Platelet volume analysis for differential diagnosis of thrombocytosis

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SUMMARY The results of the Coulter counter S plus II platelet volume analysis were studied in 100 patients with reactive thrombocytosis (platelet count $> 500 \times 10^9/l$), in 30 patients with myeloproliferative thrombocytosis, and in 32 patients with chronic myeloproliferative disease and a platelet count $< 500 \times 10^9/l$.

Patients with reactive thrombocytosis had considerably lower mean platelet volumes than those with myeloproliferative thrombocytosis, or normal subjects. The opposite was true for the platelet distribution width. This index for platelet heterogeneity was normal in reactive, but increased in myeloproliferative thrombocytosis. There were no differences in mean platelet volume or platelet distribution width between patients with myeloproliferative disease and a high or normal platelet count. The increased platelet heterogeneity in myeloproliferative disease was caused by an increase of both small and large platelets.

The platelet distribution width seemed to be the best variable for the differential diagnosis of thrombocytosis. A platelet distribution width > 17 was found in 26 of the 30 patients with myeloproliferative thrombocytosis but in only five of the 100 patients with reactive thrombocytosis. A normal platelet distribution width in a patient with a high platelet count strongly suggests reactive thrombocytosis.

Thrombocytosis can result from a myeloproliferative disease, but is more commonly found as a reactive phenomenon, not caused by a bone marrow disease, but secondary to various pathological states.¹ The differentiation between these two causes of thrombocytosis can sometimes cause difficulties. Platelets from patients with myeloproliferative thrombocytosis may differ from those with reactive thrombocytosis in morphology, platelet volume distribution pattern, surface membrane composition, arachidonic acid metabolism, and granule content and function.² From these the platelet volume analysis can easily be performed in routine daily practice, as the modern fully automated blood cell counters produce comprehensive analysis on each whole blood sample that is processed. We therefore studied the efficiency of a platelet volume analysis using the Coulter S plus II and of the derived variables mean platelet volume (MPV) and platelet distribution width (PDW) for the differential diagnosis of thrombocytosis.

Material and methods

The platelet volume analysis was made using a Coulter counter S plus II. Particles with a volume between 2 and 20 fl are classified by this instrument as platelets by definition, and a volume distribution histogram is generated and fitted to the nearest log normal curve. From this the platelet count, MPV, and PDW are computed.³ The PDW is calculated from the volumes on the 16th and 84th percentile.³

All measurements were made in whole blood anticoagulated with edetic acid. As it has been shown that edetic acid causes an increase in platelet volume during the first hour after collection, after which it remains stable for several hours,⁴ all measurements were made between one and six hours after the blood had been collected.

The histogram showing platelet volume distribution was plotted using a X-Y recorder. By measuring their relative surface area in the histogram we calculated the percentage of microthrombocytes and megathrombocytes. Microthrombocytes were arbitrarily defined as particles with a volume between 2 and 5 fl;

Table 1 Diagnoses in patients with reactive thrombocytosis (platelet count $> 500 \times 10^9/l$)

Diagnosis	Total no of cases	No of children
Infections	43	20
Postoperative or bleeding, both splenectomy	22 (7)	3
Non-haematological malignancy	11	
Iron deficiency	3	
Sickle cell disease	3	
Collagen disease	3	
Miscellaneous*	15	7
Total	100	30

*Includes healthy premature infants (4), diabetes mellitus (1), inflammatory bowel disease (2), renal insufficiency (2), congestive heart failure (2), various neurological disorders (4).

megathrombocytes, according to Garg *et al.*,⁵ as particles with a volume greater than 13 fl.

PATIENTS

All 30 patients with a thrombocytosis (platelet count $> 500 \times 10^9/l$) caused by a myeloproliferative disorder, who were either being treated or who had come under our care over two years (1983–84) were studied. Diagnoses in these patients were: essential thrombocythaemia in 14; polycythaemia vera in 10; myelofibrosis in five; and chronic granulocytic leukaemia in one. Diagnoses were made according to the criteria of the polycythaemia vera study group.² Thirty two patients with the same diagnoses, but with a platelet count between 150 and $500 \times 10^9/l$ under our care during the same period were also studied. Twelve of these patients had polycythaemia vera, 10, essential thrombocythaemia, seven, chronic granulocytic leukaemia, and three, myelofibrosis. Most of them were treated, usually with busulphan; 18 had platelet counts above $500 \times 10^9/l$.

