

# Diagnostic Value of Tests for Reticulated Platelets, Plasma Glycocalicin, and Thrombopoietin Levels for Discriminating Between Hyperdestructive and Hypoplastic Thrombocytopenia

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## Abstract

*We measured reticulated platelets (RPs) and plasma glycocalicin (GC) and thrombopoietin (TPO) levels simultaneously in 107 thrombocytopenic patients to clarify the diagnostic value of these tests for discriminating hyperdestructive from hypoplastic thrombocytopenia. The percentage of RPs and GC index (plasma GC level normalized for the individual platelet count) were markedly elevated in patients with idiopathic thrombocytopenic purpura (ITP) but normal or slightly elevated in patients with aplastic anemia (AA) or chemotherapy-induced thrombocytopenia (ChemoT). For RP percentage for diagnosing hyperdestructive thrombocytopenia the sensitivity and specificity were excellent but were lower for the GC index. Absolute RP counts and plasma GC levels were markedly decreased and plasma TPO levels markedly elevated in patients with AA or ChemoT, but absolute RP counts and plasma GC levels were moderately decreased and plasma TPO levels only slightly elevated in patients with ITP. The sensitivity and specificity of plasma TPO levels for diagnosing hypoplastic thrombocytopenia were excellent. Using the RP percentage and plasma TPO levels in combination improved specificities. Simultaneous measurement of RP percentage and plasma TPO level may help discriminate thrombocytopenia of unknown cause in routine hematologic practice.*

Thrombocytopenia is a common hematologic abnormality that must be evaluated before the proper therapeutic intervention can be chosen. The mechanism responsible for thrombocytopenia can be separated into 3 general categories: hypoproduction, hyperdestruction, and sequestration of platelets. Abnormal platelet sequestration is almost invariably due to hypersplenism and can be suspected in the presence of significant splenic enlargement. In contrast, it may be 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 diagnoses of hypoplastic thrombocytopenia, such as aplastic anemia (AA) and amegakaryocytic thrombocytopenia. However, bone marrow examinations have some shortcomings: bone marrow aspirations occasionally are subject to sampling errors and subjective interpretation. On the other hand, no simple diagnostic tests exist to diagnose hyperdestructive thrombocytopenia. The precise diagnosis requires analysis of the life span of autologous radiolabeled platelets. However, platelet life-span measurements expose the patient to radiation and are complex to perform. Thus, there is a need for a rapid, noninvasive test that would provide information about the bone marrow megakaryocyte activity and platelet life span.

Three laboratory methods have been reported to be useful for the diagnosis of thrombocytopenia. These include reticulated platelets (RPs),<sup>1</sup> plasma thrombopoietin (TPO) levels,<sup>2</sup> and plasma glycocalicin (GC) levels.<sup>3</sup> RPs are reported to be younger platelets that have been released recently into the circulation<sup>4</sup> and are probably analogous to reticulocytes, reflecting erythropoiesis. Patients with clinical criteria for idiopathic thrombocytopenic purpura (ITP), a representative disorder of hyperdestructive thrombocytopenia,

uniformly have a higher percentage of circulating RPs than do healthy subjects.<sup>1,5,6</sup> In addition, absolute RP counts are decreased in patients with hypoplastic thrombocytopenia. The absolute RP counts are considered to reflect platelet production.<sup>1</sup>

In 1994, several groups reported the cloning of the c-mpl ligand expected for TPO, which regulates megakaryocyte growth and development.<sup>7</sup> As expected, in patients with bone marrow hypoplasia, the plasma TPO levels are significantly elevated, but not elevated or only slightly elevated in patients with ITP.<sup>2,8,9</sup> From these data, the measurement of plasma TPO levels is expected to become a useful marker for the differentiation of the types of thrombocytopenia.

GC is a carbohydrate-rich hydrophilic fragment with a molecular weight of 135 kd; it represents the external portion of the alpha subunit of the platelet membrane glycoprotein Ib.<sup>10</sup> The plasma GC levels are reduced significantly in patients with hypoplastic thrombocytopenia, but not in patients with normal or increased megakaryocytes,<sup>3,11,12</sup> suggesting that the plasma GC level reflects the rate of platelet production. In addition, the GC index (plasma GC level normalized for the individual platelet count) is elevated in patients with ITP but not in patients with AA, suggesting that the GC index reflects the rate of platelet destruction. From these data, the measurements of plasma GC levels and the GC index are considered useful adjuncts for classifying thrombocytopenic disorders.

To date, there have been no reports that have measured RP, plasma TPO levels, and plasma GC levels in thrombocytopenic disorders and compared the sensitivity and the specificity of these tests for the differential diagnosis of thrombocytopenia. In the present study, to evaluate which method is most useful for the differential diagnosis of thrombocytopenia, we measured RPs, plasma TPO levels, and plasma GC levels simultaneously in patients with hyperdestructive and hypoplastic thrombocytopenia whose diagnoses already had been established. We demonstrated that the simultaneous measurements of the RP percentage and plasma TPO levels are helpful for discriminating between hyperdestructive and hypoplastic thrombocytopenia, and the measurement of the GC index or plasma GC levels may be of little diagnostic value.

