Abnormalities of Platelet Function in Patients with Polycythemia Vera

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SUMMARY

The exact mechanism(s) of thrombosis and hemorrhage in polycythemia vera has not yet been delineated. Platelet function studies were performed by examining platelet aggregation in 47 polycythemia vera patients (62 studies) with low ("spent"), normal, or elevated hematocrits and with varying platelet counts. Adenosine diphosphate, epinephrine, collagen, and thrombin were used as aggregating agents. Eighty-one % of the 62 studies were abnormal. The frequency of abnormal tests increased from those with normal hematocrits and platelet counts (75%) through to those with elevated hematocrits and platelet counts (100%). One hundred % of the studies in spent polycythemia vera were abnormal. Abnormal bleeding times and thrombohemorrhagic complications did not correlate with each other or with abnormal aggregation, hematocrits, or platelet counts. Repeat studies following therapy showed improvement in 10 of 13 patients. The incidence of abnormal aggregation in polycythemia vera is high; however, the significance of this finding in the pathogenesis of thrombosis and hemorrhage remains obscure.

INTRODUCTION

Thrombosis and hemorrhage are the most frequent causes of morbidity and mortality in polycythemia vera. Thrombotic and hemorrhagic complications occur in 26 to 63% and 16 to 35% of patients, respectively, and are the causes of death in 20 to 40% and 6 to 30%, respectively (6, 29, 30). In addition, surgical procedures are accompanied by excessive bleeding and thrombosis (2, 9, 24, 32). The incidence of complications in 1 series was almost 3 times as great in uncontrolled as controlled patients, and the longer the period of effective control, the fewer were the complications (31).

The tendency to hemorrhage and thrombosis in polycythemia vera has been ascribed to abnormalities of the vasculature, coagulation factors, and platelets. The exact mechanism(s) is, however, unknown and the relative importance of these 3 factors is not agreed upon.

Recently, interest has turned to the search for platelet function abnormalities in polycythemia vera and other myeloproliferative disorders. Little, however, has been published with respect to platelet aggregation in these disorders. Spaet et al. (26) recently reported 3 patients with essential thrombocytocemia who had marked and characteristic abnormalities in platelet aggregation. These consisted of absent epinephrine-induced aggregation, reduced ADP-induced aggregation but normal collagen-induced aggregation. In 1 of these patients, the aggregation abnormalities improved following therapy. Tangün (27) has studied platelet aggregation in 33 patients with myeloproliferative disorders, 6 of whom had polycythemia vera. Abnormal response to aggregating agents occurs in 75% of all cases and in 4 of 6 with polycythemia vera. Of the latter, 3 studies were performed prior to therapy (busulfan) and 1 while on no therapy but not in remission. Two patients in hematological remission had normal aggregation studies. Repeat studies, performed on 2 patients after (busulfan) therapy showed improvement but not complete normalization. In the entire group of patients, the effect of myelosuppressive therapy on platelet function was variable, and the reduction of a high platelet count to normal following therapy did not eradicate the platelet function abnormalities.

The results of platelet aggregation studies carried out on 40 patients with polycythemia vera in various stages of the disease are the subject of the present report. Of these, 18 patients were studied both before and after therapy.

MATERIALS AND METHODS

Subjects. Sixty-two platelet aggregation studies were performed on 47 patients with polycythemia vera. Twenty-three of the patients were male and 24 were female with an age range of 8 to 79 years (2 patients under 30 years of age). Care was taken to ensure that the patients had not recently ingested any of the drugs known to affect platelet function.

A history of clinically apparent hemorrhage or thrombosis was obtained in 15 and 30% of patients, respectively, and both complications occurred together in only 2 patients (4.3%).

Bleeding Times. Bleeding times were performed by a modified Ivy method (13) (normal = 3 to 9 min). Venous blood was collected by gravity into plastic centrifuge tubes containing 4% sodium citrate with 1 part citrate to 9 parts blood. Platelets were counted by phase microscopy (4). To obtain PRP3 the anticoagulated blood was centrifuged at 4°.

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3 The abbreviation used is: PRP, platelet-rich plasma.
for 20 min at 900 rpm on Model PR-2 International Centrifuge. Platelet-poor plasma was obtained by centrifugation for 15 min at 3500 rpm. Siliconized and plastic equipment was used throughout.

