Clinical significance of simultaneous measurement of reticulated platelets and large platelets in idiopathic thrombocytopenic purpura

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Abstract—Frequencies of reticulated platelets (RP) and large platelets (LP) among circulating platelets can now be simultaneously determined using the R-3000 automated reticulocyte counter (Sysmex, Kobe, Japan) equipped with special software. We measured frequencies of RP and LP in patients with idiopathic thrombocytopenic purpura (ITP, acute type \( n = 5 \); chronic type \( n = 39 \)), and healthy normal controls (\( n = 20 \)). In ITP patients, the platelet-associated IgG (PAIgG) level was also determined. Both RP and LP were significantly higher in chronic ITP patients than those in normal volunteers, and interestingly, the LP in acute ITP was significantly lower than that in chronic ITP although there was no significant difference in RP between acute and chronic ITP. Furthermore, we analyzed the changes in both RP and LP during the clinical course of ITP to monitor the therapeutic effect in 2 patients. An elevation of RP with a steep slope prior to a decrease in the platelet count level was observed. The RP significantly correlated with the PAIgG level. Simultaneous measurement of RP and LP may be helpful for the diagnosis of chronic ITP, for the differentiation of acute from chronic type and for the control of the efficacy of management in ITP, since RP seems to reflect the disease activity of ITP.

Key words: Reticulated platelets; large platelets; idiopathic thrombocytopenic purpura; R-3000 automated reticulocyte counter.

INTRODUCTION

In 1969, Ingram and Coopersmith [1] identified reticulated platelets (RP) as those displaying coarse, punctate condensations (reticulum) when stained supravitally with new methylene blue dye. However, this method of staining has not been routinely applied in the clinical laboratory. Circulating RP and large platelets (LP) can now be simultaneously determined using the R-3000 automated reticulocyte counter.
counter (Sysmex, Kobe, Japan) equipped with special software [2, 4, 5, 13, 15]. RP are detected after rapid staining of RNA by a fluorescent dye, auramine O. RP are defined as platelets containing large amounts of RNA. Although RP have been considered to be young platelets just derived from bone marrow [12, 19], it has not yet been determined whether the measurement of RP provides valuable clinical information regarding thrombopoiesis [11], as that of reticulocytes does for erythropoiesis [22]. LP are determined simultaneously with RP from size-frequency scattergrams obtained with the R-3000 [3, 13, 15]. LP are defined as platelets of large size without abundant RNA. However, the clinical relevance of the measurement of LP in hematological disorders has not yet been determined. We recently reported that a significant positive correlation was observed between LP and mean platelet volume, and between RP and LP in both normal and thrombocytopenic disorders, and the simultaneous measurement of RP and LP is useful for elucidating the pathophysiology of thrombocytopenic disorders [13].

In this study, we determined both RP and LP frequencies for patients with idiopathic thrombocytopenic purpura (ITP), using the R-3000 in order to evaluate the value of simultaneous measurement of RP and LP for the clinical diagnosis and the correlation of the results with the determination of the level of platelet-associated IgG.

MATERIALS AND METHODS

Patients

Twenty healthy volunteers and 44 patients with ITP (12 males and 32 females, average age 49.7 years) were entered in this study after having given informed consent. Five of the 44 patients with ITP exhibited a good response to steroid therapy and were induced into remission within six weeks. Five patients had acute ITP (one male and 4 females, average age 55.6 years) while 39 patients had chronic ITP (11 males and 28 females, average age 48.4 years). The diagnosis of ITP was based on clinical criteria [6, 24]. All ITP patients were at the onset of disease. RP, LP and platelet count (PLT) levels in two patients with chronic ITP were followed over the clinical course.

Normal controls

Normal controls were healthy adult volunteers from 20 to 50 years without abnormality for complete blood count and blood chemistry tests, according to the reference values established in our laboratory.

Measurements of RP, LP and PLT

Approximately 2 ml of peripheral venous blood was collected from each of the healthy volunteers and patients and anticoagulated with di-potassium ethylenediaminetetra-acetic acid. All blood samples were kept at room temperature until