## Materials and Methods

### Subjects

A total of 107 thrombocytopenic patients whose platelet counts were less than  $100 \times 10^3/\mu\text{L}$  ( $100 \times 10^9/\text{L}$ ) were selected for the study. They included 65 patients with ITP

(mean  $\pm$  SD age,  $51 \pm 16$  years; M/F ratio, 17:48; mean  $\pm$  SD platelet count,  $41 \pm 23 \times 10^3/\mu\text{L}$  [ $41 \pm 23 \times 10^9/\text{L}$ ]); 20 patients with AA (mean  $\pm$  SD age,  $49 \pm 15$  years; M/F ratio, 7:13; mean  $\pm$  SD platelet count,  $50 \pm 22 \times 10^3/\mu\text{L}$  [ $50 \pm 22 \times 10^9/\text{L}$ ]); and 22 patients with chemotherapy-induced thrombocytopenia (ChemoT; mean  $\pm$  SD age,  $41 \pm 18$  years; M/F ratio, 19:3; mean  $\pm$  SD platelet count,  $34 \pm 18 \times 10^3/\mu\text{L}$  [ $34 \pm 18 \times 10^9/\text{L}$ ]).

The 22 patients with ChemoT included 7 patients with acute myelogenous leukemia, 4 with chronic myelogenous leukemia, 2 with acute lymphocytic leukemia, 3 with myelodysplastic syndrome, 5 with malignant lymphoma, and 1 with multiple myeloma. The diagnosis of chronic ITP was made according to criteria described previously.<sup>13</sup> These patients with ITP had received various treatments (splenectomy without sufficient effect, 21 cases; prednisolone, 20 cases). The diagnosis of AA was based on the following criteria: pancytopenia, absence of splenomegaly or lymphadenopathy, bone marrow examination and/or needle biopsy that revealed reduction in cellularity, and no other concurrent disease or therapy that would cause pancytopenia. Four patients with AA had received antilymphocyte globulin and/or cyclosporin therapy.

Control samples were collected from 60 healthy volunteers (mean  $\pm$  SD age,  $31 \pm 8$  years; M/F ratio, 19:41; mean  $\pm$  SD platelet count,  $224 \pm 47 \times 10^3/\mu\text{L}$  [ $224 \pm 47 \times 10^9/\text{L}$ ]) after obtaining informed consent.

### Assay of RPs

RPs were measured by a previously described method.<sup>14</sup> Briefly, after platelet-rich plasma was prepared by centrifugation of blood anticoagulated with EDTA, the platelets were fixed in 1% paraformaldehyde for 5 minutes at room temperature to minimize nonspecific staining and resuspended at  $5 \times 10^7/\text{mL}$  in phosphate-buffered saline. Fifty microliters of this suspension was mixed with 5  $\mu\text{L}$  of phycoerythrin-tagged monoclonal antibody, SZ2, against glycoprotein Ib (CD42b) (Immunotech, Marseille, France) and incubated at room temperature for 30 minutes. The platelets were washed and resuspended at  $1 \times 10^7/\text{mL}$ . One hundred microliters of this suspension, 600  $\mu\text{L}$  of phosphate-buffered saline, and 100  $\mu\text{L}$  of Retic-COUNT (thiazole orange; Becton Dickinson, San Jose, CA) were mixed and incubated for 2 hours at room temperature.

The samples were analyzed on a flow cytometer (FACScan, Becton Dickinson). Live gating on platelet-sized events was performed using the distinctive forward-scatter vs side-scatter profile of platelets. Ten thousand events were measured for each sample. The second gating was performed using forward-scatter vs FL2 fluorescence (585 nm), and the CD42b phycoerythrin-tagged platelet population was selected. To exclude cell autofluorescence and instrument

background, an unstained control sample was prepared simultaneously for each sample. Thiazole-orange-stained platelets with higher fluorescence than the 99% of the unstained control samples were considered to be RPs. The absolute number of RPs was calculated by using the RP percentage and the platelet count. The intra-assay coefficient of variation analyzed on 13 successive occasions was 3.4% for samples with an RP of less than 10% and 4.8% for samples with an RP of more than 20%.

