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acute idiopathic thrombocytopenic purpura
anemia
leukemia

platelet volume
thrombocytopenias
thrombopoiesis

Differential Diagnosis of Various Thrombocytopenias in Childhood by Analysis of Platelet Volume

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Summary

Platelet volume was measured in 20 healthy children, 18 patients with acute idiopathic thrombocytopenic purpura (ITP), 24 patients with chronic ITP, 22 patients with aplastic anemia, and 17 patients with acute leukemia in childhood. The authors compared the platelet volume of these patients by use of peak platelet volume (PPV or mode of the platelet volume), mean platelet volume (MPV) and the percent of large platelets (PLP) as parameters of the platelet volume, and then studied the relationship between the platelet volume and the specific gravity of platelets from these patients.

The following was the platelet volume of normal children: PPV was 6.7 ± 1.1 μm^3, MPV was 9.9 ± 1.6 μm^3, PLP was 26.0 ± 7.7%. All parameters of the platelet volume were remarkably large in chronic ITP, but only PPV was small in acute ITP. On the other hand, all parameters were small in both aplastic anemia and acute leukemia. In acute ITP and chronic ITP, the platelet volume returned to normal value after these thrombocytopenias disappeared. The platelet volume, however, still remained small even after remission in acute leukemia.

There was better correlation between MPV and PLP than PPV and PLP.

The density analysis of the platelets obtained from platelet-rich plasma (PRP) revealed that PLP in each medium (1.010 ~ 1.052) of normal PRP increased slowly in proportion to the increase of specific density of the media. On the contrary, most of large platelets obtained from the patients with acute ITP and chronic ITP were especially contained in the medium of high specific density (1.052). Large platelets of aplastic anemia and acute leukemia, however, were found in both low and high density media.

The authors believe that analysis of the platelet volume using above mentioned three parameters would provide good weapon in early differential diagnosis of acute ITP, chronic ITP, and aplastic anemia. And these findings also may suggest the difference of thrombopoiesis between acute ITP and chronic ITP.

Speculation

It is often difficult to discriminate between acute ITP and chronic ITP, or chronic ITP and mild case of aplastic anemia at the first medical consultation. Analysis of the platelet volume of these patients is very useful in the differential diagnosis of these thrombocytopenic states; this fact may also suggest the difference of thrombokinetics and thrombopoiesis between acute ITP and chronic ITP. The analysis of the platelet volume may represent a good indicator of thrombopoiesis as well as reticulocytes in erythropoiesis.

Recently, it has been shown that the platelets in peripheral circulation are cohort of heterogeneous platelets biologically and functionally (12–15). There are several reports suggesting that the large, heavy platelets are young platelets recently released from the bone marrow (15, 17, 18) and small platelets are aged platelets with reduction of platelet function (11).

Some difficulty often has been encountered in discriminating among acute ITP, chronic ITP, and early phase or a mild case of aplastic anemia in childhood; therefore, the authors investigated whether or not the platelet volume was useful as the means of early differential diagnosis of these three diseases. The difference of thrombokinetics was also studied by comparing the platelet volume before and after treatment of the children with acute ITP, chronic ITP, and acute leukemia.

METHODS

DETERMINATION OF THE PLATELET VOLUME

Platelet counts and the platelet volume were measured by the procedures that have been previously described in detail (5, 9). Blood samples of 1 ml were drawn from the vein of forearm and mixed immediately with a drop of Anglot/ET [EDTA compound (EDTA 2K, EDTA 2Na, EDTA 3K), 8.0% heparin fraction, 0.001%] in a glass tube. PRP was obtained by gravity sedimentation of the above mentioned blood sample in plastic 3 mm-diameter tubes at room temperature for 30 min. PRP (6.66 μl) was diluted in 20 ml of diluting solution, Isoton (Coulter Electronics). Model ZBI Coulter Counter and Model P_{64} size distribution analyzer (Coulter Electronics) were employed to measure the platelet volume. A 50μ aperture tube was used and machine settings were as follows: for the Coulter Counter, 1/amplification=4, 1/aperture current=½, upper threshold=50, and lower threshold=5. And the equipment was calibrated using 2.02 μm^3 diameter latex particles (Coulter Electronics). The authors used three parameters to analyze the platelet volume in peripheral circulation (Fig. 1). PPV was used to represent a mode of platelet volumes of platelet population in test sample and MPV showed mean value of platelet volume. The PLP was calculated as ratio of platelets with volume of from 13–27 μm^3 to whole platelet population (9).