Platelet volumes were also studied in 100 consecutive patients with reactive thrombocytosis (platelet count $> 500 \times 10^9/l$). These patients were traced by reviewing the results of all blood samples analysed by the Coulter counter over six weeks. The samples

were from all medical, surgical, and paediatric specialties of our 950 bed hospital. The pattern of diagnoses (Table 1) resembles that found by others.¹ Infection was the most common cause, especially in children. Normal values were obtained from 95 healthy hospital workers.

Student's *t* test with the Bessel correction was used for statistical analysis. A difference of $p < 0.05$ was regarded as significant.

Results

Seventy three Coulter counter measurements were made from the 30 patients with myeloproliferative thrombocytosis, ranging from one to four per patient; and in the 32 patients with chronic myeloproliferative disease with a platelet count between 150 and $500 \times 10^9/l$ 58 measurements were made (one to three per patient) (Table 2).

The mean platelet volume in patients with myeloproliferative thrombocytosis was significantly higher than that of the group with reactive thrombocytosis, although the overlap was considerable (Fig. 1). Normal subjects also had a much higher mean platelet volume than patients with reactive thrombocytosis. The mean platelet volume in the patients with myeloproliferative disease, both in those with a platelet count $> 500 \times 10^9/l$ and in those with a count between 150 and $500 \times 10^9/l$, did not differ from that found in normal subjects (Fig. 1).

The opposite was true for platelet distribution width. There was no difference between normal subjects and patients with reactive thrombocytosis, but patients with myeloproliferative thrombocytosis and those with myeloproliferative disease and a platelet count between 150 and $500 \times 10^9/l$ had a considerably increased platelet distribution width (Fig. 2). In only five patients with reactive thrombocytosis (5%) was a platelet distribution width > 17 found. Two of these patients had iron deficiency, two infection, and one colonic carcinoma with metastases. In the two

Table 2 Variables of platelet volume analysis (mean (SD)) in normal subjects and different patient groups

	Normal subjects	Reactive thrombocytosis	Chronic myeloproliferative disease platelet count	
			150–500 $\times 10^9/l$	$> 500 \times 10^9/l$
No of subjects	95	100	32	30
No of counts	95	100	58	73
Platelet count ($10^9/l$)	260 (46)	611 (105)	344 (106)	852 (384)
MPV (fl)	7.5 (0.6)	6.8 (0.7)	7.4 (1.1)	7.4 (1.0)
PDW	16.1 (0.5)	16.2 (0.5)	17.9 (0.8)	17.8 (0.8)
2–5 fl particles (%)	24.3 (6.2)	30.8 (9.5)	30.7 (8.2)	30.3 (8.0)
13–20 fl particles (%)	7.6 (3.3)	4.8 (2.3)	9.6 (4.6)	8.5 (3.8)

Patients with reactive thrombocytosis differ significantly from normal subjects in MPV and percentage of 2–5 and 13–20 fl particles and from patients with myeloproliferative disease in percentage of 13–20 fl particles and PDW. Patients with myeloproliferative disease differ significantly from normal subjects in PDW and percentage of 2–5 fl particles.

Fig. 1 Mean platelet volume in normal subjects and different patient groups. Horizontal lines indicate mean (SD). ○ indicates patients with chronic granulocytic leukaemia and myelofibrosis; ● represents patients with essential thrombocythaemia and polycythaemia vera in groups with myeloproliferative disease. Mean platelet volume in patients with reactive thrombocytosis is considerably lower than that in the three other groups.

Fig. 2 Platelet distribution width in normal subjects and different patient groups. Horizontal lines indicate mean (SD). ○ indicates patients with chronic granulocytic leukaemia and myelofibrosis. ● represents patients with essential thrombocythaemia and polycythaemia vera in groups with myeloproliferative disease. Platelet distribution width in patients with myeloproliferative disease is considerably higher than that in normal subjects and those with reactive thrombocytosis.

patients with iron deficiency and the one with infection the platelet distribution width fell below 17 after treatment. For the two other patients no follow up was available. On the other hand, in only eight of the 73 measurements in myeloproliferative thrombocytosis (four patients) was a platelet distribution width below 17 found (Fig. 2). For platelet distribution width therefore, there was a much smaller overlap between reactive and myeloproliferative thrombocytosis than for mean platelet volume.

