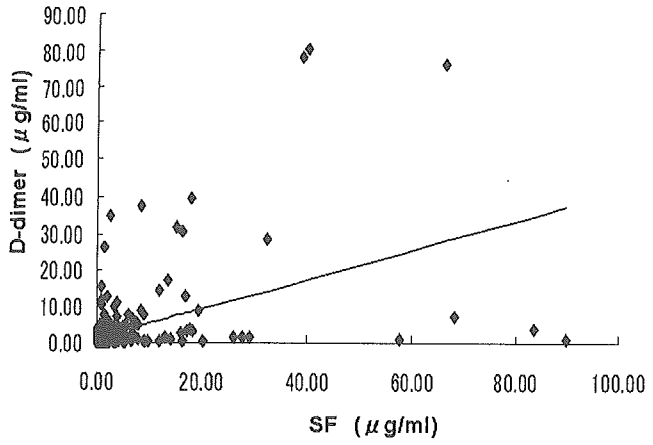

Fig. 5. Correlation between plasma concentrations of SF and D-dimer.

in patients with DVT, suggesting that the cutoff value is less than the above values. When the cutoff values were set at the average + 1 SD (D-dimer, 1.8 μg/mL; SF, 1.4 μg/mL) of those of healthy volunteers, 1 and 0 patient with DVT was overlooked, respectively. In these cases, the sensitivity was 95% for D-dimer and 100% for SF, with respective specificities of 61.9% and 53.8%. The number of patients requiring further vascular imaging studies diminished from 243 to 104 patients based on the use of D-dimer and to 123 patients based on SF. The estimated sensitivity in our study was similar to that in previous report using other D-dimer kits (0.5 μg/mL cutoff value) [6]. With regard to the difference between SF and D-dimer, SF reflects early phase of DVT/PE while D-dimer reflects secondary fibrinolysis after clot formation. In our study, plasma D-dimer concentrations correlated well with those of SF, suggesting that secondary fibrinolysis might occur immediately after clot formation. When the cutoff values were set at 2.5 μg/mL for D-dimer and 6.9 μg/mL for SF, only one DVT patient was overlooked, and the sensitivity and specificity were 95% and 69.5%, suggesting that both measurements of D-dimer and SF increase the sensitivity and specificity for the diagnosis of DVT/PE. The overlooked patient had chronic PE and he had small size thrombus.

However, as the hemostatic abnormalities are more significant in DVT patients with PE than those without PE [21], the difference between patients with PE and without PE was not significant in this study. This is probably because our study was too small and the PE was not severe.

Our data also suggest that both D-dimer and SF are useful for the diagnosis of DVT as NPV to reduce further examinations. However, the estimated cutoff values of these parameters for the diagnosis of DVT are higher in Japan than in Europe and in the U.S. Accordingly, we believe it is important to state the cutoff value of D-dimer in each measurement kit.

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Usefulness of Measurement of Reticulated Platelets for Diagnosis of Idiopathic Thrombocytopenic Purpura

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Summary: Reticulated platelets (RP) and large platelets (LP) were measured by an automated hematology analyzer (modified R-2000) in 287 healthy volunteers and 131 patients with thrombocytopenia or thrombocytosis. RP was significantly higher in patients with idiopathic thrombocytopenic purpura (ITP), especially in active phase, while RP was markedly lower in patients with essential thrombocytosis (ET) or chronic myelocytic leukemia (CML). LP was significantly higher in patients with ITP, especially in active phase, while LP was markedly lower in patients with aplastic anemia (AA), ET, or CML. In ITP, RP and LP were significantly higher in patients positive for anti-glycoprotein (Gp) IIb/IIIa antibody. RP and LP were poorly correlated with platelet-associated IgG (PAIgG). RP and LP were poorly correlated with plasma thrombopoietin levels, and negatively correlated with platelet count. These results show that RP reflects the pathology of thrombocytopenic disorders, and that measurement of RP is useful for the differential diagnosis and analysis of platelet kinetics.
Key Words: Idiopathic thrombocytopenic purpura—Reticulated platelets—Large platelets.

Thrombocytopenia is a common hematologic abnormality that is caused by decreased production, enhanced destruction, and sequestration of platelets. It is difficult to determine the pathogenesis of thrombocytopenia. The presence of normal or increased numbers of megakaryocytes in the bone marrow essentially excludes the diagnosis of hypoplastic thrombocytopenia due to aplastic anemia (AA) (1) or amegakaryocytic thrombocytopenia (2). No simple diagnostic test exists to diagnose thrombocytopenias caused by enhanced destruction of platelets such as idiopathic thrombocytopenic purpura (ITP) (3,4), disseminated intravascular coagulation (DIC) (5), and thrombotic thrombocytopenic purpura (TTP) (6). Platelets were found to have a coarse, punctuated reticulum (reticulated platelets; RP) using a new methylene blue dye in 1969 (7). It was suggested that those cells might contain increased amounts of cytoplasmic RNA, reflecting thrombopoiesis in bone marrow. Subsequently, it was reported that platelet nucleic acid could be detected with flow cytometry utilizing thiazole orange as a dye (8). RNA-rich platelets were reported to be readily measurable by flow cytometry and to provide information on thrombopoietic activity in thrombocytopenic patients (9). Analysis of RP was found to provide a good estimate of the rate of platelet production in bone marrow (10). ITP is an autoimmune disease characterized by increased platelet clearance caused by anti-platelet autoantibodies (4). In this disorder, platelet production is normal or increased but platelet destruction in the spleen is enhanced. Patients with ITP usually have increased megakaryocytes in the bone marrow, and are believed to have high RP (11,12) and

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normal TPO levels (13,14). On the other hand, patients with AA have low counts of platelets, leukocytes, and erythrocytes in peripheral blood and hypoplastic bone marrow.

In the diagnosis of immune thrombocytopenia, flow cytometric analysis of RP was reported to be better than determination of platelet-associated IgG (PAIgG) (15). The flow cytometric analysis of RP has been suggested to be useful for evaluating thrombocytopenia. Recently, an instrument for fully automated measurement of RP (modified R-2000 fully automated reticulocyte counter) capable of measuring the RP in peripheral whole blood immediately was developed.

In this study, we measured the RP in healthy volunteers and patients with underlying diseases causing thrombocytopenia, especially ITP, to examine the usefulness of RP measurement for differential diagnosis of thrombocytopenic disorders.

MATERIALS AND METHODS

RP was measured in 287 healthy volunteers (144 females and 143 males; median age, 32 years; range, 21–60 years). RP was also measured in 131 patients with thrombocytopenia (86 females and 45 males) due to various diseases: 76 with ITP, 37 with AA, 5 with systemic lupus erythematosus (SLE), 2 with myelodysplastic syndrome (MDS), and 11 patients with essential thrombocythemia (ET) or chronic myelogenous leukemia (CML). The study protocol was approved by the Human Ethics Review Committees of Mie University School of Medicine. ITP was diagnosed by thrombocytopenia, normal bone marrow with an increased number of megakaryocytes and lack of other disease causing thrombocytopenia; the disease condition was defined as follows: active phase, less than 50,000/μL platelet count; partial remission (PR), 50,000–120,000/μL platelet counts with or without treatment; complete remission (CR), more than 120,000/μL platelet count without treatment. Aplastic anemia was diagnosed by thrombocytopenia, anemia, and leukocytopenia without other disease causing pancytopenia; active phase, less than 50,000/μL platelet count; partial remission (PR), 50,000–100,000/μL platelet count with or without treatment; complete remission (CR), more than 100,000/μL platelet count without treatment.

RP was measured by a previously described method (16). Briefly, approximately 2 mL of whole blood was collected from healthy volunteers or patients and was anticoagulated with ethylene diamine tetraacetic acid (EDTA)-2K. All blood samples were kept at room temperature until analysis. Counts of RP are reported to be stable during 2–6 hours after collection (17). Total platelet counts and ratio of RP were determined by the R-2000 (Sysmex Inc., Kobe, Japan) with special software (17,18). For measurements of RP, 10 μL of blood sample was aspirated by the R-2000 and automatically mixed with 40 μL of 2.6% auramine O in 95.9% ethylene glycol solution (Ret-Search, Sysmex Inc.) to stain the RNA present in reticulocytes and RP. The samples were then irradiated by an argon laser beam at a wavelength of 488 nm. The resulting fluorescence intensity and forward light scatter were measured with flow cytometry (Fig. 1) (17). The ratio of RP was expressed as the percentage of total platelet count. The time required to complete all these measurements was approximately 90 seconds.

Serum TPO concentration was measured by a previously described sandwich ELISA (13,19). PAIgG was measured using alkaline phosphatase-conjugated anti-human IgG (Sigma Co, St Louis, MO) with a competitive enzyme immunoassay (12). Antibody to platelet glycoproteins (GP) IIb/IIIa was detected with a platelet antibody screening kit (GTI PAKPLUS, Wisconsin). Patient or donor serum was incubated in microtiter wells that had been pre-coated with platelet glycoproteins. This allows antibody, if present, to bind. Unbound immunoglobulins were then removed by washing. An alkaline phosphatase labeled anti-human globulin reagent was added to the wells. After a brief incubation period, microtiter wells were washed to remove any unbound anti-human globulin, and the enzyme substrate PNPP (p-nitrophenyl phosphate) was added. Following a short incubation, the reaction was stopped by sodium hydroxide solution, and the optical density of the color produced was measured in an ELISA reader at a wavelength of 405 or 410 nm.