PREPARATION OF PLATELET SUSPENSION FOR DENSITY ANALYSIS

Separation of platelets on density gradients was performed using Ficoll-Metrizoate solution (2, 3, 23). Two ml of venous blood anticoagulated with heparin was mixed with an equal volume of physiologic saline, and this mixed whole blood was carefully layered over 3 ml of Ficoll-Metrizoate solution [29.4 ml of 32.8% sodium metrizoate solution (the density of the solution at 20°C is 1.200 ± 0.001 g/ml; the pH is 7.3 ± 0.3) was added Ficoll solution to a total volume of 100 ml] in a 10-ml test tube. The test tube was then centrifuged for 30 min at 400 *g*. The cell suspension (lymphocytes and platelets) at the interface between plasma and separation fluid was transferred to another test tube, and some Tris-BSS (1:1 isotonic Tris buffer in balanced salt solution (8)) was added, and the suspension was centrifuged for 10 min at 240 *g*. After this supernatant fluid was centrifuged for

15 min at 960 *g*, the platelet deposits were resuspended in Tris-BSS and the same procedures were repeated twice. Finally, the platelet suspension was obtained after 1 ml of Tris-BSS was added to the deposits.

SEPARATION OF PLATELETS INTO HEAVY AND LIGHT POPULATIONS

Separation of platelets on density gradients was performed using arabic gum solution based on the modified procedure of Spear (21). One ml of the above mentioned platelet suspension was put on the top layer of arabic gum solutions of five different specific gravities in a test tube. The test tube was then centrifuged for 30 min at 1500 × *g*. The five specific gravities used to determine density distribution of the platelets were: 1.010, 1.020, 1.032, 1.042, and 1.052. These were prepared by mixing arabic gum and physiologic saline. The platelet volume was measured for the platelets of each density medium, with the above mentioned method.

MATERIALS

Twenty normal children (aged between 6 months to 14-yr-old), aplastic anemia (22 cases), acute ITP (18 cases), chronic ITP (24 cases), and acute leukemia (17 cases including 9 cases of lymphocytic, 7 cases of myelocytic, and 1 case of monocytic). Acute ITP and chronic ITP were diagnosed by criteria previously reported (1). All patients were studied on admission to this hospital and before therapy was initiated. Also studied were the platelet volumes of clinical "remission" in children with acute ITP (14 cases), chronic ITP (21 cases), and acute leukemia (25 cases).

RESULTS

COMPARISON OF THE PLATELET VOLUME IN APLASTIC ANEMIA, ACUTE ITP, AND CHRONIC ITP (TABLE 1)

Table 1 showed PPV, MPV, and PLP of normal children, patients with aplastic anemia, acute ITP, and chronic ITP. There was obvious difference in the platelet volume of these three diseases. In 20 healthy control subjects, PPV:6.7 ± 1.1 μm^3, MPV: 9.9 ± 1.6 μm^3, and PLP:26.0 ± 7.7%. In aplastic anemia (22 cases), the platelet volume was small in all of these three parameters (4.3 ± 1.2 μm^3, 8.2 ± 1.1 μm^3, 16.8 ± 6.2%), and in acute ITP (18 cases), only PPV was low value (4.0 ± 1.7 μm^3), but normal in both MPV and PLP (9.5 ± 1.3 μm^3, 26.6 ± 6.2%). On the contrary, chronic ITP had large value in all children (8.7 ± 2.2 μm^3, 12.3 ± 1.1 μm^3, 41.1 ± 7.5%).

