Microscopic evaluation of apparent platelet size and morphology was examined in a variety of hematologic disorders. The time of preparation of the blood smear was important. An artifactual increase in platelet size was noted on blood films from 20 normal individuals that were prepared either immediately or 180 min after venipuncture. The clearest differentiation of patient categories was obtained with smears prepared 60 min after venipuncture using blood anticoagulated with K$_2$EDTA. Under these conditions, normal size and morphology values were found in thrombocytopenic patients with aplasia or with increased splenic pooling. In contrast, large size values were a reliable finding in idiopathic thrombocytopenic purpura patients, whose platelet counts were <50,000/µl. Large size values were also noted in patients with infiltrated bone marrows or myeloproliferative syndromes regardless of the platelet count. The last two groups usually showed abnormal platelet morphology with greater than 10% hypogranular platelets. Normal platelet size and morphology were observed in patients with iron-deficiency and megaloblastic anemias and in patients with idiopathic thrombocytopenic purpura and systemic lupus erythematosus who had normal platelet counts.

CLINICAL EVALUATION of disorders of the platelet-megakaryocyte system rests on the quantitative measurement of platelet number, an estimation of megakaryocyte number in the bone marrow, and determination of splenic sequestration. In contradistinction to red cell disorders, where size and morphology represent important diagnostic features, cytologic evaluation of platelets has not been widely used.

Previous reports have shown that blood platelets vary in size and in functional and metabolic activity. It has been suggested that size depends upon platelet age variation, with young platelets being larger than older ones. Although there is controversy about this concept, an increased proportion of large platelets has been noted in immune thrombocytopenia. This finding is in contrast to the normal platelet size reported in hypomegakaryocytic thrombocytopenia. Previous reports failed to agree on abnormalities in platelet size in iron-deficiency and megaloblastic anemias.

The present study was conducted to determine if an estimation of apparent platelet size and morphology would be useful in the evaluation of platelet disorders. Initial results show that the time of preparation of the blood smear after venipuncture and the type of anticoagulant used were critical variables that in-
fluenced spreading artifact. Using standardized conditions, evaluation of apparent platelet size and morphology appears to be highly useful in assessing a variety of hematologic disorders.

MATERIALS AND METHODS

All specimens were obtained in connection with clinical tests. Platelet counts were performed by the method of Brecher and Cronkite. Platelet survival studies were done after labeling the platelets in vitro with radioactive chromium as previously described.

Microscopic measurement of platelet diameters on Wright-stained "push" peripheral blood smears was done by one observer without knowledge of the source of the slide. Care was taken to measure platelets away from the periphery of the smear in areas where the red cells showed central pallor and did not overlap. One hundred platelet diameters were measured using a calibrated Leitz ocular micrometer at 1000× and recorded as the percentage larger than 3 μm. These studies were performed in patients whose platelet counts did not significantly change within a week of study.

The measured platelets were also designated as being "granular," "hypogranular," or "activated." A platelet was considered to be granular if most of the platelet was deeply stained. Hypogranular platelets were defined as those consisting of a rim of cytoplasm containing less than 20 discrete granules. The platelets were considered activated if they were surrounded by a veil of blue-staining cytoplasm with centralization of the granules. Illustrations of the last two types are shown in Fig. 1.

In order to assess whether or not the time of preparation of the blood smear was important, peripheral blood smears were prepared from 20 normal individuals at 0, 10, 60, and 180 min after venipuncture from B-D vacutainer tubes containing dry powdered Na₂EDTA (ethylenediaminetetraacetate) and liquid K₃EDTA at a final concentration of 1.5 mg/ml.

Patients

Platelet size values were obtained on 20 normal volunteers (10 male, 10 female). None was of Mediterranean origin; thus Mediterranean macrothrombocytosis was not considered.

Thirty-nine patients with acquired destructive thrombocytopenia, on a presumed immunologic
MICROSCOPIC EVALUATION OF PLATELETS

basis, were studied. Splenectomies were performed in 32 of these patients during the course of the study for chronic idiopathic thrombocytopenic purpura (ITP). Shortened platelet lifespan was confirmed by $^{51}$Cr platelet survival studies in 14. In addition, 10 patients with systemic lupus erythematosus (SLE) and 10 of the ITP patients who were not thrombocytopenic at the time of the study were also evaluated.

Six patients with increased splenic pooling, defined as peripheral thrombocytopenia (38,000-117,000/μl), splenomegaly, tagged platelet recovery values of less than 40%24 and platelet survival half-life values of 2.3-5.5 days (normal range 3.0-5.5 days), were included. The primary diagnoses were cirrhosis (three patients), paroxysmal nocturnal hemoglobinuria (two), and lymphoma (one).

Nine patients with chronically decreased platelet production and no bone marrow infiltration were analyzed. Five had chronic idiopathic aplasia; one had phenylbutazone-induced aplasia; three had aplasia associated with antineoplastic therapy.

Fourteen patients with infiltrated bone marrows and platelet counts ranging from 4,000 to 136,000/μl were evaluated. Patient categories were multiple myeloma (one patient), lymphoma (three), and acute leukemia (ten).