### TPO Assay

EDTA-anticoagulated whole blood was centrifuged at 1,000g for 15 minutes, and the platelet-poor plasma then was recentrifuged at 10,000g for 15 minutes; the supernatant plasma was stored at  $-20^{\circ}\text{C}$  until assay. The plasma TPO concentration was determined by using an enzyme-linked immunosorbent assay kit (Quantikine Human TPO Immunoassay, R&D Systems, Minneapolis, MN). Briefly, 200  $\mu\text{L}$  of recombinant human TPO standard plasma samples or phosphate-buffered saline was added in duplicate to the wells of a microtiter plate precoated with an anti-TPO monoclonal antibody. The plate was incubated for 3 hours at  $4^{\circ}\text{C}$ . After washing, 200  $\mu\text{L}$  of horseradish peroxidase-conjugated anti-TPO antibody was added and incubated for 1 hour at  $4^{\circ}\text{C}$ . The color was developed by using tetramethylbenzidine as the substrate. The reaction was stopped by adding 50  $\mu\text{L}$  of acid solution to each well, and the absorbance was recorded at 450 nm. The sample TPO concentration was calculated from the corresponding standard curve. The mean  $\pm$  SD intra-assay values for 10 determinations of 2 samples (AA and healthy subject) were  $425.0 \pm 30.3$  and  $85.6 \pm 4.7$  pg/mL, respectively, and the coefficients of variation were 7.1% and 5.5%, respectively.

### GC Assay

The GC level was measured by using an enzyme immunoassay kit (Glycocalicin EIA Kit; Takara Biomedical, Ohtsu, Japan). Briefly, 200  $\mu\text{L}$  of GC standard plasma samples that were prepared by the same procedure as for the TPO assay or phosphate-buffered saline were added in duplicate to the wells of a microtiter plate precoated with an anti-glycocalicin monoclonal antibody. The plate was incubated for 1 hour at room temperature. After washing, 200  $\mu\text{L}$  of horseradish peroxidase-conjugated anti-GC antibody was added and incubated for 1 hour at room temperature. The color was developed by the same procedure as for the TPO assay. The sample GC concentration was calculated from the corresponding standard curve. The GC index also was calculated as proposed by Steinberg et al<sup>3</sup>: GC index = GC  $\times$  250  $\times$  10<sup>3</sup>/individual platelet count ( $\mu\text{L}$ ). The mean  $\pm$  SD intra-assay values for 10 determinations of 2 samples from healthy subjects were  $0.72 \pm 0.04$  and  $0.57 \pm 0.02$   $\mu\text{g}/\text{mL}$ , respec-

tively, and the coefficients of variation were 5.6% and 3.5%, respectively.

### Statistics

Standard statistical methods were used for the calculation of means and SD, and the mean  $\pm$  SD is reported. The differences between mean values were evaluated using the 2-tailed Student *t* test for unpaired data, and a *P* value less than .05 was considered statistically significant. The Spearman test was used for nonparametric correlation. The predictive value of a positive test result was determined by dividing the number of true-positive samples in the disorder groups to be diagnosed by the total number of true-positive plus the number of false-positive samples. The negative predictive value of a negative test result was determined by dividing the number of true-negative samples in the control disorder group by the total number of negative samples plus the number of false-negative samples.

## Results

### RP Percentage and GC Index

We measured the RP percentage and GC-index in 65 patients with well-characterized ITP, a representative disorder of hyperdestructive thrombocytopenia, and in 20 patients with AA and 22 patients with ChemoT, both of which are representative disorders of hypoplastic thrombocytopenia. The diagnoses of these patients had been made based on complete hematologic examination, including bone marrow aspiration or biopsy. Values for the RP percentage and the GC index are shown in **Table 1** and **Figure 1**. The RP percentage in healthy subjects was  $7.7\% \pm 2.7\%$ . As shown in Figure 1A, patients with ITP had markedly increased RP percentages ( $23.8\% \pm 11.6\%$ ) that differed significantly from those of the healthy subjects ( $P < .001$ ). There was a significant correlation between platelet counts and RP percentages ( $R = 0.52$ ;  $P < .001$ ) in patients with ITP (Figure 1B). In contrast, the RP percentages in patients with AA or ChemoT were normal.

The GC index in healthy subjects was  $1.30 \pm 0.45$ . As shown in Figure 1C, patients with ITP had markedly increased GC indexes ( $10.37 \pm 12.51$ ) that differed significantly from those for healthy subjects ( $P < .001$ ). There was a significant correlation between platelet counts and GC indexes ( $R = 0.63$ ;  $P < .001$ ) in patients with ITP (Figure 1D). The GC indexes were not elevated in patients with AA but were elevated significantly in patients with ChemoT. There was a significant correlation between the RP percentage and the GC index ( $R = 0.66$ ;  $P < .001$ ) in patients with ITP.