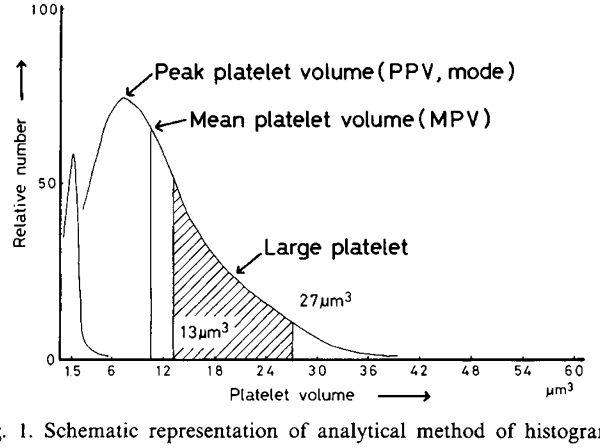

Fig. 1. Schematic representation of analytical method of histogram of platelet volume.

RELATIONSHIP BETWEEN PPV AND PLP (FIG. 2)

The authors investigated the reciprocity between PPV and PLP in the above mentioned 64 cases with aplastic anemia, acute ITP, and chronic ITP. There was poor correlation in PPV and PLP (r = 0.71).

RELATIONSHIP BETWEEN MPV AND PLP (FIG. 3)

A close correlation between MPV and PLP was found in these three diseases (r = 0.95). As for the above two parameters, acute ITP had slight overlapping with chronic ITP or aplastic anemia, but there was no overlap in chronic ITP and aplastic anemia.

COMPARISON OF THE PLATELET VOLUME BEFORE AND AFTER TREATMENT OF CHILDREN WITH ACUTE ITP, CHRONIC ITP, AND ACUTE LEUKEMIA (FIG. 4)

PPV of acute ITP returned to normal value (6.7 ± 0.8 μm^3) after thrombocytopenia disappeared (14 cases). In chronic ITP, all of the abnormal parameters observed before therapy became normal (PPV:7.2 ± 0.8 μm^3, MPV:9.9 ± 1.9 μm^3, PLP:27.3 ± 6.4%) after the platelet counts recovered more than 100 × $10^3/mm^3$ by splenectomy or corticosteroid therapy (21 cases). In acute leukemia, the platelet volume was small (4.1 ± 1.5 μm^3, 8.5 ± 1.4 μm^3, 17.7 ± 5.9%) regardless of various types of acute leukemia; and PPV returned to normal (6.4 ± 0.9 μm^3) in complete remission (25 cases), but MPV and PLP remained unchanged (8.7 ± 1.6 μm^3, 19.7 ± 5.5%).

COMPARISON OF THE PLATELET VOLUME IN VARIOUS PHASES OF TREATMENT IN CHRONIC ITP (FIG. 5)

The platelet volume returned to normal value (PPV:7.1 ± 1.0 μm^3, MPV:9.2 ± 2.0 μm^3, PLP:26.0 ± 6.6%) in 10 splenectomized

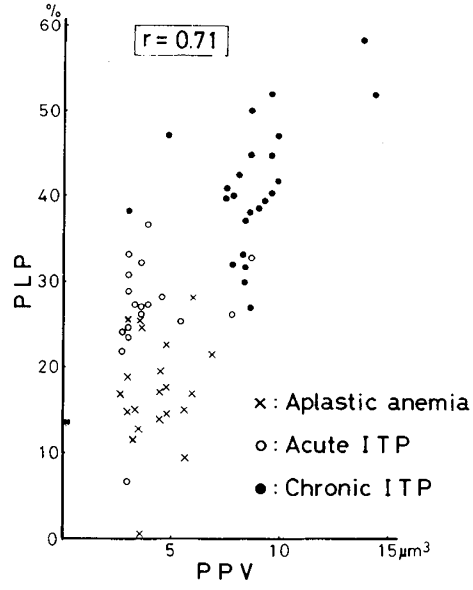

Fig. 2. Relationship between PPV and in thrombocytopenic children.