Statistical Analysis

Results are expressed as mean ± SD. Differences between groups were evaluated by one-way ANOVA and the Bonferroni test. For assessment of correlations, coefficients of correlation were determined by Pearson's correlation coefficient and linear regression analysis. In both cases, a level of $p < 0.05$ was considered significant.

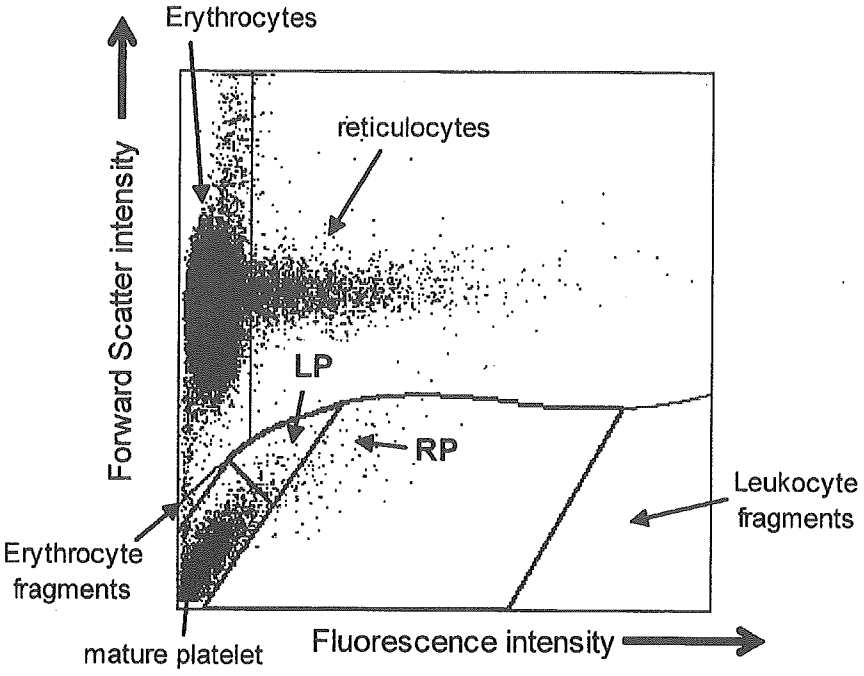
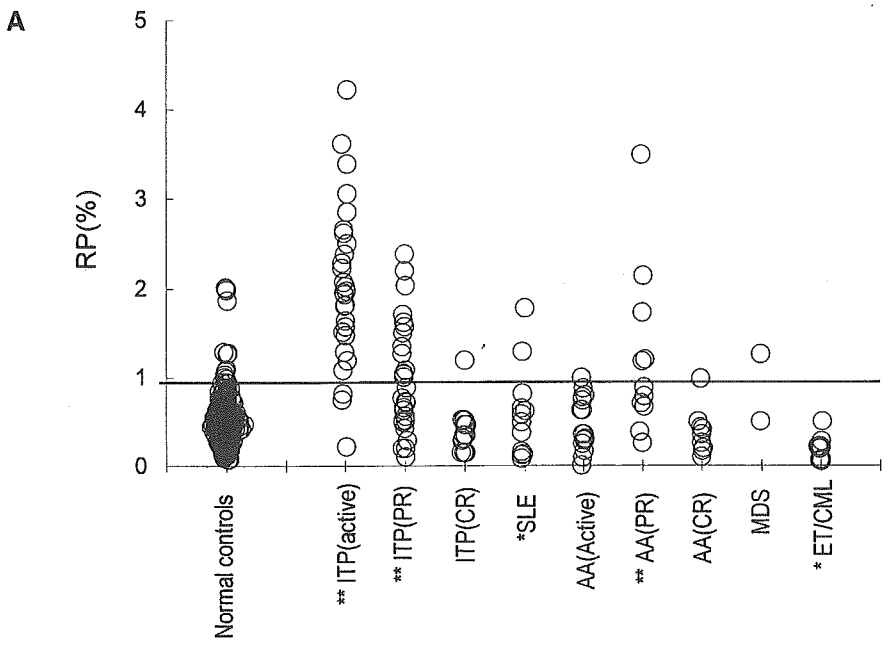


FIG. 1. Dot plot showing fluorescence intensity versus forward scatter intensity. Each line automatically discriminates RP, mature red blood cells, reticulocytes, mature platelets, and white blood cell fragments.

TABLE 1. RP, LP, and Platelet Counts in Healthy Volunteers and Patients with Thrombocytopenia

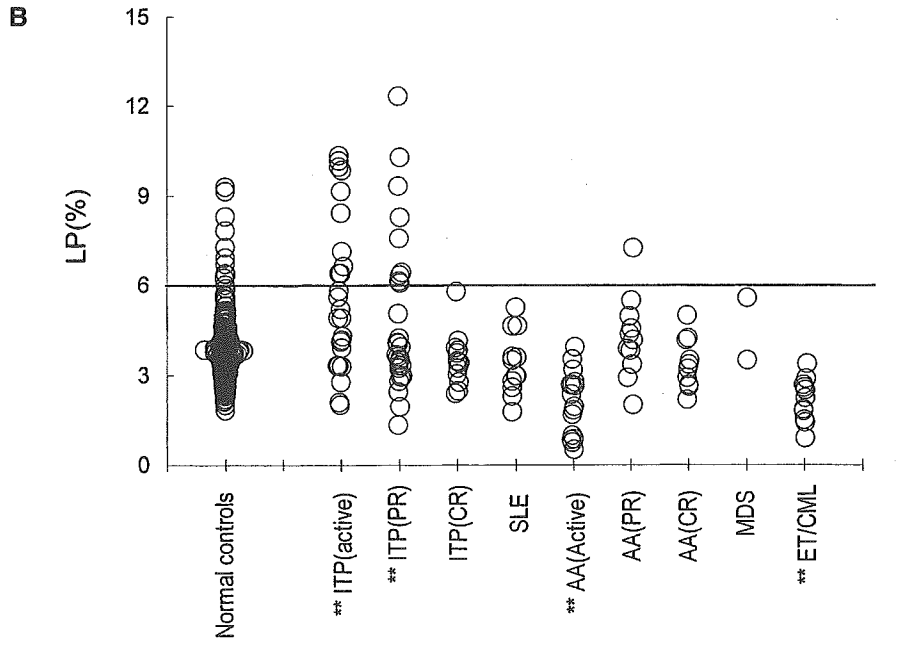
Subjects	N	RP (%) (mean ± 1SD)	Platelet Count ($\times 10^9$/L) (mean ± 1SD)	LP (%) (mean ± 1SD)
Normal controls	287	0.48 ± 0.29	247.5 ± 48.6	3.78 ± 1.14
ITP (all)	76	1.21 ± 0.94	119.0 ± 89.1	5.17 ± 3.44
Active phase	28	2.03 ± 0.88	45.5 ± 20.6	6.80 ± 4.42
PR phase	28	0.98 ± 0.61	116.8 ± 57.6	4.85 ± 2.60
CR phase	20	0.39 ± 0.25	225.1 ± 78.1	3.33 ± 0.89
AA (all)	37	0.66 ± 0.65	91.7 ± 60.0	3.03 ± 1.44
Active phase	17	0.46 ± 0.29	34.5 ± 13.6	2.04 ± 1.00
PR phase	11	1.21 ± 0.89	122.2 ± 35.2	4.24 ± 1.32
CR phase	9	0.38 ± 0.25	162.6 ± 24.4	3.42 ± 0.85
SLE	5	0.92 ± 0.56	298.4 ± 69.5	3.87 ± 0.92
MDS	2	0.88 ± 0.38	119.0 ± 8.0	4.54 ± 1.05
ET/CML	11	0.20 ± 0.12	740.8 ± 603.2	2.15 ± 0.70



* $p < 0.05$

** $p < 0.01$

The horizonal line indicates mean+2SD.



** $p < 0.01$

The horizonal line indicates mean+2SD.

FIG. 2. RP and LP in healthy volunteers and patients with thrombocytopenia or underlying diseases. ITP, idiopathic thrombocytopenic purpura; AA, aplastic anemia; SLE, systemic lupus erythematosus; **/*, significantly different (**$p < 0.01$, *$p < 0.05$). **A:** RP, **B:** LP.

