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Changes in Platelet Volume in Various Patients with Thrombocytopenia and Thrombocytosis as Observed by the Platelet Saponin Test

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ABSTRACT. Platelet volume, the expansion ratio (ER) and the shrinkage ratio (SR) were measured by the platelet saponin test in various patients with thrombocytopenia and thrombocytosis.

In patients with platelet counts of less than $10\times10^4/\mu l$ the platelet volume increased to above the normal range ($p<0.001$, t-test) with reduction of the ER ($p<0.001$, t-test). The SR was heightened in thrombocytopenias due to diminished megakaryopoiesis resulting from enhanced platelet aggregation. On the other hand, in thrombocytopenias due to increased platelet destruction the SR level was normal notwithstanding and enlarged platelet volume presumably due to lowered platelet activity.

In thrombocytosis with platelet count above $40\times10^4/\mu l$, both the platelet volume ($p<0.05$) and the ER ($p<0.01$, t-test) were smaller than in normal subjects. The numerical results obtained by the platelet saponin test reflect the qualitative platelet abnormalities in patients with thrombocytopenia and thrombocytosis.

Key words : Platelet volume — Expansion ratio — Shrinkage ratio — Platelet saponin test — Thrombocytopenia — Thrombocytosis

It was reported in the previous paper that there was an inverse correlation between platelet volume and platelet count or expansion ratio (ER) when platelets were examined by means of the platelet saponin test.[1] Giant platelets were encountered in patients with cerebrovascular accidents at the acute stage and in burned patients.[1]

The purpose of the present study was to investigate the relation between platelet count and platelet volume changes occurring following the addition of saponin (ER) and that arising subsequent to exposure to hypotonic saline solution (Shrinkage ratio ; SR) in thrombocytopenia (below $10\times10^4/\mu l$) and thrombocytosis (above $40\times10^4/\mu l$) caused by various kinds of disorders.

MATERIALS AND METHODS

The twenty-four cases of thrombocytopenia observed in our study are

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collected in Table 1, and fourteen patients with thrombocytosis examined by us are shown in Table 2. Normal subjects were healthy volunteers between the ages of 20 and 35 (male 15, female 15) with normal complete blood counts. Their platelet counts were of course within normal range. The platelet saponin test was performed according to a method previously described.[1,2]

The results were assessed as to the significance of difference by Student's t-test.

TABLE 1. Cases of thrombocytopenia (below $10\times10^4/\mu l$)

Disseminated intravascular coagulation	8	9
Idiopathic thrombocytopenic purpura	4	5
Cirrhosis of the liver	4	5
Aplastic anemia	2	4
Multiple myeloma	2	3
Acute leukemia	2	2
Malignant lymphoma	1	1
Myelodysplastic syndrome	1	3
Total	24 cases	32 cases

TABLE 2. Cases of thrombocytosis ($>40\times10^4/\mu l$)

Reactive thrombocytosis	6	6
Myeloproliferative disorders		
Polycythemia vera	2	4
Chronic myelogenous leukemia	2	2
Postsplenectomic condition	2	2
Thrombosis	1	1
Others	1	1
Total	14 cases	16 occasions

RESULTS

1. Comparison of thrombocytopenia with thrombocytosis in the platelet saponin test is shown in Table 3. It is evident that there are significant differences

TABLE 3. Platelet volume, ER and SR in patient with thrombocytopenia and thrombocytosis (mean±SD)

Subgroup	(n)	Platelet count ($\times10^4/\mu l$)	I-MPV*1 (μ^3)	ER*2	SR*3
$10\times10^4/\mu l>$	(32)	5.6± 2.4	10.0±1.3	1.20±0.11	31±14
$40\times10^4/\mu l<$	(16)	86.6±56.8 *	7.5±1.1 *	1.30±0.12 §	16± 6 *
Normals	(30)	24.2± 4.4	8.1±0.7	1.41±0.07	24±4

*1 : Initial mean particle volume
*2 : Expansion ratio
*3 : Shrinkage ratio
§ : Significantly different ($p<0.01$)
* : Significantly different ($p<0.001$)

between the two groups with regard to platelet count, I-MPV, SR ($p<0.001$, t-test) and ER ($p<0.01$, t-test).

2. Diminished platelet production, enhanced platelet destruction and sequestration of platelets, which are seen in patients with thrombocytopenia, are compared in Table 4. In thrombocytopenia due to depressed megakaryopoiesis, the SR values, which are considered to be parameters of platelet aggregation,[2] were larger than in thrombocytopenia caused by enhanced platelet destruction or sequestration of platelets.