**Table 1**  
**Reticulated Platelet (RP) Percentage and Count and Plasma Glycocalicin (GC) and Thrombopoietin (TPO) Levels\***

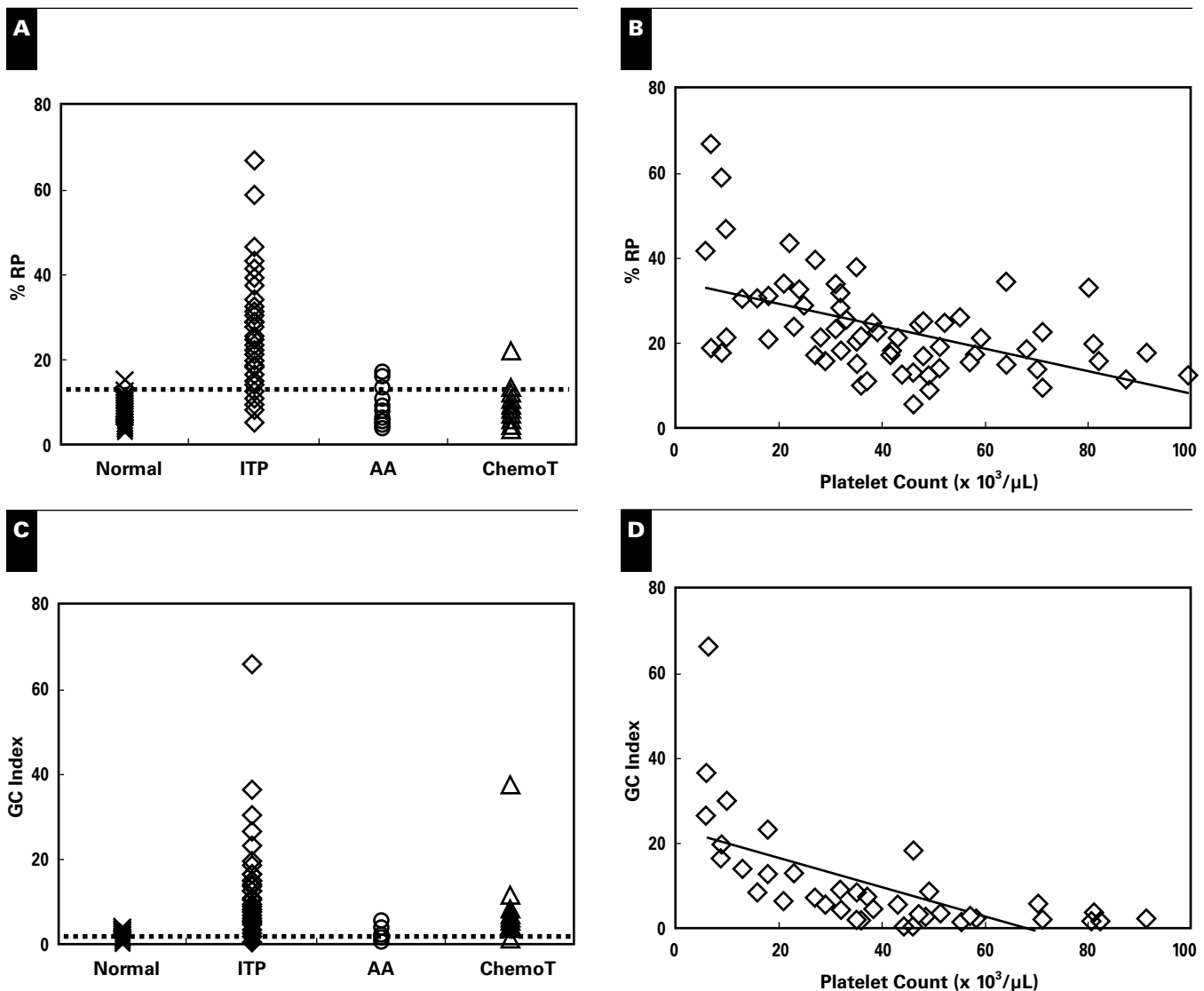
	RP (%)	GC Index	Absolute RP Level (10 <sup>3</sup> /μL)	Plasma GC Level (μg/mL)	Plasma TPO Level (pg/mL)
Healthy subjects	7.7 ± 2.7	1.30 ± 0.45	170 ± 6.6	1.13 ± 0.33	43 ± 29
Idiopathic thrombocytopenic purpura	23.8 ± 11.6 <sup>†</sup>	10.37 ± 12.51 <sup>†</sup>	8.3 ± 4.6 <sup>†</sup>	0.91 ± 0.59 <sup>‡</sup>	74 ± 66 <sup>§</sup>
Aplastic anemia	8.9 ± 3.7	1.95 ± 1.16	4.2 ± 2.0 <sup>†</sup>	0.36 ± 0.23 <sup>†</sup>	349 ± 210 <sup>†</sup>
Chemotherapy-induced thrombocytopenia	8.2 ± 4.2	7.64 ± 9.95 <sup>†</sup>	2.7 ± 2.1 <sup>†</sup>	0.73 ± 0.58 <sup>†</sup>	402 ± 329 <sup>†</sup>

\* Values are given as mean ± SD. The average results for each group are compared with those for healthy subjects. The GC index is the plasma GC level normalized for the individual platelet count and is calculated as follows: GC × 250 × 10<sup>3</sup>/individual platelet count (μL).