The patients with myeloproliferative syndromes (MPS) included those with polycythemia vera (five), idiopathic thrombocytopenia (two), and myelofibrosis (seven). Their platelet counts ranged from 17,000 to 1,600,000/μl.

Also studied were fifteen patients with iron-deficiency anemia. Nine had platelet counts in excess of 400,000/μl; six had normal platelet counts. Eight patients with megaloblastic anemia were studied; two were modestly thrombocytopenic, with platelet counts of 67,000 and 89,000/μl.

RESULTS

Initial results indicated the potential for an artifactual increase in platelet size in normal subjects using K$_3$EDTA-anticoagulated blood. This increase was apparent on smears prepared immediately and 3 hr following venipuncture but was not present on smears prepared between those intervals (Fig. 2). On films made 10 and 60 min after venipuncture the percentage of platelets >3 μm in diameter averaged 4.7% and 4.3%, respectively, with a range of 0-14%. These same measurements on immediate and 3-hr smears were 10% and 9%, respectively. This apparent increase in size was associated with an increased cyto logically abnormal fraction of platelets, as evidenced by either a hypogranular or an activated appearance. Lowest values averaged 4% at 10 and 60 min, whereas 17% and 22%, abnormal cells were noted in the immediate and 180-min...
Platelet size and morphology using 10- and 60-min blood films in patients with aplasia, infiltrated bone marrow, and ITP. Disparate size and morphology results obtained in eight patients with aplasia at 10 and 60 min. Comparable size values seen at these two time intervals in patients with infiltrated bone marrow and ITP. Excess hypogranular platelets (○) were found in those with infiltrated bone marrows and large size values.

smears, respectively. A similar artifactual increase was observed using 3-hr Na2EDTA blood films.

Because of these effects in vitro, care was taken to prepare blood films 10 and 60 min following venipuncture in a variety of clinical disorders. Figure 3 compares the results of microscopic platelet diameter and morphologic analysis in three patient groups (aplasia, infiltrated bone marrows, and ITP). A marked difference in platelet size was seen in the patients with aplasia at the two time intervals. The 10-min value average of 23% ± 3.5% (SEM) was significantly greater than the 60-min average of 7.0% ± 1.5% (p < 0.001). Moreover, the 60-min values were normal with respect to size and morphology; the 10-min films had an excess of hypogranular platelets. These changes were apparent with both Na2EDTA and K3EDTA as anticoagulant.

In contrast to the patients with aplasia, increased size values were found at both time intervals in thrombocytopenic patients with ITP and in those patients with infiltrated bone marrows. A morphologic difference was noted in these two groups: excess numbers of hypogranular platelets were found in the patients with infiltrative disease. Platelets from patients with ITP did not show this abnormality.

Figure 4 shows a comparison of platelet size to peripheral platelet count in 39 patients with ITP. Of 19 patients, 17 whose platelet counts were less than 50,000/μl had elevated size values. Large size values were found in only 4 of 15 moderately thrombocytopenic patients (50,000–150,000/μl); normal size values were observed in all ten nonthrombocytopenic patients with ITP.

Twelve patients with iron-deficiency anemia were studied using 10- and 60 min smears and both the sodium and potassium salts of EDTA as the anticoagulant. Using 60-min K3EDTA smears, size and morphology values were

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Fig. 3. Platelet size and morphology using 10- and 60-min blood films in patients with aplasia, infiltrated bone marrow, and ITP. Disparate size and morphology results obtained in eight patients with aplasia at 10 and 60 min. Comparable size values seen at these two time intervals in patients with infiltrated bone marrow and ITP. Excess hypogranular platelets (○) were found in those with infiltrated bone marrows and large size values.

Fig. 4. Relationship between platelet size and peripheral platelet count in 39 patients with ITP. The more severe the thrombocytopenia, the greater the likelihood of finding an increased size value.
normal. This finding differed from the other time values and from the 60-min Na₂EDTA values, where an admixture of normal and large sizes and of normal and abnormal morphology values was seen.

Figure 5 depicts size and morphology values using K₃EDTA 60-min blood films in a variety of hematologic disorders. Under these conditions platelet diameter and morphology values were normal in 8 patients with megaloblastic anemia, 15 nonthrombocytopenic patients with iron-deficiency anemia, 5 thrombocytopenic patients with aplasia, 6 thrombocytopenic patients with increased splenic pooling, and 20 nonthrombocytopenic patients with ITP and SLE. Large size values were noted in thrombocytopenic patients with ITP and in patients with infiltrated bone marrow or myeloproliferative syndromes without relationship to platelet count. In contrast to the findings in ITP, large size values in the latter two groups were usually associated with an excess number of hypogranular platelets.

**DISCUSSION**

The results of this study show that an assessment of apparent platelet size and granularity is of value in determining the etiology of platelet disorders. As shown by these data, the assessment must be performed under standardized conditions. The most important variables are the times of preparation of the blood smear and the anticoagulant. Optimal separation of patient categories is obtained using K₃EDTA (liquid) blood smears prepared 1 hr following venipuncture.