In the myeloproliferative groups there were no differences in mean platelet volume or platelet distribution width between patients with essential thrombocythaemia and polycythaemia vera and those with myelofibrosis or chronic granulocytic leukaemia (Figs. 1 and 2).

Table 2 shows the results of determining the percentage of microthrombocytes (2–5 fl) and megathrombocytes (13–20 fl) from the platelet volume distribution histogram. Compared with normal subjects the patients with reactive thrombocytosis and those with myeloproliferative disease had a substantially increased number of small platelets. The percentage of megathrombocytes in the patients with myeloproliferative disease was considerably greater than that in patients with reactive thrombocytosis, and was also greater, although not significantly so, than in normal subjects. The percentage of megathrombocytes in patients with reactive thrombocytosis was significantly lower than that in normal subjects (Table 2). The overlap in the percentage of megathrombocytes between reactive and myeloproliferative thrombocytosis was greater than that found for platelet distribution width.

Discussion

In patients with myeloproliferative disease there are often abnormalities in platelet morphology in blood smears, including an increased percentage of megathrombocytes and giant platelets.^{6–8} In our patients with chronic myeloproliferative disease we found an increased percentage of megathrombocytes by Coulter counter S plus II platelet volume analysis. The percentage of microplatelets, however, was also increased. This leads to the increased platelet heterogeneity in such patients, which has been reported in several studies.^{8–10} It is probably a reflection of the megakaryocyte abnormalities that are often found in patients with myeloproliferative disease.¹¹ The increased platelet heterogeneity finds expression in an increased platelet distribution width computed by the Coulter counter.

Patients with reactive thrombocytosis also had an increased percentage of microplatelets but a lower number of megathrombocytes. This results in a nor-

mal platelet distribution width but a mean platelet volume that is lower than that found in normal subjects. This observation agrees with the findings of others.^{1,4} In normal subjects a non-linear inverse relation between platelet count and mean platelet volume has been found: the higher the platelet count, the lower the mean platelet volume.¹² The low mean platelet volume in reactive thrombocytosis might be an extrapolation of this relation. Consequently, the mean platelet volume in myeloproliferative thrombocytosis, although not different from that in normal subjects, might still be abnormally increased.

Although the mean platelet volume and the percentage of megathrombocytes in patients with myeloproliferative thrombocytosis were considerably higher than in patients with reactive thrombocytosis, these variables are of little clinical use in differential diagnosis because of the large overlap. The clinical importance of the platelet distribution width in this regard seems to be greater. Only five of the 100 patients with reactive thrombocytosis had a platelet distribution width > 17, whereas this was found in 26 of the 30 patients with myeloproliferative thrombocytosis. When a platelet distribution width > 17 was chosen as the dividing point the sensitivity and specificity for diagnosing myeloproliferative thrombocytosis was 87% and specificity 95%, respectively. The prevalence of myeloproliferative thrombocytosis was, however, much lower than that of reactive thrombocytosis. During the six weeks it took us to collect the 100 cases of reactive thrombocytosis no new myeloproliferative thrombocytosis was diagnosed. In analysing 372 cases of thrombocytosis Robbins and Barnard found a myeloproliferative disorder to be the cause in only 1.6%.¹ According to the theory of Bayes, this leads to a low positive predictive value.¹³ With a prevalence of 1.6% the positive predictive value for myeloproliferative thrombocytosis of a platelet distribution width > 17 is only 22%. On the other hand, the negative predictive value is 99.8%.

In conclusion, an increased platelet heterogeneity is found not only in most patients with myeloproliferative thrombocytosis but also in patients who have chronic myeloproliferative disease and normal platelet counts. In reactive thrombocytosis platelet heterogeneity is only sporadically increased. A normal platelet distribution width in patients with a platelet count > $500 \times 10^9/l$ strongly suggests reactive thrombocytosis.

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*Differential diagnosis of thrombocytosis***References**

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