<sup>†</sup> P < .001.

<sup>‡</sup> P < .05.

<sup>§</sup> P < .005.



**Figure 1** Reticulated platelet (RP) percentage and glycocalicin (GC) index in healthy subjects and thrombocytopenic patients and correlation between platelet counts and RP percentage or GC index. **A**, RP percentage in patients with idiopathic thrombocytopenic purpura (ITP), aplastic anemia (AA), or chemotherapy-induced thrombocytopenia (ChemoT). **B**, Correlation between the platelet counts and the RP percentage in patients with ITP. **C**, GC index in thrombocytopenic patients. **D**, Correlation between the platelet counts and the GC index in patients with ITP. The dotted line represents the mean + 2 SD of results for healthy subjects.

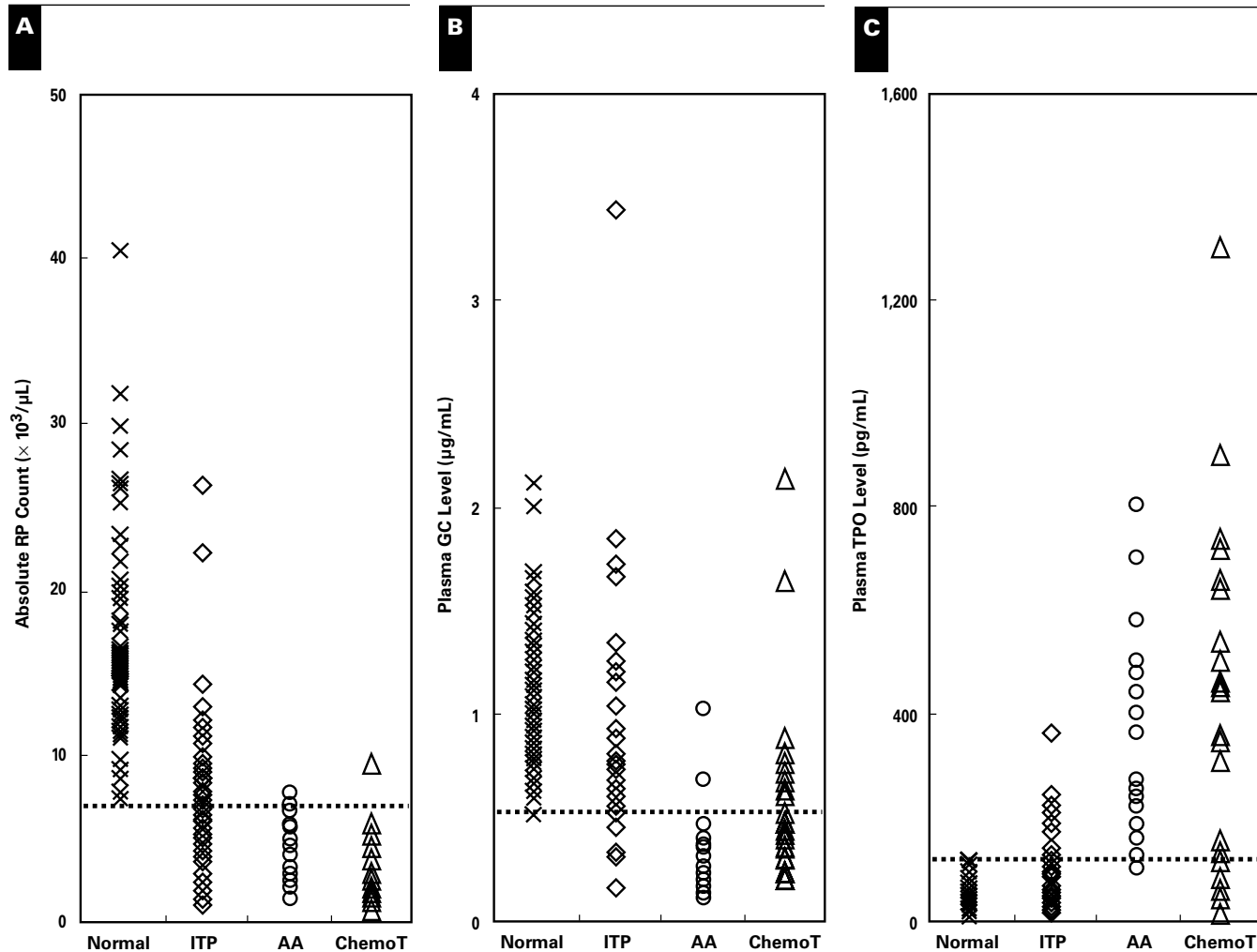
### Absolute RP Count, Plasma GC Level, and Plasma TPO Level