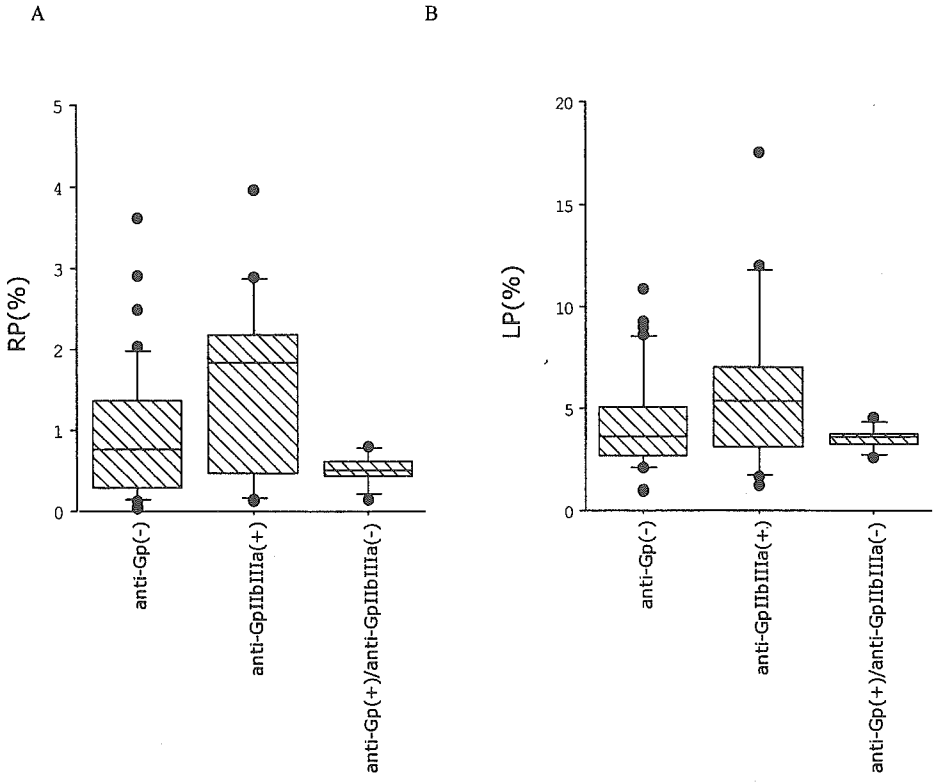


FIG. 3. RP and LP in patients with antibodies. anti-Gp(-): patients negative for any GP antibody (N=37); anti-GpIIbIIIa(+): patients positive for anti-GP IIb/IIIa antibody (N=17); anti-Gp(+)/GpIIbIIIa(-): patients positive for some anti-GP antibodies; exception with anti-GP IIb/IIIa antibody (N=7). **A:** RP, **B:** LP.

RESULTS

RP was significantly higher in patients with ITP (1.21 ± 0.94%, $p < 0.01$), especially in active phase (2.03 ± 0.88%, $p < 0.01$) and PR phase of AA (1.21 ± 0.89%, $p < 0.01$), than in healthy volunteers (0.48 ± 0.29%), the patients with ITP and AA in CR phase showed low RP rate; however, it was not significant (Table 1). RP was markedly lower in patients with ET or CML (0.20 ± 0.12%, $p < 0.05$) (Fig. 2A). LP was significantly higher in patients with ITP (5.17 ± 3.44%, $p < 0.01$) than in healthy volunteers (3.78 ± 1.14%), especially in active phase (6.80 ± 4.42%, $p < 0.01$), while LP was markedly lower in patients in the active phase of AA (2.04 ± 1.00%, $p < 0.01$) and with ET or CML (2.15 ± 0.70%, $p < 0.01$). On the other hand, RP was not significantly different among healthy volunteers and patients with ITP or AA in CR phase. There were no difference in LP rate between healthy volunteers and patients with SLE (Fig. 2B). As shown in Fig. 3, RP was significantly higher in the patients positive for anti-GpIIb/IIIa antibody (1.53 ± 1.10%) than in those negative for it ($p < 0.05$), and LP tended to be higher in the patients positive for it (5.89 ± 4.13%) (Fig. 3). RP and LP were poorly correlated with PAIgG ($R = 0.14$ and 0.12) (Fig. 4), and poorly correlated with plasma TPO level (Fig. 5). We next investigated the association with number of platelet, RP, and LP on the patients with ITP in active phase. RP and LP were negatively correlated with platelet count ($R = 0.302$ and 0.197) (Fig. 6).

The case report patient was 35 years old, female. She was diagnosed with ITP at 24 years of age. She has been treated with low-dose corticosteroid. She has medicated by intravenous immunogloblin for her delivery. The progression of platelet count and RP and LP before and after medication is shown in Fig. 7. She had low platelet counts (38 × 10^9/L), high RP (4.3%)

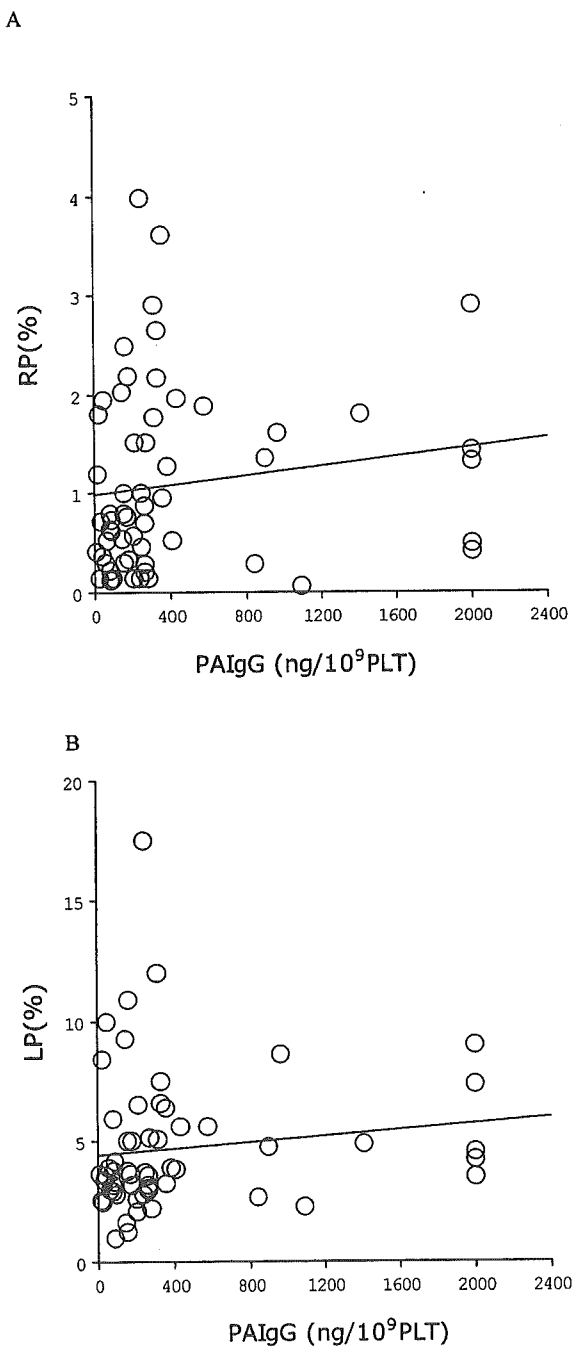


FIG. 4. Relationship between PaIgG and RP or LP in patients with ITP. **A:** RP, **B:** LP.