Table 1. *Comparison of platelet volume of children with aplastic anemia, acute ITP, and chronic ITP (mean value ± SD)*

	Normal	Aplastic anemia	Acute ITP	Chronic ITP
Cases	20	22	18	24
PPV(μm^3)	6.7 ± 1.1	4.3 ± 1.2	4.0 ± 1.7	8.7 ± 2.2
MPV(μm^3)	9.9 ± 1.6	8.2 ± 1.1	9.5 ± 1.3	12.3 ± 1.1
PLP(%)	26.0 ± 7.7	16.8 ± 6.2	26.6 ± 6.2	41.1 ± 7.5

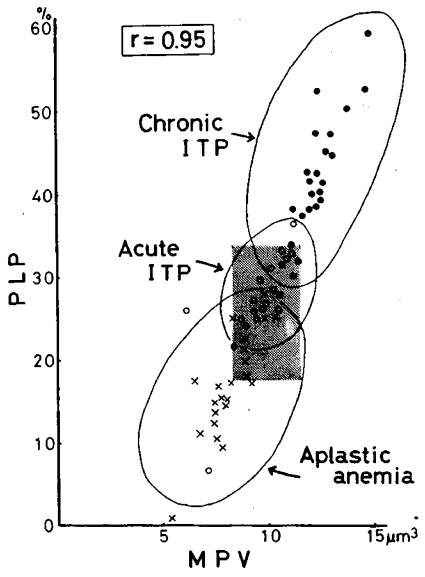

Fig. 3. Relationship between MPV and PLP in thromboyctopenic children. The shaded area denotes normal range (mean value ± S. D.).

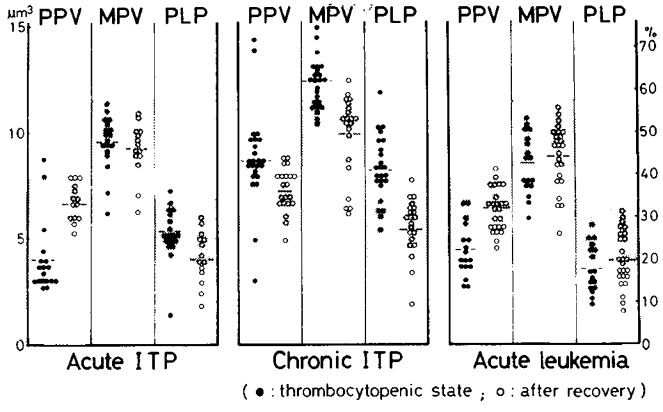

Fig. 4. Changes in platelet volume after treatment of thrombocytopenic children. The shaded area denotes normal range (mean value ± S. D.).

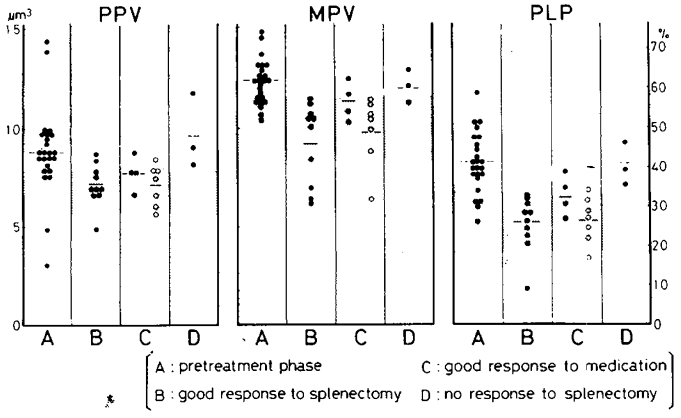

Fig. 5. Comparison of platelet volume in various phases of treatment in chronic ITP. Platelet counts became more than $150 \times 10^3/mm^3$ (C. O) and $100 \times 10^3/mm^3$ up to $150 \times 10^3/mm^3$ (C. ●) by corticosteroid therapy. The shaded area denotes normal range (mean value ± S. D.).