The absolute RP count and the plasma GC level are considered to reflect the rate of platelet production.<sup>1,3,11,12</sup> Values for the absolute RP count and the plasma GC level are shown in Table 1 and **Figure 2**. The absolute RP counts in healthy subjects were  $17.0 \pm 6.6 \times 10^3/\mu\text{L}$ . As shown in Figure 2A, patients with AA and patients with ChemoT had markedly reduced absolute RP counts ( $4.2 \pm 2.0 \times 10^3/\mu\text{L}$  and  $2.7 \pm 2.1 \times 10^3/\mu\text{L}$ , respectively) compared with the healthy subjects ( $P < .001$ ). In patients with ITP, absolute RP counts were slightly reduced but higher than those of patients with AA and patients with ChemoT.

The plasma GC level in healthy subjects was  $1.13 \pm 0.33 \mu\text{g/mL}$ . As shown in Figure 2B, patients with AA had markedly reduced plasma GC levels ( $0.36 \pm 0.23 \mu\text{g/mL}$ ) compared with those for healthy subjects ( $P < .001$ ), while in

patients with ChemoT, plasma GC levels were not so reduced ( $0.73 \pm 0.58 \mu\text{g/mL}$ ;  $P < .001$ ). In patients with ITP, plasma GC levels were slightly reduced but higher than those of patients with AA.

Plasma TPO levels are reported to be elevated markedly in cases of hypoplastic thrombocytopenia.<sup>2,8,9</sup> Therefore, it is considered that the marked elevation of the plasma TPO level indicates hypoproduction of platelets. The plasma TPO level in healthy subjects was  $43 \pm 29 \text{ pg/mL}$  (Figure 2C). Patients with AA or ChemoT had markedly increased plasma TPO levels ( $349 \pm 210$  and  $402 \pm 329 \text{ pg/mL}$ , respectively) that differed significantly from the levels for healthy subjects ( $P < .001$  for both). Patients with ITP had slightly increased plasma TPO levels ( $74 \pm 66 \text{ pg/mL}$ ;  $P < .005$ ) compared with levels for healthy subjects. There was no correlation between plasma TPO levels and platelet counts in patients with AA or ChemoT.



**Figure 2** Absolute reticulated platelet (RP) counts, plasma glyocalicin (GC) levels, and plasma thrombopoietin (TPO) levels in healthy subjects and patients with idiopathic thrombocytopenic purpura (ITP), aplastic anemia (AA), or chemotherapy-induced thrombocytopenia (ChemoT). **A**, Absolute RP counts. **B**, Plasma GC levels. **C**, Plasma TPO levels. The dotted line represents the mean + 2 SD of the results for healthy subjects.

### Sensitivity, Specificity, and Predictive Values for the RP Percentage and GC Index for Diagnosing Hyperdestructive Thrombocytopenia

To calculate the sensitivity, the specificity, and the predictive values for the RP percentage and GC index for diagnosing hyperdestructive thrombocytopenia, patients with AA and those with ChemoT were combined in a hypoplastic group. There was no significant difference between the mean platelet counts of patients with ITP ( $41 \times 10^3/\mu\text{L}$ ) and patients in the hypoplastic group ( $42 \times 10^3/\mu\text{L}$ ). By using 2 SDs above the mean for healthy subjects to define an upper limit, the sensitivity and the specificity of the RP percentage for diagnosing hyperdestructive thrombocytopenia were 85% and 86%, respectively, while those for the GC index were 87% and 40%, respectively (Table 2). In addition, the positive and negative predictive values for the RP percentage (90% and 78%, respectively) were superior to those of the GC index.

### Sensitivity, Specificity, and Predictive Values for Absolute RP Count, Plasma GC Level, and Plasma TPO Level for Diagnosing Hypoplastic Thrombocytopenia

The sensitivity, the specificity, and the predictive values for the absolute RP count, plasma GC level, and plasma TPO level are given in Table 3. The sensitivity and the specificity of the plasma TPO level for diagnosing hypoplastic thrombocytopenia were 82% and 80%, respectively. The sensitivity of plasma GC level and the specificity of absolute RP count were low compared with the sensitivity and the specificity of the plasma TPO level.