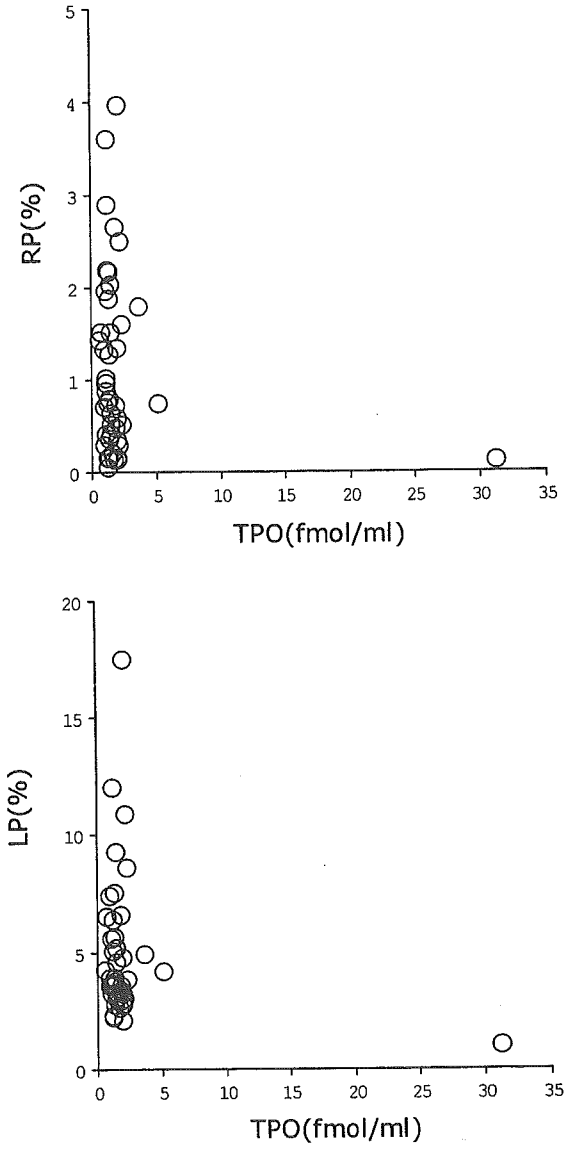


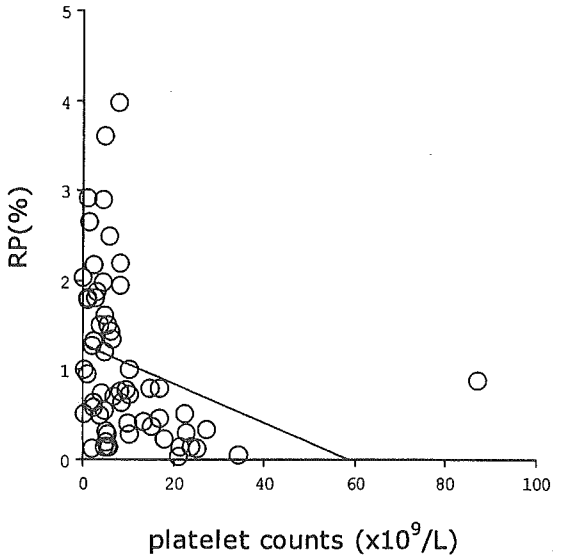
FIG. 5. Relationship between TPO and RP or LP in patients with ITP. **A:** RP, **B:** LP.

and LP (16.7%) before treatment with high-dose gamma globulin. She was given gamma-globulin 20 g per day for 5 days intravenously (day 0–day 5). After treatment, platelet count (134×10^9/L) increased, while RP (1.15%) and LP (5.56%) decreased. Platelet count (26×10^9/L) decreased again 40 days after treatment, while RP (2.36%) and LP (9.42%) increased again (Fig. 7).

DISCUSSION

Recently, RP measurement (7,16,17) has been developed to evaluate or diagnose the pathophysiology of thrombocytopenia, but is still not established. We found that RP in healthy volunteers was approximately 0.48%, with no significant differences between females and males or

A



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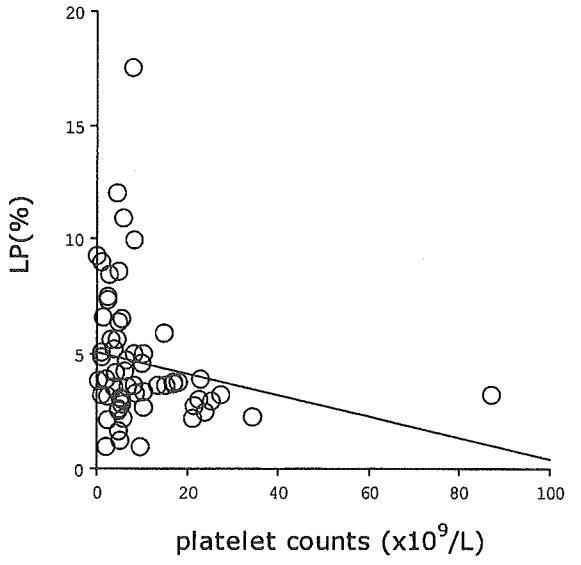

FIG. 6. Relationship between platelet counts and RP or LP in patients with ITP. **A:** RP, **B:** LP.

among various age groups. Platelet count and RP were negatively correlated in healthy volunteers and patients with thrombocytopenia, suggesting that RP may reflect platelet generation in normal bone marrow (16,17).

In our study, RP was significantly higher in ITP, especially in the active phase, and PR phase of AA, while RP was markedly lower in patients with ET or CML. These results suggest that RP may reflect clinical phase of ITP and that clearance of platelet is not enhanced in CML and ET. Although RP in AA was within normal range in active phase and CR, it was high in PR phase of AA. Increase in RP may reflect recovery of platelet production in bone marrow in patients in PR phase of AA. RP are reported to be younger

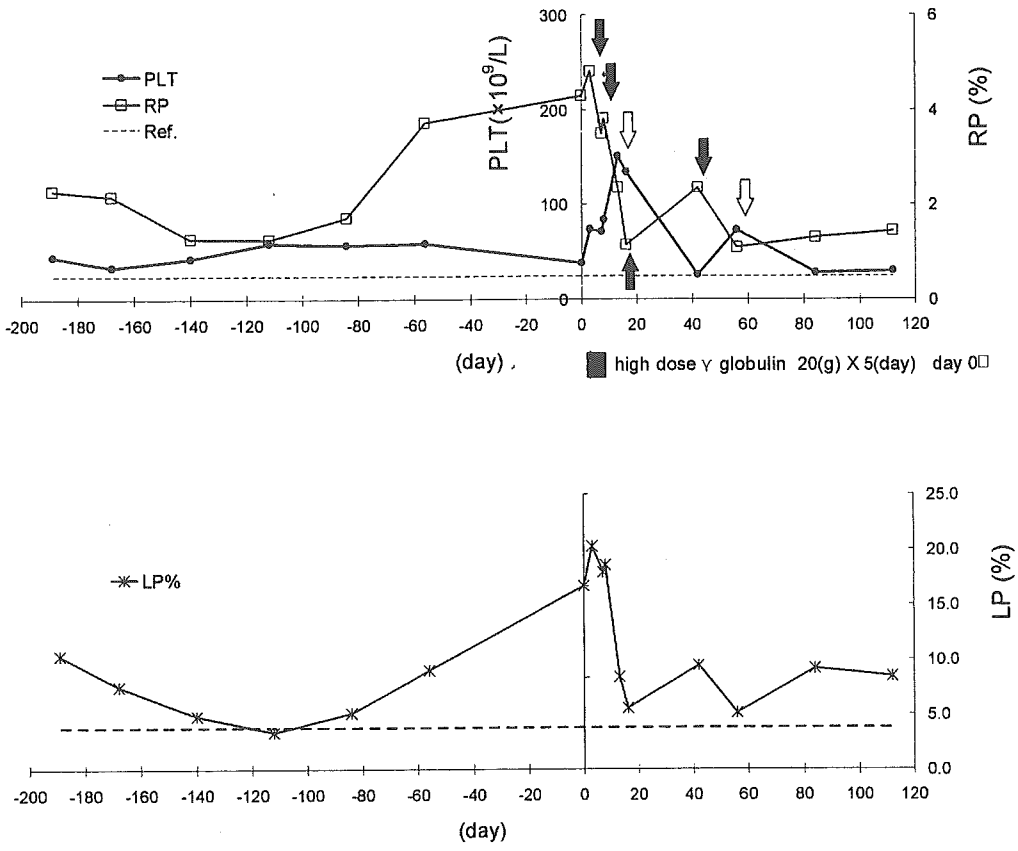

FIG. 7. ITP case.

platelets that have been newly released into the circulation (19). High levels of RP are more valuable but low levels of RP are less valuable. LP measurement (11,16) has also been developed to evaluate thrombocytopenia. In this study, LP was significantly higher in ITP, especially in active phase, while LP was markedly lower in active phase of AA and ET or CML. LP measurement is useful for the diagnosis of thrombocytopenia, especially AA. Both measurements of RP and LP may be more sensitive for diagnosis for thrombocytopenia.

In ITP, RP and LP were significantly higher in patients positive for anti-GpIIb/IIIa antibody, suggesting that this antibody may be essential for the onset of ITP. As shown in several reports (20,21) that immunodominant epitopes on GpIIb-IIIa are recognized by autoreactive T cells in ITP, anti-GpIIb/IIIa antibodies would be most important in ITP. RP and LP were poorly correlated with PAIgG in our ITP cases. Although PAIgG (15,22) was as an anti-platelet antibody developed to diagnose ITP, its sensitivity and specificity were low. RP and LP were in this study poorly correlated with plasma TPO level, although it was reported that TPO level was negatively correlated with platelet counts and megakaryocytes in bone marrow (12–14,23). Comparison between TPO and RP or LP in our study was performed for only patient with ITP. It is reported as another reason that autoantibodies against either platelet GPIb or GPIIb/IIIa in ITP plasma are not only involved in platelet destruction, but may also contribute to inhibition of platelet production (24). In the clinical course, RP in our cases reflected platelet count in ITP. Recombinant TPO has been reported to increase RP before increase in platelet count in patients with ITP (25).

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Usefulness of Fully Automated Measurement of Reticulated Platelets Using Whole Blood

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Summary: Reticulated platelets (RP) were measured with an automated hematology analyzer (modified R-2000) in 287 healthy volunteers and in 212 patients with thrombocytopenia. In healthy volunteers, the RP was 0.48 ± 0.26% in men and 0.48 ± 0.32% in women. No significant difference in the RP values due to gender or age (21–60 years) was observed. Furthermore, the reverse correlation was observed between platelet counts and RP. The RP was high in patients with idiopathic thrombocytopenic purpura (ITP), those with high fibrinogen and fibrin degradation products (FDP), and those with high C-reactive protein (CRP), but low in patients after chemotherapy. The RP was highest in active phase of ITP, and relatively high in the partial remission phase of aplastic anemia. In patients after chemotherapy, the patients had a minimum phase of RP and then a maximum phase of RP before platelet counts increased. RP was significantly high in the maximum phase and significantly low in the minimum phase. The relationships between platelet count and RP were negatively correlated in patients with ITP, high FDP, or high CRP, but were not correlated in patients with aplastic anemia, liver disease, or after chemotherapy. These results show that RP reflects the pathology of thrombocytopenic disorders and the measurement of RP is useful for the differential diagnoses and analysis of platelet kinetics.
Key Words: Reticulated platelet—Chemotherapy—ITP—Thrombocytopenia—Aplastic anemia.

