HUS, the level was moderately high; and in myelodysplastic syndrome (MDS), the difference between the patients was larger. In hepatic cirrhosis, IPF was obviously lower than the other diseases.
The results for ITP were similar to those reported previously. However, because there must be involvement of anti-platelet antibodies reaching to megakaryocytes, [28] other factors need to be examined as a cause other than stimulation of platelet production in bone marrow due to platelet destruction in peripheral blood. On the contrary, Nishiyama et al. [22] concluded from their study on absolute IPF count that ITP did not necessarily exhibit increased platelet production. It suggests a necessity to examine further from different aspects.
In MDS, IPFs differed largely among patients, which suggested complexity of its diagnosis. In this disease, megathrombocytes are sometimes produced. Because IPFs are determined by RNA content per platelet, they can vary a great deal. A program to calculate RNA content per unit volume might be needed in future. In fact, Wang et al. [25] suggested that it was useful to utilize mean fluorescence intensity (MFI) combinedly for predicting platelet recovery following chemotherapy. For patients of our study, we could not calculate the number per unit volume because it was not possible to determine mean platelet volume (MPV) due to small platelet count.
With regard to decreased platelet count associated with hepatic cirrhosis, it has been described conventionally that increased splenic function, i.e., decreased platelet count due to platelet destruction in the periphery was a central factor to cause it. However, it appears to be consistent with the result that decreased function of bone marrow is strongly associated with it, as Panasiuk et al. [30] suggested. In fact, bone marrow examinations of patients with hepatic cirrhosis revealed marrow hypoplasia. [31] Thus, the fact that IPF is low in hepatic cirrhosis cases is considered to be useful for differential diagnosis.

Results in collagen diseases [27]

In patients with collagen disease, decreased blood cells and/or platelets are often observed. Some of the diseases include them in the diagnostic criteria. As shown in ***Fig. 9***, a study on 51 cases in the Department of Clinical Pathology and Immunology of Kobe University suggested that IPF could vary largely, ranging from several percent to nearly 40% when platelet count was decreased among the patients.
The changes of IPF and platelet count in two typical cases are shown in ***Fig. 10*** and ***Fig. 11***. In ***Fig. 10***, the SLE patient exhibited significantly high IPF. Because IPF was decreased when the platelet count temporarily recovered after splenic artery embolization, the disease took a similar course to ITP.
In ***Fig. 11***, the patient with eosinophilic fasciitis had a relatively high IPF (15 - 20%) initially. The parameter was considered to indicate ITP-like disorder, and the patient was treated but with no response. Subsequently, AA-like marrow hypoplasia was confirmed in a bone marrow clot section.
Although there are also complicated clinical conditions other than the diseases presented here due to the effects of TMA, hemophagocytic syndrome, multiple drugs and others, it was demonstrated that IPF could be effective for differential diagnosis of decreased platelets. Ongoing

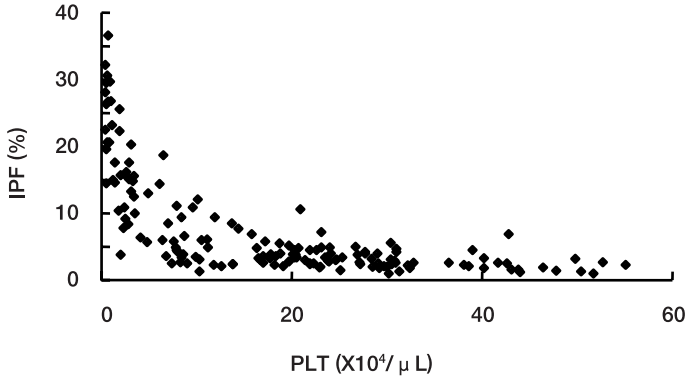

Fig. 9 *Association of platelet count and IPF (%) in patients with various collagen diseases (from Reference 27)*