### Analysis Using the RP Percentage and Plasma TPO Level for Discriminating Thrombocytopenic Disorders

Since the sensitivity and the specificity of the RP percentage for diagnosing hyperdestructive thrombocytopenia and those of the plasma TPO level for diagnosing hypoplastic thrombocytopenia were excellent, we evaluated whether the sensitivity or the specificity for discriminating thrombocytopenic disorders would improve if we measured the RP percentage and plasma TPO levels simultaneously and analyzed both results in combination. Figure 3 is the graph of plasma TPO levels plotted against the RP percentage. Of 65 patients with ITP, results for 45 were plotted in the region of elevated RP percentage and normal plasma TPO level, and results for none of the patients in the hypoplastic group were plotted in this region. In contrast, results for 29 of 42 patients in the hypoplastic group were plotted in the region of elevated plasma TPO level and normal RP percentage, and results for only 3 patients with ITP were plotted in this region. The sensitivity and the specificity of the result showing elevated RP percentage and normal plasma TPO level for diagnosing hyperdestructive thrombocytopenia were 69% and 100%, respectively (Table 2), and the positive predictive value was 100%. The sensitivity and the specificity of the results showing normal RP percentage and elevated plasma TPO level for diagnosing hypoplastic thrombocytopenia were 68% and 95%, respectively (Table 3), and the positive predictive value was 91%.

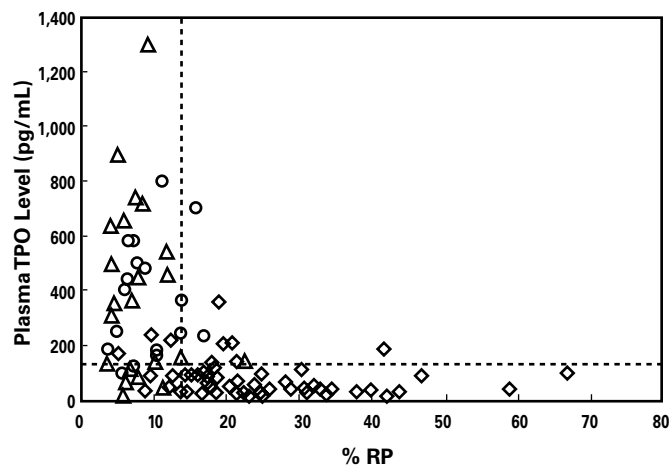
**Table 2**  
Sensitivity, Specificity, and Predictive Values of Reticulated Platelet (RP) Percentage, Glycocalicin (GC) Index, and Thrombopoietin (TPO) Level for Diagnosing Hyperdestructive Thrombocytopenia\*

	Sensitivity (%)	Specificity (%)	Predictive Value (%)	
			Positive	Negative
Elevated RP percentage	85	86	90	78
Elevated GC index	87	40	70	68
Elevated RP percentage and normal TPO level	69	100	100	68

\* The GC index is the plasma GC level normalized for the individual platelet count and is calculated as follows:  $\text{GC} \times 250 \times 10^3 / \text{individual platelet count} (\mu\text{L})$ .

**Table 3**  
Sensitivity and Specificity of Absolute Reticulated Platelet (RP) Count, Plasma Glycocalicin (GC) Level, and Plasma Thrombopoietin (TPO) Level for Diagnosing Hypoplastic Thrombocytopenia

	Sensitivity (%)	Specificity (%)	Predictive Value (%)	
			Positive	Negative
Decreased absolute RP count	90	60	59	91
Decreased plasma GC level	60	87	76	77
Elevated plasma TPO level	82	80	72	87
Normal RP percentage and elevated plasma TPO level	68	95	91	83



**Figure 3** Plasma thrombopoietin (TPO) level plotted against reticulated platelet (RP) percentage in thrombocytopenic patients. Diamonds indicate patients with idiopathic thrombocytopenic purpura; circles, patients with aplastic anemia; triangles, patients with chemotherapy-induced thrombocytopenia; the dotted lines represent the mean + 2 SD of the results for healthy subjects.

## Discussion

ITP is caused by the destruction of autoantibody-sensitized platelets by the reticuloendothelial system.<sup>15</sup> Despite some 40 years of study, there is no single diagnostic test for ITP. The diagnosis of ITP is based on the finding of thrombocytopenia with normal bone marrow megakaryocytes and the exclusion of other causes of the thrombocytopenia. Assays that measure platelet-associated IgG (PAIgG) are not useful diagnostic tests because patients with immune or nonimmune thrombocytopenia have elevated levels of PAlGg.<sup>16</sup> According to the American Society of Hematology guideline, bone marrow evaluation and PAlGg assay are unnecessary.<sup>17</sup> Some investigators have suggested that techniques capable of measuring immunoglobulins bound to individual platelet glycoproteins can be used to diagnose ITP.<sup>18-20</sup> The specificity of this method for diagnosing ITP was very high, 91%<sup>21</sup>; however, the sensitivity was low, 39%. Therefore, this method is actually not convenient for use in the routine hematologic laboratories.