With controlled conditions, destructive thrombocytopenia could be distinguished from aplasia with platelet counts <50,000/μl. In the former condition, low platelet counts were associated with an increase in size. By contrast, there was no size increase in the thrombocytopenia associated with aplasia.
Large size values, irrespective of platelet counts, were found in patients with the myeloproliferative syndromes or infiltrated bone marrows. These disorders were also associated with an excess number of hypogranular platelets. Platelet size was not increased in patients with ITP or SLE and normal platelet counts. Although such patients may have a modest shortening of platelet lifespan, these studies were unable to detect a potential compensated thrombocytolytic state. Furthermore, apparent size and morphology values were normal in patients with iron-deficiency anemia, megaloblastic anemia, and thrombocytopenia associated with increased splenic pooling.

The excess number of hypogranular platelets observed in patients with the myeloproliferative syndromes and with acute leukemia may reflect one or several defects. There may be an abnormal population of platelets deficient in granules, as has been described by Maldonado and co-workers. Alternatively, this finding may be due to accentuated spreading in vitro. Regardless of the mechanism, an excess number of hypogranular platelets in a thrombocytopenic subject with a large size value was evidence against the diagnosis of ITP.

The results of this study largely confirm the findings of Karpatkin and co-workers, who first pointed out the clinical significance of megathrombocytes in conditions of increased platelet turnover and aplasia. Our results differed in terms of the sensitivity of the technique. In the present study, only a small percentage of patients with destructive thrombocytopenia and modest platelet depressions (50,000–150,000/µl) had elevated size values. Therefore a normal size value at a platelet count above 50,000/µl was compatible with either a destructive or a production defect.

Our findings in ITP relative to platelet count closely parallel those of Paulus, who employed electronic measurement of platelet volume. Moreover, platelet size was not increased in nonthrombocytopenic patients with ITP or SLE or in thrombocytopenic patients with increased splenic sequestration. This finding contrasts with the findings of Karpatkin and co-workers, who found an increased fraction of large platelets in patients with hypersplenism and in a variety of nonthrombocytopenic patients. These included patients with ITP, SLE, disseminated intravascular coagulation, diabetic retinopathy, and valvular heart disease. The reason for these differences was unclear. The present visual technique may not be sensitive enough to define differences from normality in patients with only modest degrees of increased platelet turnover. However, the only substantial difference between the two techniques was the strict attention to time between venipuncture and slide preparation.

Large size values have been reported in conditions other than ITP. These have included hereditary thrombocytopenias, Mediterranean macrothrombocytopenia, and alcoholic thrombocytopenia. Platelet size has been reported to be normal in megaloblastic anemia; normal size and morphology values were found in this disorder in the present study. However, only two of our patients were thrombocytopenic, whereas the great majority of those analyzed by Garg et al. and who had elevated size values were thrombocytopenic. Those reported by Paulus had normal values and were not thrombocytopenic. The apparent discrepancies in megaloblastic anemia may be dependent on severity of the thrombocytopenia. Certainly the present results con-
firm the findings of Paulus in nonthrombocytopenic individuals with megaloblastic anemia.

Similarly, both decreased\textsuperscript{19} and increased\textsuperscript{15} size values have been reported in iron-deficiency anemia. Normal values were found in this study in 15 patients with iron-deficiency anemia, 9 of whom were thrombocytotic; the remainder had normal platelet counts. Garg et al.\textsuperscript{15} found increased size values in iron-deficient patients with low or normal platelet counts and normal size values in those who had elevated counts. In this study it was noted that platelets from this group of patients appeared to have an increased tendency to spread on the glass slide, especially when Na\textsubscript{2}EDTA was the anticoagulant. Perhaps this finding may account for our discrepant findings in iron-deficient patients with normal platelet counts. We have no ready explanation for this phenomenon, or for the abnormal but reproducible differences between 10- and 60-min smears in patients with aplasia (Fig. 3).

Ultimately, one has to consider that the size and morphology of all cells on blood smears are artifacts. This thesis is particularly true for the platelet, which is so reactive to non-physiologic stimuli, such as glass surfaces. The anticoagulants in this study were chosen on the basis of availability. The hypogranular platelets seen at 0 and 180 min in normals and at 10 min in patients with aplasia and iron deficiency are considered as artifacts. These platelets resemble the spread platelets usually seen on smears prepared without anticoagulation. We assumed that the 60-min smears had minimal spreading artifact because they had the fewest hypogranular forms and allowed the clearest separations among patient groups.

It is probably naive to make confident correlations between apparent platelet size on peripheral smears and platelet volume in vivo. It might be hoped that mechanical devices based on the Coulter principle would yield a more accurate reflection of volume in vivo, but there are many difficulties in this type of analysis as well.\textsuperscript{22}

With these reservations, this study outlines a simple, inexpensive technique that can be used by clinicians for evaluation of altered thrombopoiesis.

REFERENCES

Microscopic platelet size and morphology in various hematologic disorders

Z Zeigler, S Murphy and FH Gardner