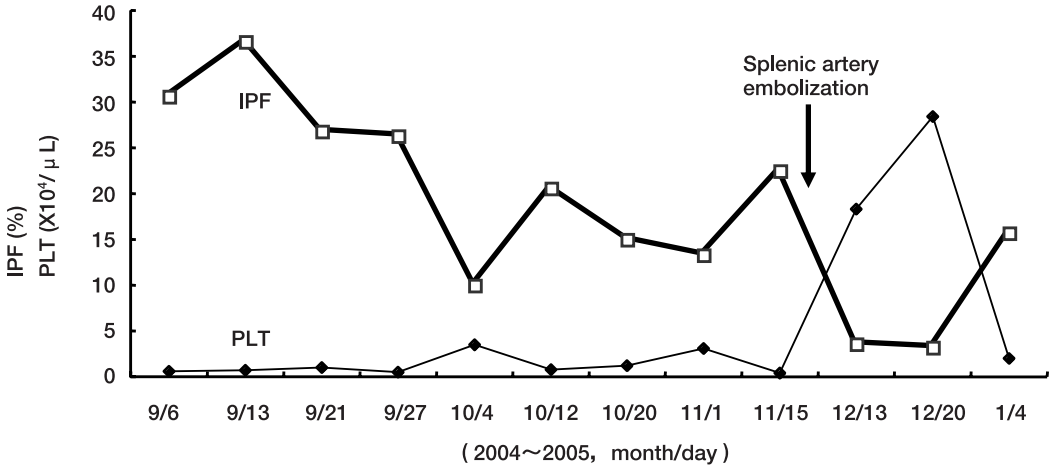

Fig. 10 *Clinical course of an SLE patient with decreased platelets (from Reference 27)*

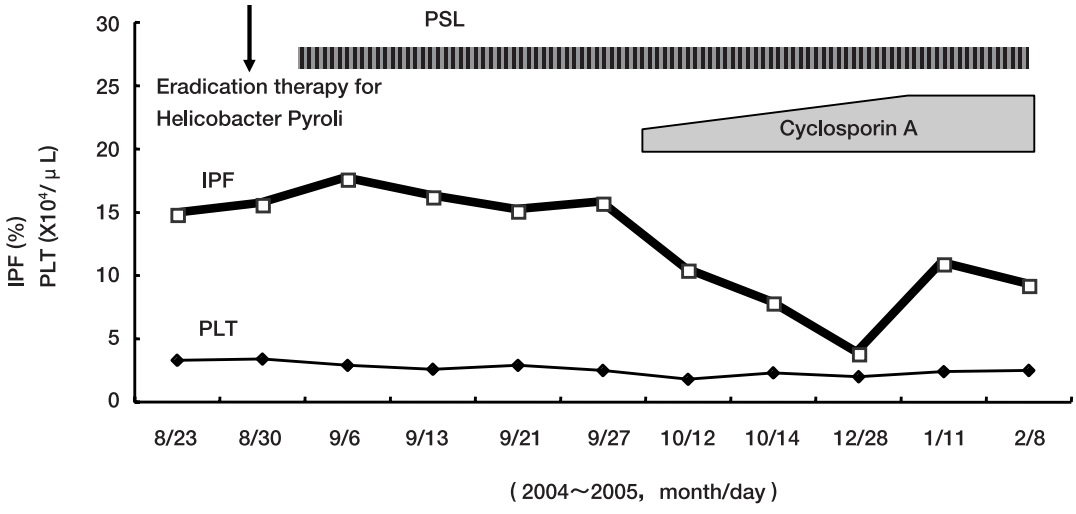

Fig. 11 *Clinical course of an eosinophilic fasciitis patient with decreased platelets (from Reference 27)*

accumulation of cases will be necessary for elucidating the respective clinical condition of various disorders, especially of commonly observed disorders such as hemophagocytic syndrome.