The measurement of RP, GC, and TPO was reported to be useful for discriminating between hyperdestructive and hypoplastic thrombocytopenia.<sup>1-3,8,9,11,12,22</sup> The sensitivity and the specificity of the RP percentage for diagnosing ITP were reported to be 91% to 96% and 67% to 100%, respectively.<sup>22,23</sup> The GC index also is thought to reflect platelet life span<sup>3,11,12</sup>; however, the sensitivity and the specificity of the GC index for diagnosing ITP have not been reported. We compared the sensitivity and the specificity of these 2 tests

for diagnosing hyperdestructive thrombocytopenia. To date, no data exist comparing the sensitivity and the specificity of 2 tests. Our data indicated that the sensitivities of the RP percentage and the GC index were almost the same, but the specificity of the RP percentage was superior to that of the GC index (Table 2).

The absolute RP count and the plasma GC level were reported to reflect the rate of platelet production.<sup>1,3,11,12</sup> The absolute RP count and the plasma GC level were decreased dramatically in patients with AA (Table 1 and Figures 2A and 2B). These results were consistent with previously reported data.<sup>1,3,11,12</sup> In addition, plasma TPO levels were elevated dramatically in patients with hypoplastic thrombocytopenia (Table 1 and Figure 2C), in accord with previously reported data.<sup>2</sup> On the other hand, patients with ITP had moderately decreased absolute RP counts and plasma GC levels and slightly elevated plasma TPO levels. These observations are consistent with the previous report that the platelet production in patients with ITP is decreased moderately compared with that for healthy subjects.<sup>24</sup> When the sensitivity and the specificity of these 3 tests were compared for diagnosing hypoplastic thrombocytopenia, those of plasma TPO level were excellent (82% and 80%, respectively). In contrast, the sensitivity of the plasma GC level and the specificity of the absolute RP count were low (60% and 60%, respectively) compared with those for the plasma TPO level (Table 3).

The sensitivity and the specificity of the RP percentage for diagnosing hyperdestructive thrombocytopenia and those for the plasma TPO level for diagnosing hypoplastic thrombocytopenia were excellent, as mentioned. Therefore, we sought to determine whether the discrimination of thrombocytopenia improved when we analyzed the data in combination (Figure 3). The specificities for diagnosing destructive and hypoplastic thrombocytopenia improved to 100% and 95%, respectively (Tables 2 and 3); however, the sensitivities decreased slightly. We believe it is important to confirm the diagnosis by 2 methods based on different principles. Koike et al<sup>6</sup> also measured the RP percentage and plasma TPO level simultaneously in a small group of patients with ITP and AA and showed that the simultaneous measurement may help distinguish between hyperdestructive and hypoplastic thrombocytopenia.

The results for 3 patients with ITP were plotted in the region of elevated plasma TPO level and normal RP percentage. We performed reevaluation, including bone marrow examination, in these 3 patients and found that 2 of 3 patients had relative lymphocytosis in peripheral blood and hypoplastic bone marrow, suggesting AA. The measurements of the RP percentage and plasma TPO level are easy to perform in the routine hematologic laboratories. There will be a number of occasions to diagnose thrombocytopenia

of unknown cause or secondary to a variety of disorders in the routine hematologic practice. Valuable information will be obtained, without invasive examination, from the results of the RP percentage and plasma TPO levels.

In this study, we selected thrombocytopenic patients whose platelet counts were less than  $100 \times 10^3/\mu\text{L}$  ( $<100 \times 10^9/\text{L}$ ). As shown in Figures 1B and 1D, there were significant negative correlations between the platelet counts and the RP percentage or GC index. A majority of patients with ITP whose platelet counts were more than  $100 \times 10^3/\mu\text{L}$  ( $>100 \times 10^9/\text{L}$ ) had normal RP percentages and GC indexes (data not shown). Therefore, the present suggestion that the measurement of the RP percentage and GC index may help discriminate thrombocytopenia could apply to thrombocytopenic patients whose platelet counts were less than  $100 \times 10^3/\mu\text{L}$  ( $<100 \times 10^9/\text{L}$ ).

The specificity of the GC index for diagnosing hyperdestructive thrombocytopenia and the sensitivity of the plasma GC level for diagnosing hypoplastic thrombocytopenia were low because some patients with ChemoT had higher GC indexes or plasma GC levels than expected from their platelet counts. Beer et al<sup>11</sup> reported that patients with leukemia had elevated plasma GC levels because cell destruction may result in the liberation of enzymes such as leukocyte elastase from the leukemic cells, which easily cleaves GC from platelets. Our patients with ChemoT might have higher plasma GC levels than expected from their platelet counts because of the same mechanism since the samples were obtained at the peak of destruction of tumor cells. Therefore, the measurement of plasma GC level may have little diagnostic value for distinguishing thrombocytopenias.<sup>25</sup>

We showed that the measurements of the RP percentage and plasma TPO levels are of diagnostic value for discriminating between hyperdestructive and hypoplastic thrombocytopenia. Particularly when we measured the RP percentage and plasma TPO levels simultaneously and analyzed both results in combination, these tests were helpful for discriminating thrombocytopenia of unknown cause in the routine hematologic practice.

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