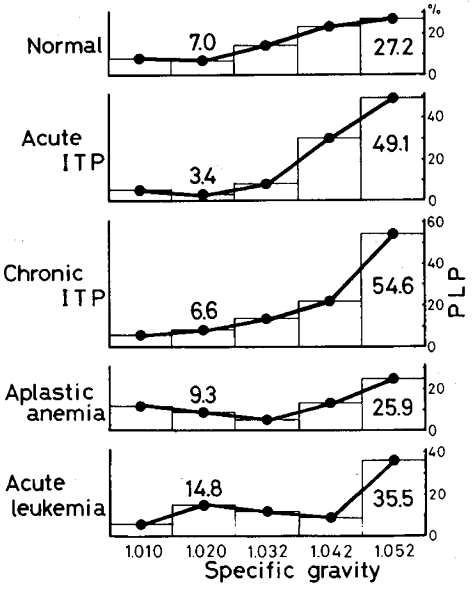

Fig. 6. Relationship between PLP and specific gravity in thrombocytopenic children.

patients (B) whose platelet counts increased more than $150 \times 10^3/mm^3$. The platelet volume also returned to normal value (7 cases, PPV:7.1 ± 0.9 μm^3, MPV:9.8 ± 1.6 μm^3, PLP:26.2 ± 5.4%) when the platelet counts became more than $150 \times 10^3/mm^3$ by corticosteroid therapy (C. O). The platelet volume still tended to be large (PPV:7.7 ± 0.7 μm^3, MPV:11.3 ± 0.8 μm^3, PLP:32.8 ± 4.1%) in four cases whose platelet counts are $100 \times 10^3/mm^3$ up to $150 \times 10^3/mm^3$ (C. ●). In only three patients whose platelet counts remained unchanged in spite of splenectomy, these platelet volumes were remarkably high value (PPV:9.6 ± 1.5 μm^3, MPV:12.1 ± 0.6 μm^3, PLP:40.3 ± 4.1%), but bleeding tendency of all patients improved in comparison with preoperated stage.

PLATELET VOLUME:DENSITY ANALYSIS OF THE PLATELETS IN THE CHILDREN WITH VARIOUS THROMBOCYTOPENIAS (FIG. 6)

PLP in each medium of normal children and these patients with thrombocytopenias were shown in Figure 6. Large platelets were contained in low density medium, too. In general, PLP in each medium increased in normal children, acute ITP, and chronic ITP as density of gum solution increases. In normal children, the increment rate of PLP associated with an increase of specific gravity from 1.010–1.052 was 3.8, but in acute ITP or in chronic ITP, the increment rate of PLP associated with the same specific gravity range was 14.4, 8.3, respectively. Thus, the increment rate of PLP in acute ITP and chronic ITP, especially in heavy media, was remarkably high as compared with normal subject. On the contrary, high PLP of aplastic anemia and acute leukemia was found in both low and high density media.

DISCUSSION

Often seen are the patients with aplastic anemia whose hematologic abnormalities are mild, for example, they had remarkable thrombocytopenia, but the intesity of anemia and leukopenia remained slight for many months. At that time, there were difficulties in the diagnosis of chronic ITP or mild aplastic anemia or early stage of aplastic anemia. Furthermore, the differential diagnosis between acute ITP and chronic ITP in childhood at the first visit is not always easy if preceding infection as cause of thrombocytopenia could not be found in past history or onset of

bleeding episodes was obscure. Retrospective diagnosis has been made as for acute type or chronic type for such patients. Platelet survival studies must be good weapon to discriminate these thrombocytopenias, but this test is the time consuming procedure. Therefore, the authors tried to investigate the usefulness of platelet volume as the means of early differential diagnosis of these different kinds of thrombocytopenias. As mentioned above, striking contrast was found in the platelet volume among these three diseases. For example, the apparent differences were found in the platelet volume of the patients with aplastic anemia and chronic ITP in even early stage, and it was shown that these thrombocytopenias could be easily discriminated by analysis of the platelet volume. On the other hand, all patients with ITP who were diagnosed as acute type by analysis of the platelet volume at the first medical consultation never transformed to chronic ITP retrospectively. Other investigators (4, 10) as well as these data described that there were many large platelets in chronic ITP. On the other hand, Khan *et al.* (16) describe that a striking increase in microthrombocytes, as well as megathrombocytes, is noted in 86% of 21 patients with idiopathic/autoimmune thrombocytopenic purpura on one or more occasions, particularly in the presence of severe thrombocytopenia, and that electron microscopic studies of the patients with thrombocytopenia reveal that microthrombocytes are composed of intact small platelets as well as platelet fragments. In the results of this study, only two patients of chronic ITP showed significantly low value in PPV (3.0 μm^3, 4.8 μm^3), in whom the microthrombocytes and the destroyed platelets may increase remarkably as well as those reported by Khan et al. (16) but they had high value in both MPV and PLP (MPV:11.1 μm^3, PLP:38.3%; MPV:13.0 μm^3, PLP:47.1%, respectively).