Applicability of IPF to the decision on the timing of platelet transfusion

Practice of platelet transfusion

In many cases, such as hematologic malignancies, platelet transfusion is performed as a prophylaxis when platelet count is decreased after chemotherapy. Although it is difficult in practice, ideally platelet transfusion is performed as indicated by the data of morning blood test, or the transfusion trigger. [32)]

One of the reasons is that the minimum level of platelet to prevent bleeding is not clear. Another major reason is that platelet preparations should be ordered the day before use because the shelf life of the preparations is only 72 hours by Japanese regulation. For the minimum level of platelet, several control studies were conducted in the U.S. and Europe. Some of them reported that there was no change in bleeding risk when the transfusion trigger was set to 10,000/μL compared to 20,000/μL, and naturally, the transfusion cost was reduced when the trigger was set to be lower. The blood transfusion guideline in Japan states that platelet transfusion is indicated for the platelet count of less than 10,000 - 20,000/μL after chemotherapy.

Feasibility of IPF as a marker for the decision on the timing of platelet transfusion

Stohlawetz et al. [33)] reported that in 7 patients with acute myeloid leukemia, the rate of reticulated platelets (%) increased on Day 17 - 24 of chemotherapy, and that after 1 - 3 days platelet recovery was observed, using reticulated platelet measurement by FCM. Wang et al. [29)] concluded that it was possible to predict platelet recovery within 42 hours based on reticulated platelet measurement by FCM method, combined with MFI as mentioned above. The positive predictive value was 82%, and the negative predictive value was 91%.

Chaui et al. [34)] monitored auto-PBSCT patients using FCM, and reported that increase of platelet count could be expected in 76% of the patients within 4 days because of recovered bone marrow function if the rate of reticulated platelets reached a certain level on Day 8. Briggs et al. [35)] reported their application of XE-2100's IPF to hematologic diseases. They reported that after chemotherapy an elevation of IPF rate was observed several days before platelet recovery and that IPF elevated (7.7 - 18.9%) 1 - 2 days before platelet recovery in auto-PBSCT patients, suggesting applicability of IPF to the decision on the timing of platelet transfusion. In addition, they suggested that while the patient had sepsis, an elevation of IPF without platelet recovery was observed probably through the mediation of cytokines including interleukin-6, and that following platelet transfusion, a decrease of IPF was observed probably because of reduced production of TPO. These findings need to be considered at clinical evaluation. Indeed, it was reported that blood TPO concentration decreased significantly following effective platelet transfusion. [36)]

A report from Japanese researchers can be found in that of Takami et al. [37)], Kanazawa University, on IPF rate following allogeneic SCT. IPF exceeded 3% on 11th day in PBSCT, 18th day in bone marrow transplantation (BMT), and 19th day in cord blood transplantation (CBT). It was 1, 4, and 14 days, respectively, before platelet engraftment. The study suggested an applicability of IPF to the evaluation of bone marrow function. However, it also seems that its application to the decision on the timing of platelet transfusion in PBSCT or CBT were not clinically relevant.

Observation in children treated with chemotherapy

We followed 11 patients of the Department of Pediatrics, Kobe University, following chemotherapy (17 courses in total), and examined the applicability of IPF to the decision on the timing of platelet transfusion. The age of the patients ranged from 0 to 15 years. Six patients had acute leukemia, and the other had solid tumor of various organs. After chemotherapy, as shown in ***Table 5***, the patients were classified into 4 groups based on whether there was a decrease of platelet count down to 20,000/μL or less and on whether there was a peak IPF elevation observed. In Group 2 (6 courses of chemotherapy), the platelet counts recovered to 30,000/μL or more within 3 days, whereas they took longer in Groups 3 and 4. The mean of peak IPF in Group 2, 3, and 4 was 12.1%, 8.3%, and 4.6%, respectively. For chemotherapy in pediatrics, if peak IPF exceeds 10%, it can be predicted that platelet recovery may be observed within several days. Our study suggested that it was highly possible to avoid useless platelet transfusion based on IPF data.