Platelets were identified to have a coarse, punctuated reticulum (reticulated platelets; RP) by using a new methylene blue dye in 1969 (1). It was suggested that those cells might contain increased amounts of cytoplasmic RNA, reflecting thrombopoiesis in bone marrow. Subsequently, it was reported that platelet nucleic acid could be detected with flow cytometry utilizing thiazole orange as dye (2). RNA-rich platelets were reported to be readily measured by flow cytometry and to provide information on thrombopoietic activity in thrombocytopenic patients (3). Analysis of RP was indicated to provide a good indication of the rate of platelet production in bone marrow (4). In the diagnosis of immune thrombocytopenia, flow cytometric analysis of RP was reported to be better than platelet-associated IgG (5). Furthermore, a significant difference in reticulated platelet count between normal men and women had been shown using erythrocyte thiazole orange fluorescence in whole blood as an internal standard (6).

Idiopathic thrombocytopenic purpura (ITP) is caused by an immunologic mechanism of antiplatelet antibodies (7). In this state, platelet production is normal or increased but platelets are hyper-destroyed in spleen. On the other hand, patients with aplastic anemia have low platelet, leukocyte, and erythrocyte counts in peripheral blood and hypoplastic bone marrow. The flow cytometric analysis of RP is suggested to be a useful method for estimating thrombocytopenia. Recently, the instruments for fully automated measurement of RP (modified R2000 fully automated reticulocyte counter), capable of measuring the RP in the peripheral whole blood immediately, was developed.

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In this study, we measured the RP in healthy volunteers, thrombocytopenic patients, and in patients with underlying diseases due to thrombocytopenia to examine the usefulness of the RP count for different diagnosis of thrombocytopenia.

MATERIALS AND METHODS

RPs were examined in 287 healthy volunteers (144 female and 143 male; median age, 32 years old; range, 21–60 years old). RP was also measured in 212 patients with thrombocytopenia and underlying diseases of thrombocytopenia: 58 ITP, 23 aplastic anemia (AA), 7 systemic lupus erythematosus (SLE), 49 liver diseases, 8 leukemic patients after chemotherapy, 22 patients with high FDP (> 10 μg/mL), and 45 patients with high CRP (more than 4 mg/dL). The study protocol was approved by the Human Ethics Review Committees of Mie University School of Medicine. ITP was diagnosed by thrombocytopenia, normal bone marrow with more than normal count of megakaryocytes and no other disease due to thrombocytopenia; active phase, less than 50,000/μL of platelet counts; partial remission (PR), 50,000–120,000/μL of platelet counts with or without treatment; complete remission (CR), more than 120,000/μL of platelet counts without treatment. Aplastic anemia was diagnosed by thrombocytopenia, anemia, and leukocytopenia without other disease due to pancytopenia; active phase, less than 50,000/μL of platelet counts; partial remission (PR), 50,000–100,000/μL of platelet counts with or without treatment; complete remission (CR), more than 100,000/μL of platelet counts without treatment.

RP was measured by a previously described method (8). Briefly, approximately 2 mL of whole blood was collected from healthy volunteers or patients and was anticoagulated with ethylenediamine tetraacetic acid (EDTA)-2K. All blood samples were kept at room temperature until analysis. Counts of RP are reported to be stable during 2–6 hours after collection (9). Total platelet counts and frequency of RP were determined by the modified R2000 (Sysmex Inc., Kobe, Japan) with special software (9–11). For measurements of RP, 10 μL of blood was aspirated by the modified R2000 and automatically mixed with 40 μL of of 2.6% auramine O in 95.9% ethylene glycol solution (Ret-Search dye, Sysmex Inc.) to stain the RNA present in reticulocytes and RP. The samples were then irradiated by an argon laser beam at the wavelength of 488 nm. The resulting fluorescence intensity and forward light scatter were measured with flow cytometry (Fig. 1) (9). The frequency of RP was expressed as the percentages of total platelet

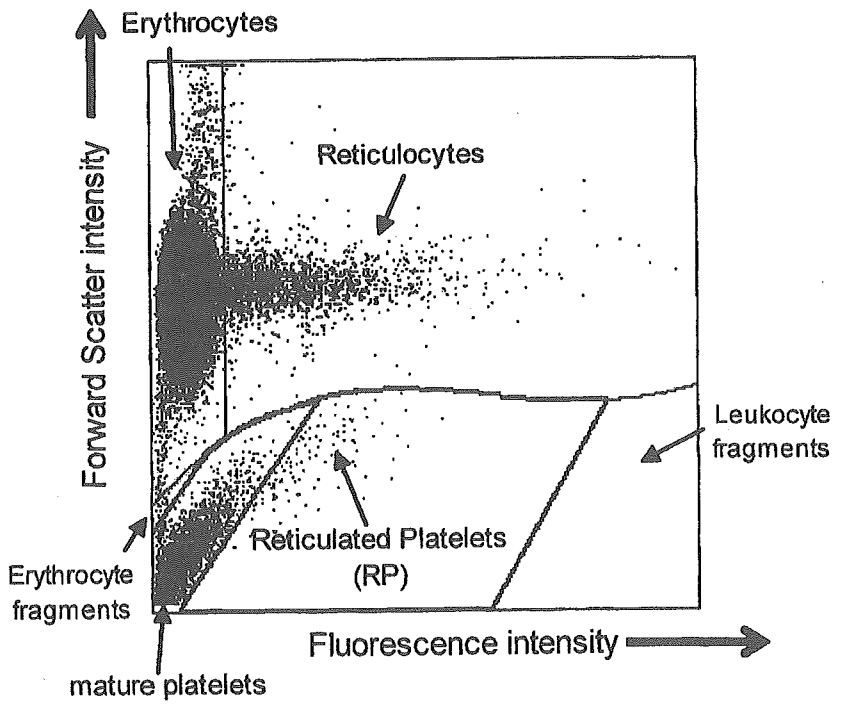

FIG. 1. Dot plot showing fluorescence intensity versus forward scatter intensity. Each line discriminates automatically. RP, mature red blood cell, reticulocyte, mature platelets, white blood cell fragments.

count. The time required to complete all these measurements was approximately 90 seconds. C-reactive protein (CRP) was measured using N-assay TIA CRP-S (Nittobo Company, Tokyo, Japan). Fibrin and fibrinogen degradation products (FDP) were measured using LPIA-FDP (Dia-Iatron Company, Tokyo, Japan). Prothrombin time was measured using a Thromborel S (Dade Behring, Tokyo, Japan).

Statistical Analysis

Results are expressed as mean ± SD. Differences between groups were evaluated by one-way ANOVA and the Bonferroni test. For assessment of correlations, coefficients of correlation were determined by Pearson's correlation coefficient and linear regression analysis. In both cases, a p value less than < 0.05 was considered significant.

RESULTS

Mean value of platelet count was 251.5 ± 50.0 × 10^9/L in female and 243.6 ± 46.8 × 10^9/L in male. Mean value of RP was 0.48 ± 0.32% in female and 0.48 ± 0.26% in male, and median of RP was 0.40 (range, 0.07%–2.01%) in female and 0.44 (range, 0.09%–2.00%) in male, respectively; there was no difference of RP between female and male (Fig. 2). There was no significant difference in the RP values due to age differences (Fig. 3). The relationship between platelet count and RP was negatively correlated in healthy volunteers ($r = 0.348$, $p < 0.01$) (Fig. 4). RP was significantly higher in patients with ITP ($p < 0.01$), those with high CRP ($p < 0.01$), those with high FDP ($p < 0.01$), those with SLE ($p < 0.05$) and those with liver disease ($p < 0.05$) than in healthy volunteers (Fig. 5). In ITP, RP was highest in active phase and that in CR was similar to normal controls (Table 1). In aplastic anemia, RP was within normal range at CR or active phase, but was significantly increased at PR phase ($p < 0.01$). RP was high in PR phase of aplastic anemia and in those with SLE. In patients after chemotherapy, there were two phases: minimum and maximum of RP. After chemotherapy, the patients had firstly minimum phase of RP and then had maximum phase of RP before increase of platelet counts. RP was significantly higher in the maximum phase ($p < 0.01$) and significantly lower in minimum phase ($p < 0.05$) than in healthy volunteers. The relationship between platelet count and RP was negatively correlated in patients with thrombocytopenia and healthy volunteers ($r = 0.404$, $p < 0.01$) (Fig. 6). The relationship between platelet count and RP was negatively correlated in patients with ITP ($r = 0.489$, $p < 0.01$) (Fig. 7A) but were