The platelets of aplastic anemia were small. It is probable that aged platelets may increase in peripheral circulation of this disease or these platelets may represent abnormal aberrant platelet population due to defective thrombopoiesis.

In acute leukemia, PPV was remarkably low value and both MPV and PLP also tended to be low value, as a rule. In complete remission, PPV returned to normal value, but MPV and PLP remained unchanged as compared with pretreatment phase. This fact may show that platelet function or thrombopoiesis in acute leukemia does not always normalize completely even after complete remission. In fact, it is possible that the bone marrow and the platelets are impaired under the influence of chemotherapy as reported by Steinherz *et al.* (22). In separation of the platelets by density gradients, the authors found that the platelet population of acute leukemia contained many large, light platelets. Cowan and Graham (7) describe the platelets of acute leukemia as giant platelets with marked variability in the number and size of cytoplasmic granules, dilatation of the open channel system, cytoplasmic vacuolization, and poorly delineated microtubular system, and that metabolic defects of the platelets include reduced cellular concentrations of ATP, ADP and selective reduction of the storage pool (nonmetabolic) nucleotides. Many large, light platelets were found in aplastic anemia as well as acute leukemia, and so it is thought that there is something in common between the pathogenesis of these thrombocytopenias in a certain aspect.

From the investigation of the platelet volume of 64 children with aplastic anemia, acute ITP, and chronic ITP, the correlation between MPV and PLP showed better than the one of PPV and PLP, and a positive linear relationship was apparently shown to exist when MPV of each individual was plotted against their PLP. It is emphasized that the correlation diagram of MPV and PLP is very useful in differential diagnosis of various thrombocytopenias, and also it is presumed that MPV or PLP are good parameters to reflect the status of thrombopoiesis.

There is still controversy whether large platelets always means young platelets or not (11, 15, 17–19). These data that the population of large platelets increases in ITP patients whose thrombopoiesis is considered to be stimulated and reduces in aplastic anemia with defective thrombopoiesis, however, apparently confirm close relationship between the platelet volume and thrombopoiesis. The experiments using arabic gum solution with various densities, however, showed that there were cohorts large, light platelets and small, heavy platelets in the circulating platelets, because large platelets were found in every medium of various specific gravities. Penington *et al.* (20) describe that small platelets present in every density fraction at density analysis of the platelets without exception. And their findings by no means rule out the possibility of diminution of the platelet size with aging within the several density fractions, nor that the megathrombocytes of the intermediate and dense fractions are, on average, the younger cells in these fractions. Corash *et al.* (6) demonstrate that the platelet subpopulations separated by density overlapped each other but that the platelets showed a general trend of increasing volume with increasing density. In the results of this study, PLP of each fraction in normal children increased slowly in proportion to the specific gravity of separating solution, too. And the increment rate of PLP in each fraction in acute ITP and chronic ITP was remarkably high as compared with normal children; and the platelet population of acute ITP, as well as chronic ITP, was found to have many large, heavy platelets by density gradient analysis; on the other hand, the platelet volume of acute ITP revealed small PPV and normal MPV or PLP value by sedimentation method. Accordingly, there may be not only small, heavy platelets, but also large, heavy platelets in peripheral circulation in acute ITP. On the contrary, in chronic ITP, large, heavy platelets may predominantly increase in peripheral circulation as shown by both density gradient analysis and sedimentation method, and so these findings may suggest that large platelets are not always young platelets and there is the difference of thrombopoiesis between acute ITP and chronic ITP.

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