Table 5 *Classification of children based on clinical course after chemotherapy*

	Group 1	Group 2	Group 3	Group 4
Decreased platelet count *1	Not observed	Observed	Observed	Observed
IPF elevation	—	++	+	—
Time to platelet recovery (days) *2	—	< 3	5 - 6	3 - 7
Mean of peak IPF (%)		12.1	8.3	4.6

*1 < 20,000/μL
*2 Time from the day of IPF peak to the day when the platelet count reached 30,000/μL without transfusion.

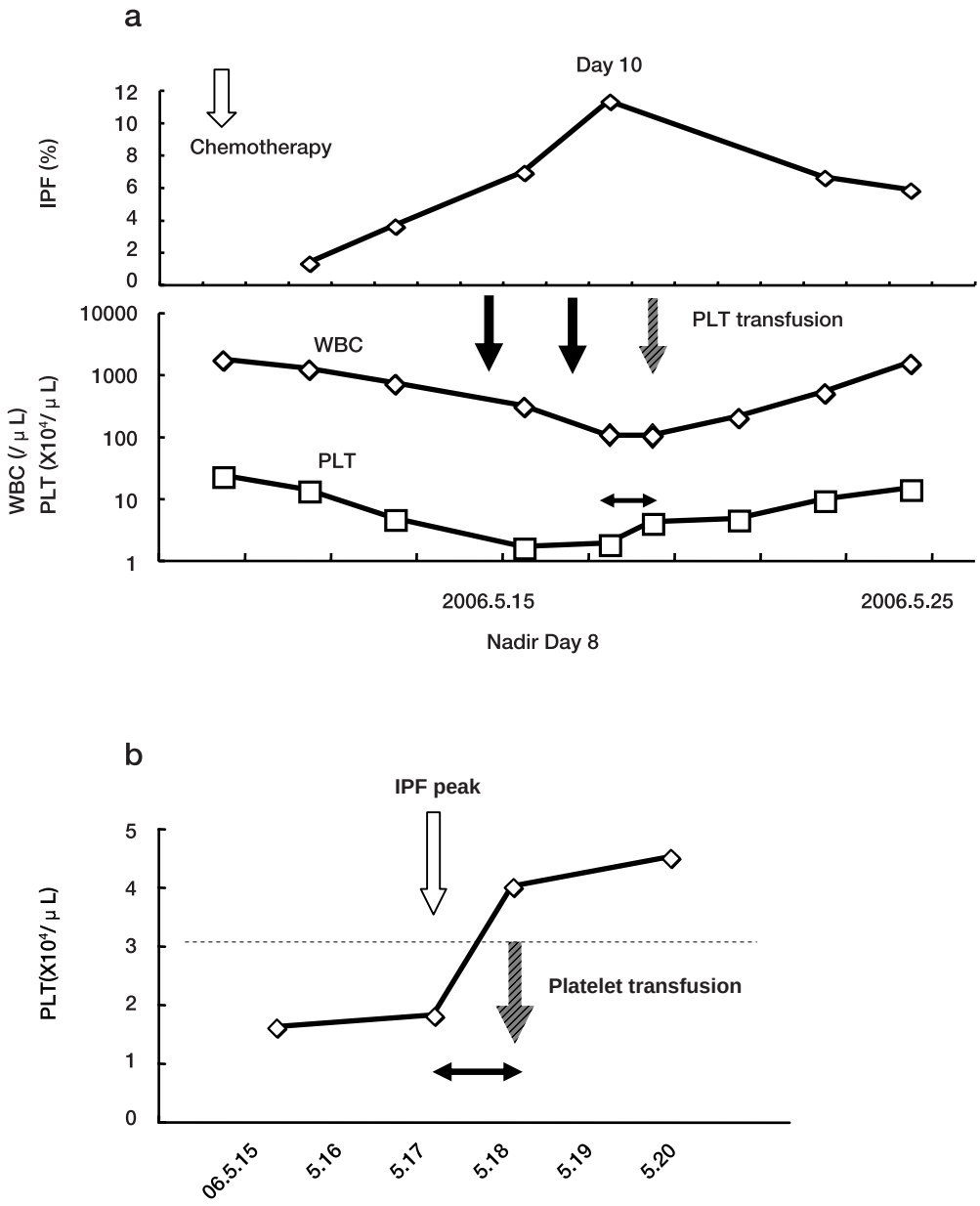

Fig. 12 *Clinical course of a patient with child rhabdomyosarcoma observed by IPF, platelet count, and platelet transfusion (from Reference 31)*
a; overall changes, b; time of decreased platelet count

Case report (Fig. 12)

The patient was 13 years old who had rhabdomyosarcoma. On Day 10 after the initiation of chemotherapy, IPF reached the peak and indicated 11.3%. During this period, three platelet transfusions were performed as shown in the figure. However, the third one should be considered to be unnecessary because of being carried out after IPF had exceeded 10% and platelet recovery had already started. In this case, the transfusions were performed as scheduled previously based on the prediction of platelet decrease. Such situations are common in clinical settings. However, if the patient does not have other bleeding risks such as fever and/or DIC, it is recommended to refer to IPF in real-time for the purpose of effective use of platelet transfusion, in order to achieve appropriate use of resources, prevention of adverse reactions, and reduction of medical cost.

We are planning to evaluate the effects of IPF used in clinical practice on the differences in the volume of platelet transfusion as well as bleeding risks in future.

Applicability of parameters to thrombotic disorders

Mean platelet volume (MPV)

We were interested in platelet size distribution, and reported the clinical relevance of the mean platelet volume (MPV). As mentioned above, immature platelets are larger and contain relatively large amounts of intraplatelet physiologically active substances while the cell surface charge is low. Thus, we assumed that larger platelets would be used first at an onset of a thrombotic event. When we treated chronic cerebral infarction patients with antiplatelet agent and observed the change of MPV, 50% of them did exhibit a decrease in MPV. This result led us to think that the parameter might be usable as an assessment scale of antiplatelet therapy. [38] Although there were some reports on topics similar to our idea, [39] whether MPV is truly effective or not from such perspective is still unknown.