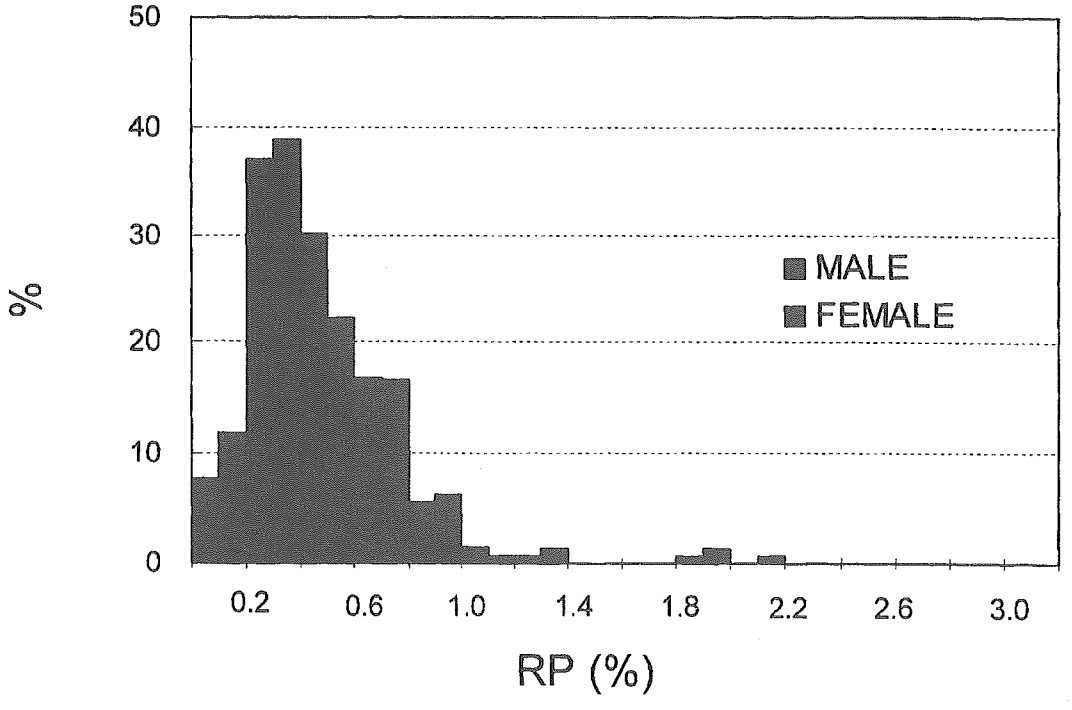

FIG. 2. Histogram of RP in healthy volunteers. Gray bar, female; black bar, male.

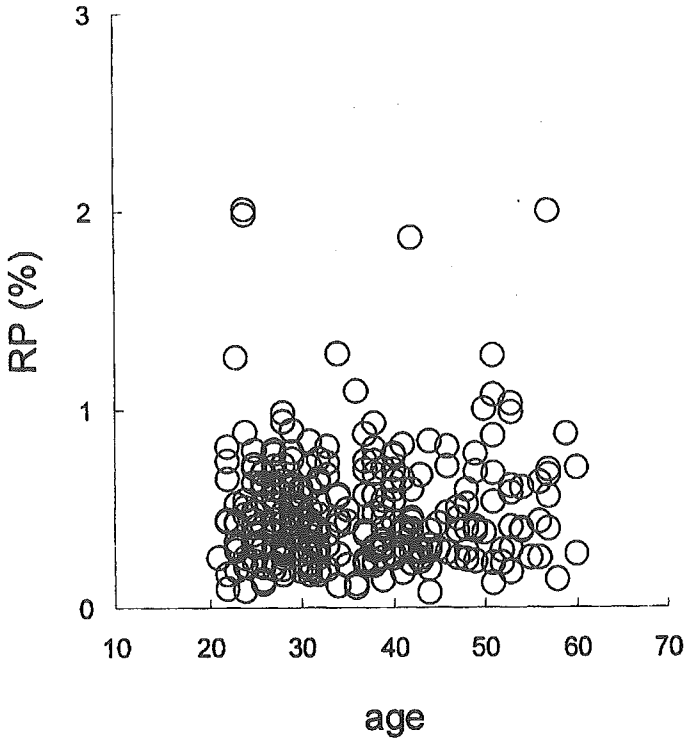

FIG. 3. Relationship between RP and age. There was no significant difference of RP among ages (from 21 to 60 years old).

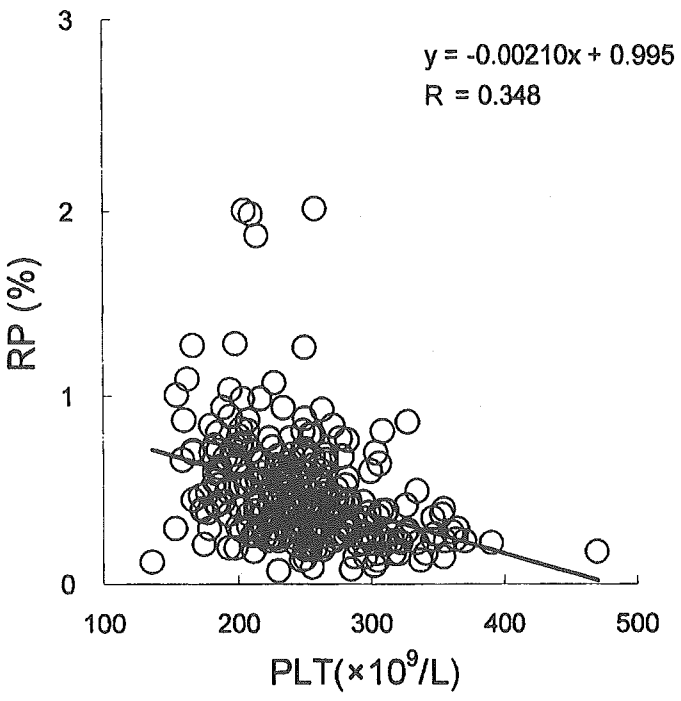

FIG. 4. Relationship between platelet count and RP in healthy volunteers.

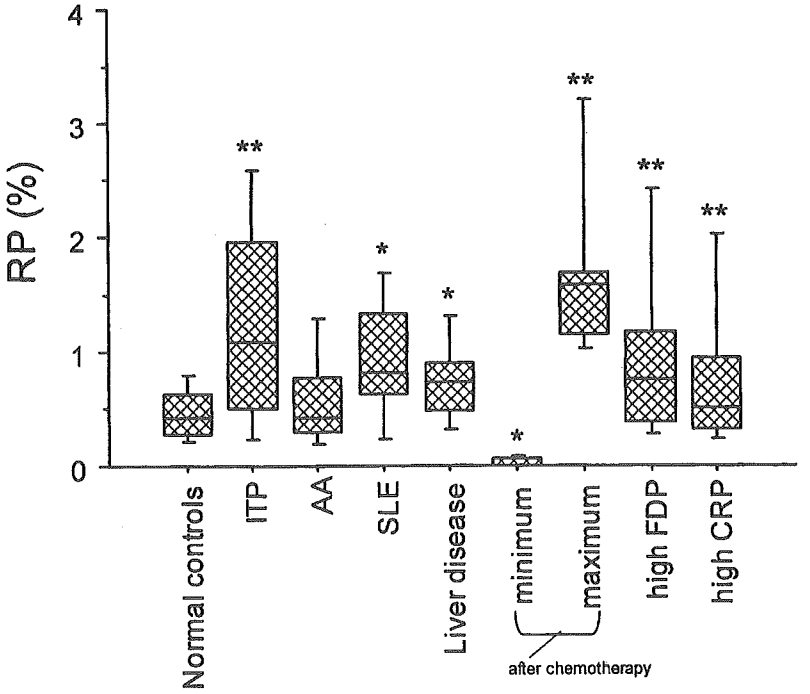

FIG. 5. RP in healthy volunteers and the patients with thrombocytopenia or the underlying diseases. ITP, idiopathic thrombocytopenic purpura; AA, aplastic anemia; SLE, systemic lupus erythematosus; **/*, significantly different (**$p < 0.01$, *$p < 0.05$). The box plots show the standard elements. The lowest boundary of the box indicate the 25th percentiles and the highest boundary of the box indicate the 75th percentiles. The upper whiskers indicate the 90th percentiles and the lowest whiskers indicate 10th percentiles. The solid line in the box represents the median value.