**Platelet Aggregation Studies.** Platelet aggregation studies were performed by a turbidimetric method (3) using a Chrono-Log Corporation Aggregometer with a Bausch and Lomb recorder. Studies were done at 37° on stirred PRP adjusted to a final platelet count of 300,000 per cu mm with autologous platelet-poor plasma within 1 hr of blood collection. ADP disodium salt (Sigma Chemical Company, St. Louis, Mo.) was stored as an 0.01 M stock solution at −20° and used, after dilution, at a final concentration of 2 × 10⁻⁴ M. Topical bovine thrombin (Parke, Davis and Company, Detroit, Mich.) was stored as a stock solution of 100 units/ml at −20°. It was diluted immediately before use to a final concentration of 0.2 units of PRP per ml. Epinephrine HCL (4.6 × 10⁻³ M; Parke, Davis and Company) was kept at 4° and was used after dilution at a final concentration of 5 × 10⁻⁶ M. Human collagen prepared from dried collagen (Sigma Chemical Company) was used at a final suspension containing 0.72 mg protein per ml PRP. By using 0.4% sodium citrate it is not necessary to correct for the hematocrit. Multiple studies were carried out varying the 0.4% citrate concentration without any significant difference in results.

**RESULTS**

**Hematoctrit.** Of the 62 studies, 38 were done at normal (≤50%) hematocrits, 19 at elevated hematocrits, and 5 at low hematocrits (≤37%) in patients with “spent” polycythemia.

**Platelet Count.** The platelet count was normal (150 to 350,000 per cu mm) during 41 studies and elevated in the remaining 21 studies. The degree of elevation was generally modest, being over 1,000,000 per cu mm (1,600,000) in only 1 patient. The mean platelet count for the 62 studies was 374,000 per cu mm.

**Platelet Aggregation Studies.** Results expressed are in units on the Bausch and Lomb recording paper and are a measure of light transmittance. Normal values for this laboratory are as follows (23); ADP, 5.2 ± 1.0 units (primary aggregation, mean ± 1 S.D.; epinephrine, 2.9 ± 0.6 units (primary aggregation; collagen, 6.4 ± 0.5 units; and thrombin, 4.6 ± 0.7 units. Results were considered abnormal if they were less than the mean − 2 S.D.’s. Secondary waves of aggregation were recorded as abnormal if present and normal if absent. ADP and epinephrine studies were considered abnormal if either the primary or the secondary wave of aggregation was abnormal.

**ADP-induced Aggregation.** Forty-seven % of the 62 studies were abnormal. The group with elevated hematocrits had a greater percentage of abnormalities (74%) than those with normal (32%) or low (60%) hematocrits. Primary wave abnormalities occurred approximately as frequently as secondary wave abnormalities. More abnormalities were present within a hematocrit group if there was a concomitant elevation of platelet count (Chart 1) and the greatest number of abnormal results (80%) were obtained in the group (10 patients) with both high hematocrits and platelet counts.

**Epinephrine-induced Aggregation.** Epinephrine-induced aggregation was abnormal in 52% of the studies, but in contrast with ADP-induced aggregation, there was essentially no difference in the percentage of abnormal studies in the different groups with normal, elevated, or low hematocrits. In contrast to ADP-induced aggregation, an abnormal primary wave occurred almost twice as frequently as an absent secondary wave. As seen in Chart 1, within the elevated hematocrit group, there was a higher percentage of abnormal studies in those with concomitant elevation of platelet count. In the normal hematocrit group, there was no significant difference. Similar to ADP-induced aggregation, the highest percentage of abnormal studies occurred in the group with both elevated hematocrits and platelet counts.

**Collagen-induced Aggregation.** Collagen-induced aggregation was abnormal in 37% of the studies. The patients with elevated hematocrits had more than twice the number of abnormal studies than those with normal hematocrits. One hundred % of the studies in the spent group were abnormal. In the remainder of the patients, those with high platelet counts and high hematocrits had the highest percentage of abnormal studies (Chart 1).

**Thrombin-induced Aggregation.** Thrombin-induced aggregation was abnormal in 37% of the 54 times it was performed. Those with spent polycythemia and those with elevated hematocrits had abnormal tests in approximately 50% of cases. Elevation of the platelet count did not increase the risk of abnormal studies within each hematocrit group (Chart 1).