Reticulated platelets and thrombotic disorders

Rinder et al. [40] demonstrated that there was no change in platelet count but the rate of reticulated platelets measured by FCM was high in the group of patients with thrombocytosis and thrombotic complication. They reported that the change was also observed in the patients 24 hours prior to the complication of thrombosis. In addition, the report suggested that reticulated platelet counts decreased significantly in the aspirin treatment group, although the association with the MPV change mentioned in the previous section is unclear. However, these symptoms have not been observed in deep venous thrombosis or arterial thrombosis patients with normal platelet count. Nakamura et al. [41] reported that the rate of reticulated platelets elevated in patients with cardiogenic stroke.

On the contrary, McCabe et al. [42] concluded that the rate of reticulated platelets did not demonstrate a substantial usefulness. Although the rate was significantly higher in the patient group ($p = 0.047$) if the result was adjusted by age, overall changes were not significant when they determined it in patients with cerebral infarction or transient ischemic attack.

As shown above, the results of the reports so far are not consistent. However, we expect that these parameters can be utilized as new monitoring method of thrombotic disorders when used combinedly with other parameters.

Other applications

Stiegler et al. [43] measured reticulated platelet count by FCM in patients with hyperthyroidism and demonstrated that the numbers of their reticulated platelets elevated apparently and platelet turnover was increased in the state of hyper thyroid function. The report suggests a possibility of the reticulated platelet count to be used for the evaluation of drug effect and is very interesting.

CONCLUSION

In this article we introduced new functions of XE-2100 using its RET Channel mainly based on our experiences. Clinical applications of these parameters can extend more and more, and further progress of the studies on laboratory hematology is awaited. Meanwhile, we would like to search for and develop another new parameter.

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