TABLE 1. Reticulated Platelet in Healthy Volunteers and Patients with Thrombocytopenia or the Underlying Diseases

Subjects	Number	RP (%)	Platelet Count (×10⁹/L)
Healthy volunteers	287	0.48 ± 0.29	247.5±48.6
ITP (all)	58	1.28 ±0.94*	113.3±91.5
Active phase	26	1.99±0.85*	48.7±25.3
PR phase	19	0.89±0.56†	111.7±42.6
CR phase	13	0.41±0.26	245.0±87.8
Aplastic anemia (all)	23	0.69±0.71	102.8±53.8
Active phase	9	0.51±0.20	42.1±16.9
PR phase	7	1.36±0.94*	135.4±32.0
CR phase	7	0.25±0.10	148.1±15.7
SLE	7	0.94±0.51†	250.7±96.6
Liver disease	49	0.77±0.37†	76.2±24.6
After chemotherapy	8		
Minimum‡	8	0.02±0.04†	38.1±11.4
Maximum§	8	1.60±0.80*	61.4±34.1
High FDP**	22	1.18±1.63*	202.3±143.1
High CRP††	45	0.84±0.92*	232.6±122.4

‡ Minimum phase of RP after chemotherapy.

§ Maximum phase of RP after chemotherapy.

** A group of the patients with high FDP (> 10 μg/mL).

†† A group of the patients with high CRP (> 4 mg/dL).

*p< 0.01; †p < 0.05.

tend to be positively correlated in patients with aplastic anemia (Fig. 7B). The relationship between platelet count and RP was negatively correlated in patients with high FDP ($r = 0.425$, $p < 0.05$) and those with high CRP ($r = 0.402$, $p < 0.01$) but not in patients with liver diseases ($r = 0.196$) and in patients after chemotherapy (Fig. 8). However, RP was not significantly correlated with CRP, FDP, or PT (Table 2).

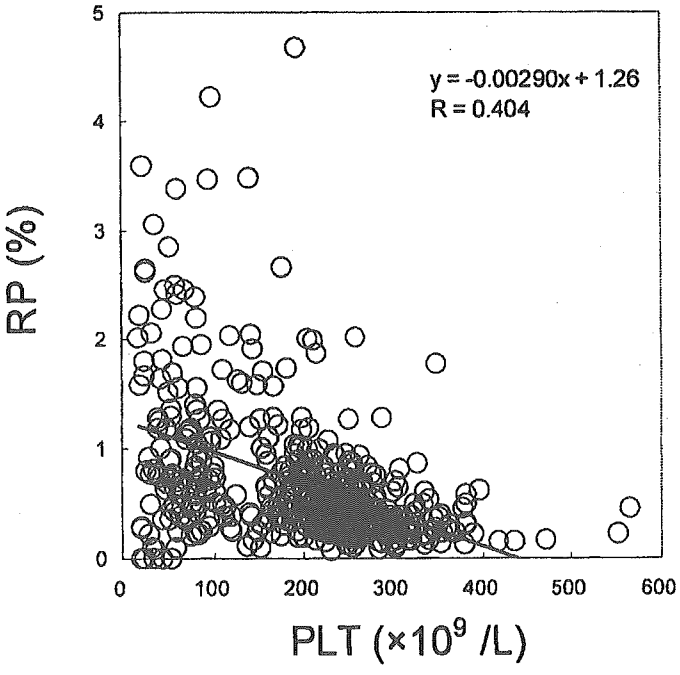

FIG. 6. Relationship between RP and platelet counts in healthy volunteers and patients with thrombocytopenia.

DISCUSSION

Thrombocytopenia is a common hematologic abnormality that is caused by hypoproduction, hyperdestruction, and sequestration of platelets. It is difficult to distinguish between hypoplastic and hyperdestructive forms of thrombocytopenia. The presence of normal or increased numbers of megakaryocytes in the bone marrow essentially excludes the diagnosis of hypoplastic thrombocytopenia due to aplastic anemia or amegakaryocytic thrombocytopenia. To date, no simple diagnostic test exists to diagnose hyperdestructive thrombocytopenia such as ITP and thrombotic thrombocytopenic purpura (TTP).

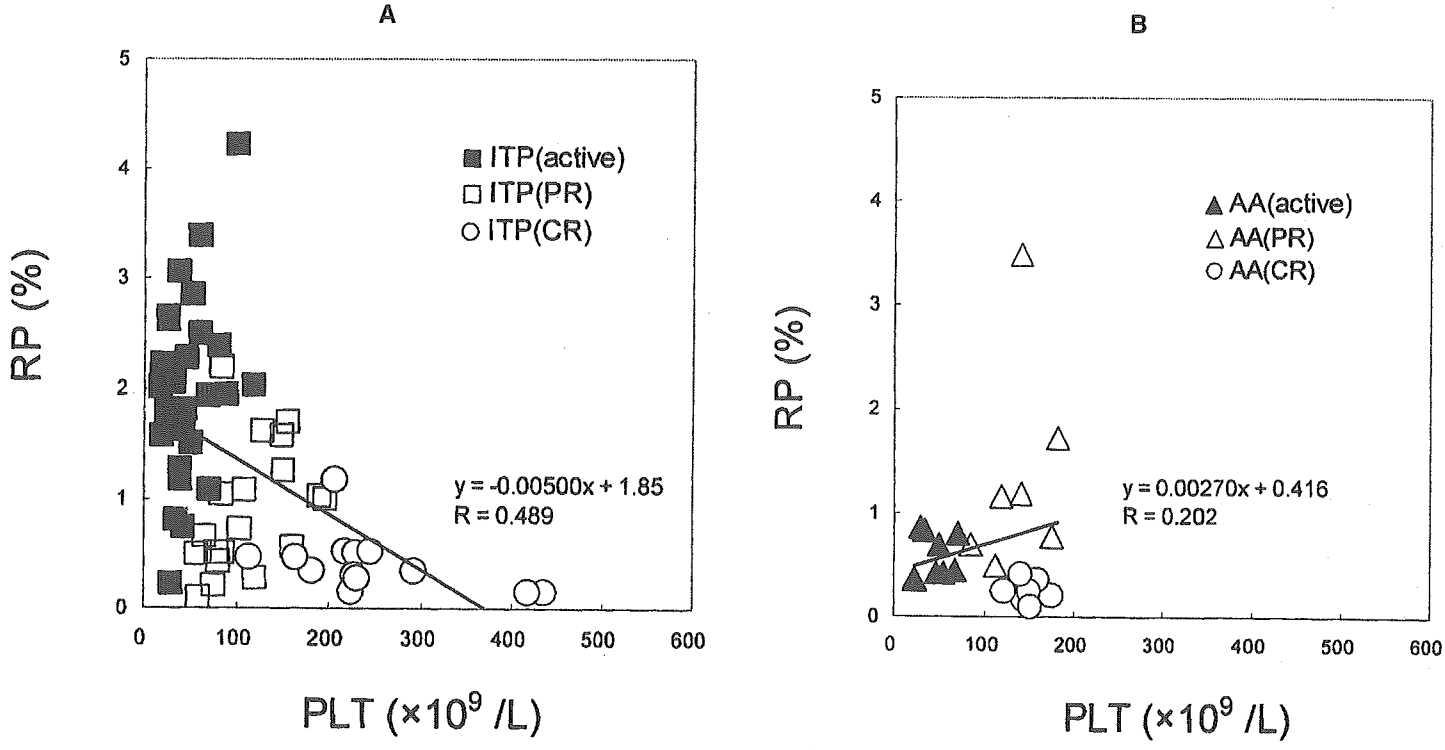

FIG. 7. Relationship between RP and platelet count in patients with ITP (**A**) and aplastic anemia (**B**). (**A**) active phase; ■, PR phase; □□ CR; ○ (B) active phase; ▲, PR phase; □ □ CR; ○.

TABLE 2. Relationship Between RP and CRP, FDP or PT

	CRP	FDP	PT
RP	R = 0.294	R = 0.0447	R = 0.200

Although platelet-associated immunoglobulin G (PAIgG) (5,12) as anti-platelet antibodies was developed to diagnose ITP, the sensitivity or specificity was low. Recently RP (1,8,9) and thrombopoietin (TPO) (13,14) have been developed to evaluate for thrombocytopenia. RP are reported to be younger platelets that have been newly released into circulation (15).

We found that, in healthy volunteers, RP was approximately 0.48%, with no significant difference between female and male, and among various ages, which was different from the previous report (6). The relationships between platelet count and RP were negatively correlated in healthy volunteers, suggesting that RP may reflect the platelet generation in normal bone marrow.