**Absence of Aggregation in Response to Aggregating Agents.** Absence of aggregation occurred most commonly with epinephrine followed in decreasing order by collagen, ADP, and thrombin in 8, 5, 4, and 1 studies, respectively. Four studies showed an absent response to 2 aggregating agents. Absence of aggregation occurred in 34 studies (54%). Absence of aggregation was seen in 27% of elevated hematocrits, 51% of normal hematocrits, and 47% of low hematocrits. Absence of aggregation was seen in 14% of those with high platelet counts. In contrast, the highest rate of absence of aggregation of any of these agents was 44% in the spent polycythemia patients with increased platelet counts, and the lowest rate of absence of aggregation was 4% in the group with low hematocrits. There was no significant difference in the percentages of studies with absent aggregation for the different groups with normal, elevated, or low hematocrits.
agents. There was no relationship, however, between absence of response and platelet count, elevation of bleeding time, or history of thrombohemorrhagic phenomena. In contrast to this, some aggregation responses were increased, i.e., they showed maximal deflections of the absorbance curves greater than 2 S.D.'s above the mean. Again, however, there was no relationship to platelet count, bleeding time, or incidence of thrombohemorrhagic phenomena.

Platelet Aggregation: Summary of Findings. The most frequent abnormalities occurred with epinephrine (52%) and ADP (47%), followed by collagen and thrombin (37% each). In general, patients with elevated hematocrits had a greater percentage of abnormal studies than did those with spent polycythemia, who in turn had more abnormalities than those with normal hematocrits. The commonest abnormality was a decrease in aggregation, followed by a complete absence of the secondary wave of aggregation.

Considering all the aggregating agents collectively, 81% of the studies were abnormal. No patient with either spent polycythemia or an increased hematocrit plus platelet count had a completely normal study. Eighty-nine % of those with an increased hematocrit but normal platelet count had abnormal studies. Approximately 75% of the patients with normal hematocrits had abnormal studies whether or not the platelet count was abnormal.

Bleeding Time. The Ivy bleeding time was prolonged (greater than 9 min) in 10 of the 49 studies. In only 1 was the platelet count elevated (496,000 per cu mm). None of the patients had a platelet count below 100,000 per cu mm. All 4 patients with spent polycythemia had prolonged bleeding times when studied. Their hematocrits and platelet counts ranged from 21 to 35% and 110,000 to 298,000 per cu mm, respectively. The hematocrit was not elevated during any study with a prolonged bleeding time. Two of the patients had hemorrhagic complications and 1 had a history of thrombosis. Four of the 10 studies showed completely normal platelet aggregation. Thus abnormal bleeding times in our patients with polycythemia occurred much less frequently than platelet aggregation abnormalities and did not correlate with an elevation of hematocrit or platelet count or a history of thrombohemorrhagic complications.

Thrombohemorrhagic Complications. Thrombotic and hemorrhagic complications occurred in 30 and 15% of the patients, respectively. When these groups of patients were compared to the group as a whole, there was no significant difference between the number with elevated platelet counts, elevated hematocrits, and abnormal aggregation studies.

Response to Therapy (Table 1). Thirteen patients had repeat aggregation studies. Five had tests done first at an elevated hematocrit and again, following treatment, at a normal hematocrit. Two, who originally had abnormal studies, completely normalized after treatment that consisted of phlebotomies only in one and chemotherapy in the other. The remaining 3 patients, treated with various combinations of phlebotomy and chemotherapy, had their aggregation studies return toward normal. Two had absent epinephrine-induced aggregation prior to therapy which improved after therapy. One had absent collagen-induced aggregation which became normal.

Two patients were studied first at normal hematocrits and then following p.o. iron therapy at elevated hematocrits. In one, the studies normalized, but in the other, they became abnormal.

A total of 6 patients had repeat studies performed before and after therapy but with hematocrits that remained in the same range. One of these 6, in the spent phase of the disease and on no treatment, showed no change in platelet aggregation. Four of the remaining 5 showed improvement, including 1 treated with phlebotomies only for 7 months. The 5th patient's studies were unchanged 4 months after therapy with phlebotomies and chemotherapy.