TABLE 4. Comparison of diminished platelet production (A) with enhanced platelet destruction or platelet sequestration (B) in patient with thrombocytopenia

Group	No.	Platelet ($\times 10^4/\mu l$)	I-MPV (μ^3)	ER	SR
A	13	4.2±1.5 §	10.1±1.7*	1.25±0.12*	39±19* §
B	19	6.5±2.5 §	9.9±0.9*	1.24±0.13*	24± 9 §
Normal	30	24.2±4.4	8.1±0.7	1.41±0.07	24± 4

A : aplastic anemia 4, multiple myeloma 3, refractory anemia with excess of blasts 3, acute leukemia 2, malignant lymphoma 1
B : idiopathic thrombocytopenic purpura 5, cirrhosis of the liver 5, disseminated intravascular coagulation 9
§ : significantly different (t-test, $p<0.01$)
* : significantly different when compared with normal (t-test, $p<0.001$)

3. Comparison of reactive thrombocytosis with myeloproliferative disorders as observed by the platelet saponin test is presented in Table 5. Causative

TABLE 5. Platelet saponin test in patient with reactive thrombocytosis and myeloproliferative disorders

Subgroups	(n)	Platelet	I-MPV	ER	SR
Reactive thrombocytosis▲	(6)	57.4±18.4	7.3±1.1■ NS	1.28±0.05 § NS	13±6 NS
Myeloproliferative disorders△	(6)	132.7±69.9	7.1±0.8■	1.33±0.05 §	18±6

▲ : Reactive thrombocytosis included burn 1, multiple injury 1, cerebrovascular accident 2 and recovery stage of disseminated intravascular coagulation 2
△ : Myeloproliferative disorders included chronic myelogenous leukemia 2 and polycythemia vera 4
Values are expressed in means ± SD. NS denotes "not significant".
§ : compared with normal ($p<0.01$, t-test)
■ : compared with normal ($p<0.05$, t-test)

disorders of thrombocytosis were divided into two groups. Severe burns, multiple injuries and the reconvalescent stage of disseminated intravascular coagulation, which cause reactive thrombocytosis, fall into one group. Myeloproliferative disorders, such as seen in chronic myelogenous leukemia and polycythemia vera fall into the other. No differences were observed between the two groups.

4. The clinical course of a patient with DIC was followed up by platelet saponin test only one patient mentioned here. The results are shown in Table 6.

TABLE 6. Day-sequential changes of volume, ER and SR of platelets in a DIC patient under treatment

Date	Platelet ($\times 10^4/\mu l$)	I-MPV (μ^3)	ER	SR
2/ 2	5.4	10.8	1.04	26
2/ 6	32.2	8.1	1.25	/
2/17	45.3	6.4	1.36	16

A case of DIC associated with hemorrhagic shock was treated with gabexate mesilate (84-0698). As shown in Table 6, the platelet count was restored to the normal level within a week and later it increased to above the normal level. There was a tendency toward reduction in platelet volume (I-MPV) and the shrinkage ratio (SR) in accordance with the clinical improvement of DIC. There was an inverse correlation between I-MPV and ER ($r=-0.9988$).

DISCUSSION

Recent studies[1,9] performed by the authors and Giles have disclosed that there exists an inverse relationship between the number of circulating platelets and their platelet volume. Some investigators postulate that the mean platelet volume is larger in some types of thrombocytopenia, and in such a condition the platelets are functionally more active.[5,6,11] However, controversy still exists regarding the mean platelet volumes in patients with aplastic anemia, acute leukemia or idiopathic thrombocytopenic purpura.[7–9]

Therefore, this study was designed to see whether or not any differences or variations exist in the characters of platelet volume and the functional relationship among thrombocytopenias and thrombocytoses. Thrombocytopenia was divided into two groups based on etiology, namely group (A) due to diminished platelet production and group (B) ascribed to enhanced platelet destruction or sequestration of platelets in the spleen. There was no difference between the two groups with respect to the level of platelet volume (I-MPV) and the expansion ratio (ER), though, the shrinkage ratio (SR), which is one of the possible parameters of platelet aggregation,[2] was more greatly elevated in group A than in group B ; the t-test demonstrating a significant difference at $p<0.01$. Thompson and his associates[10] asserted on the basis of their experimental data that LDH activity per 10^{10} platelets and dense-body content per platelet correlated significantly with platelet volumes, and that larger platelets were not always young, but were destined to have a longer lifespan. The fact that SR is lower in large platelets, as shown in Group B in Table 4, indicates that the functional disturbance of platelets in thrombocytopenia is caused by enhanced platelet destruction and stimulated megakaryopoiesis.

It is well known that the platelet count is elevated[12] in "myeloproliferative disorders", the post-splenectomy condition, infection, some malignant diseases and the reconvalescent stage of disseminated intravascular coagulation (DIC). Abnormal platelet function and prolonged bleeding time occur in varied frequency in myeloproliferative disorders,[13] but the pathogenetic mechanism by which thrombocytosis causes hemostatic disturbance in these diseases has not yet been clarified. It is apparent from Table 5 that a decrease in platelet volume, a

greater reduction in ER than expected, and lowered platelet reactivity represent the main abnormalities inherent in thrombocytosis. However, the thrombotic complication frequently seen in the thrombocytosis of myeloproliferative disorders cannot be explained by this lowered platelet reactivity. It is therefore necessary to assume that there is heterogeneity of the circulating platelet population to understand thrombocytosis of these disorders. Small and his associates[8] reported affirmatively that distribution analysis of the platelet volume was useful for the detection of myeloproliferative diseases. Functional studies of indiscriminate platelet populations on the whole may not accurately disclose platelet dysfunction.

The vicissitude of the platelet count seen for a short period in the course of DIC (Table 6) was the most intriguing problem to us. The SR tended to be low despite the large platelet volume. In any case, this connotes the possibility of platelet dysfunction in thrombocytopenia without bone marrow suppression.

The platelet saponin test, in our opinion, may be recommended as a simple and reliable functional test of platelets.

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