In various diseases, RP was significantly high in patients with ITP, in those with high CRP, and in those with high FDP. ITP is an autoimmune disease characterized by increased platelet clearance caused by anti-platelet auto-antibodies (16). The patients with ITP usually have increased megakaryocytes in the bone marrow, and are considered to have high RP (10,17) and normal TPO levels (14,18). In ITP, RP was highest in active phase and reduced similar to normal controls in CR, suggesting that RP may reflect to clinical phase of ITP. Although RP was within normal range in patients with active phase or CR of aplastic anemia, RP was high in PR phase. The increase in RP may reflect to the recovery of platelet production in bone marrow in patients with aplastic anemia. RP was also increased in patients with SLE, those with high FDP, and those with high CRP. SLE also has an autoimmune mechanism for thrombocytopenia such as ITP. In those with high FDP and high CRP, aggregation of platelets may be caused by thrombin or an inflammatory reaction and platelet production may be enhanced. In patients after chemotherapy, the patients had a minimum phase of RP and then had a maximum phase of RP before platelet counts increased. RP was sig-

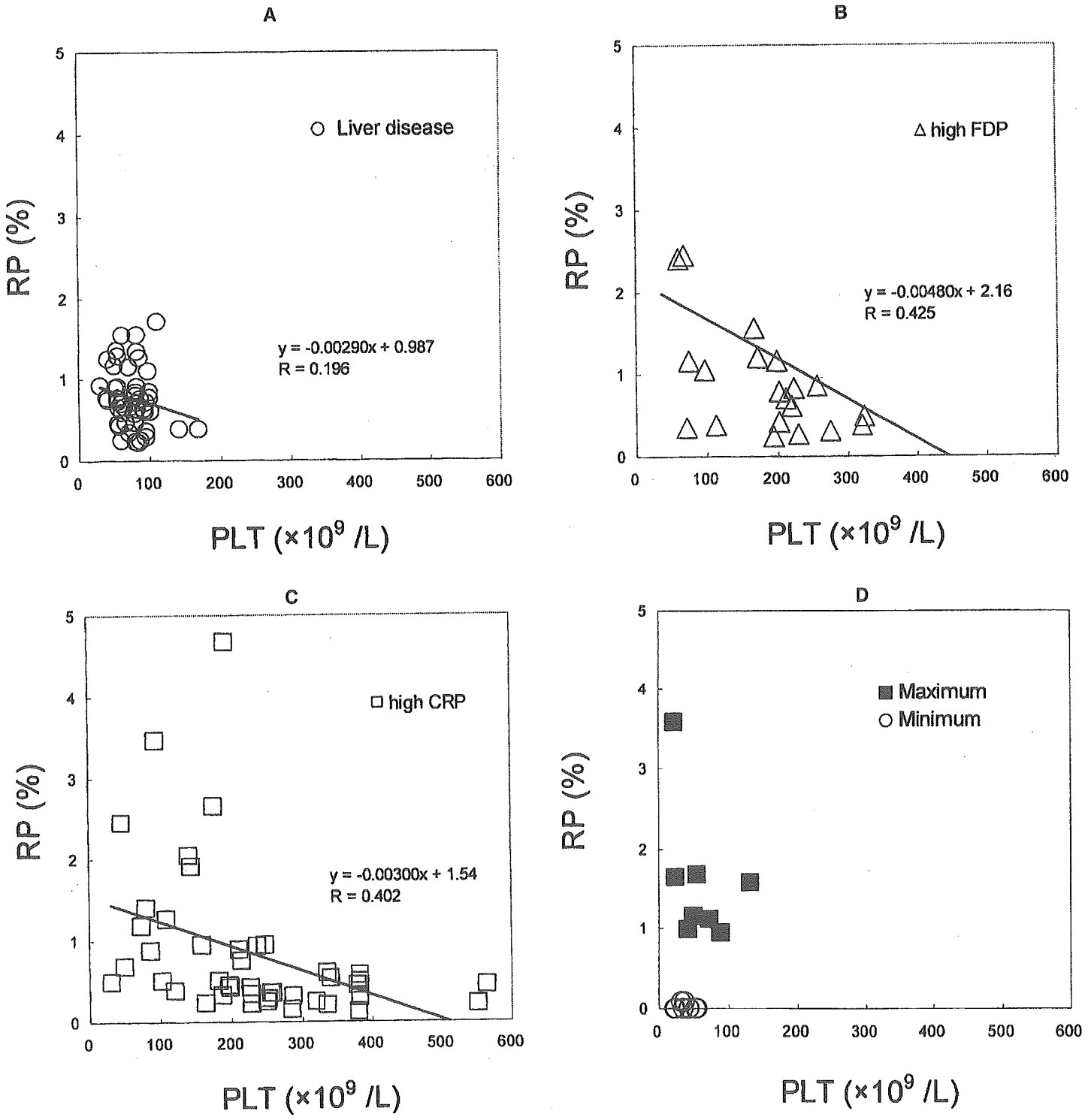

FIG. 8. Relationship between RP and platelet count in patients with liver diseases (**A**) and patients with high FDP (**B**) and patients with high CRP (**C**) and patients after chemotherapy (**D**).

nificantly low in minimum phase and significantly high in maximum phase, suggesting that RP may reflect platelet production in bone marrow after chemotherapy. Measurement of RP may be useful for determining the necessity and/or timing of platelet transfusion in patients with thrombocytopenia after chemotherapy. RPs were negatively correlated in patients with ITP,

in patients with high FDP, and in those with high CRP, but were not correlated in patients with aplastic anemia and those after chemotherapy. These results suggest that RP is negatively correlated with platelet count in hyperdestructive thrombocytopenia but not correlated with that in amegakaryocytic thrombocytopenia.

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Severe secondary deficiency of von Willebrand factor–cleaving protease (ADAMTS13) in patients with sepsis-induced disseminated intravascular coagulation: its correlation with development of renal failure

Tomoko Ono, Jun Mimuro, Seiji Madoiwa, Kenji Soejima, Yuji Kashiwakura, Akira Ishiwata, Katsuhiro Takano, Tsukasa Ohmori, and Yoichi Sakata

Deficiency of ADAMTS13 is found in patients with thrombotic thrombocytopenic purpura (TTP), and the genetic defects in the *ADAMTS13* gene or the autoantibody against ADAMTS13 is thought to be responsible for the development of TTP. The clinical correlation and mechanisms of secondary ADAMTS13 deficiency in other disease states were investigated. In addition to TTP, ADAMTS13 levels were severely decreased in patients with sepsis-induced disseminated intravascular coagulation (DIC). The incidence of acute renal failure and serum creatinine levels in patients with ADAMTS13 activity levels lower than 20% (incidence, 41.2%; creatinine, 160 ± 150 μM [1.81 ± 1.70 mg/dL]) ($P < .05$) were significantly higher than they were in patients with ADAMTS13 activity levels higher than 20% (incidence, 15.4%; creatinine, 84 ± 67 μM [0.95 ± 0.76 mg/dL]) ($P < .01$). Additionally, unusually large von Willebrand factor multimers were detected in 26 (51.0%) of 51 patients with ADAMTS13 activity levels lower than 20%. Lower molecular weight forms of ADAMTS13 were found in the plasma of patients with sepsis-induced DIC, suggesting that the deficiency of ADAMTS13 was partially caused by its cleavage by proteases in addition to decreased synthesis in the liver. These data suggested that severe secondary ADAMTS13 deficiency can be associated with sepsis-induced DIC and may contribute to the development of renal failure. (Blood. 2006;107: 528-534)

Introduction

Deficiency of the von Willebrand factor (VWF)–cleaving protease,[1-5] ADAMTS13 (a disintegrin-like metalloprotease with thrombospondin type 1 repeats) is found in most patients with thrombotic thrombocytopenic purpura (TTP), and this deficiency is thought to be responsible for platelet aggregation and microthrombi formation in the circulation, which in turn cause typical thrombotic microangiopathies (TMAs) to develop.[6-9] Deficiency of ADAMTS13 in patients with TTP is caused by genetic defects in the *ADAMTS13* gene (familial TTP, Upshaw-Schulman syndrome) or by autoantibodies against ADAMTS13. Although hemolytic uremic syndrome (HUS) is clinically similar to TTP, the role of ADAMTS13 deficiency in the development of HUS is controversial because reports conflict about whether ADAMTS13 activity remains unchanged[6-8] or decreases.[10-13] It also is possible that secondary deficiency of ADAMTS13 may account for the development of microthrombi formation in disease states other than TTP. To search for the clinical correlation of secondary ADAMTS13 deficiency in disease states, we measured ADAMTS13 activity levels by the standard method[14] and determined antigen levels by our newly developed monoclonal antibody–based enzyme-linked immunosorbent assay (ELISA) for ADAMTS13 in patients with TTP and in patients with sepsis-induced disseminated intravascular coagulation (DIC). We found that severe secondary ADAMTS13 deficiency could occur in patients with sepsis-induced DIC and that it had a clinical correlation with the development of renal failure.

Patients, materials, and methods

Blood samples

All samples were obtained with informed consent from patients according to the Declaration of Helsinki. Blood was drawn from 113 patients (65 men, aged 17-83; 44 women, aged 21-81; idiopathic TTP, 3 patients; Upshaw-Schulman syndrome, 1 patient; sepsis-induced DIC, 109 patients). The diagnosis of TTP was made with note of the presence of typical clinical features (fever, bleeding tendency, neurologic symptoms) laboratory examination results (thrombocytopenia, hemolytic anemia with red blood cell fragmentation, increased levels of LDH, increased levels of serum creatinine), and effectiveness of plasma exchange treatment. Patients with definite infection, such as bacteremia, pneumonia, urinary tract infection, biliary tract infection, or pathogenic *Escherichia coli* O-157 infection, were excluded from the TTP group. The patient with Upshaw-Schulman syndrome had TTP, and plasma transfusion was effective in preventing recurrence.

The diagnosis of DIC was made according to the criteria established in 1988 by the Japanese Ministry of Health and Welfare. Criteria for DIC were

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