DISCUSSION AND CONCLUSIONS

The platelet has been incriminated in the thrombohemorrhagic complications of polycythemia vera with respect to both increased numbers and abnormal function. An elevated platelet count is a common occurrence in polycythemia vera and is a prerequisite of essential thrombocythemia, another myeloproliferative disorder. Some have found no influence (31), while others have reported significantly increased complications rates in patients with elevated platelet counts in polycythemia vera (8, 17), secondary thrombocytosis (12, 17), and thrombocythemia (10, 22). Our data showed no relationship between elevated platelet counts and complication rate. However, the platelet counts in our patients, as is usual in polycythemia vera (32),

<table>
<thead>
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<th>Change in hematocrit after therapy</th>
<th>No. of patients</th>
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<th>Aggregation studies</th>
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<tr>
<td>I to N*</td>
<td>5</td>
<td>P, C</td>
<td>2</td>
</tr>
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<td>2</td>
<td>Iron</td>
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<tr>
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<td>P, C</td>
<td>1</td>
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<tr>
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</tr>
<tr>
<td>Total</td>
<td>13</td>
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* I, increased; N, normal; P, phlebotomy; C, chemotherapy.
were only mildly to moderately elevated (<1,000,000 per cu mm) except in 1 patient (1,600,000 per cu mm). The latter had no thrombohemorrhagic complications. This discrepancy may be due to the fact that over 90% of our patients were studied as outpatients at a time when they were not suffering from complications. It is possible that our findings relating platelet count (and function) with complication rate would have been significant were we able to study these patients immediately prior to and/or during a thrombohemorrhagic event.

Qualitative platelet abnormalities of several types have been reported in polycythemia vera and other myeloproliferative disorders. Variable results have been reported for several platelet function tests in patients with polycythemia vera. In vitro platelet adhesiveness studies have shown both increased (25) and decreased (17) values but the test is difficult to standardize and involves several variables including the content of the red blood cells in the sample (11), the hematocrit (25), the volume of anticoagulant (25), but apparently not the platelet count (7, 17, 25).

Recently, much attention has turned to the in vitro measurement of another platelet function, platelet aggregation (1, 3, 18). Abnormalities have been described in the myeloproliferative disorders (7, 16, 19, 26, 27) but there are no reports of platelet aggregation in a large group of patients with polycythemia vera and on minimal data on the effect of therapy. Our patients exhibited a large number of abnormalities regardless of whether they were "controlled" or "uncontrolled." Multiple aggregation abnormalities were more common than single in any given study. In general, the number of abnormalities increased from the group with normal hematocrit and platelet count through to the group with elevated hematocrit and platelet count. The latter, as well as the spent group, showed abnormal platelet aggregation studies 100% of the time. Prolonged Ivy bleeding times and thrombohemorrhagic complications were not related to each other or to the other parameters measured.

Repeat aggregation studies in patients whose hematocrits normalized following therapy showed improvement. Similarly, improvement also occurred in other patients in whom therapy did not alter the hematocrit. The findings were not influenced by the mode of therapy (myelosuppressive therapy versus phlebotomy).

These studies further add to the information gathered regarding hemostasis in polycythemia vera. There are several possible explanations both for what appears to be a discrepancy between the findings in vitro and the clinical histories, and also for the possible mechanisms producing abnormal platelet function. It is possible, first, that these measurements in vitro have in fact little relation to the in vivo situation. Similarly, it is now well recognized that in other conditions, for example, acetylsalicylic acid-induced platelet function abnormality, there exists a discrepancy between the high percentage of abnormalities in vitro and the very few clinical complications in otherwise hemostatically normal persons (28, 33).

The aggregation abnormalities may be due to platelets that are refractory to aggregating agents (21). Recently, human plasma has been noted to have an inhibitor to connective tissue-induced aggregation which may play a role in maintaining normal hemostasis (20). Polycythemia vera patients may have more of this or other types of inhibitors than the normal population, perhaps lacking an unknown required plasmatic factor. Peripherial platelets represent a heterogeneous population, the young platelets being more active metabolically and the more responsive to aggregating agents (14). Recently, emphasis has been placed on the study of platelet kinetics in the myeloproliferative disorders. In polycythemia vera, platelet life span appears to be slightly decreased and production increased (5, 15). These data point up the dynamics of maintaining a peripheral platelet count in this disorder. In myeloproliferative disorders, platelet function abnormalities may thus represent a lack of heterogeneity of platelets in the peripheral blood. Although their morphology in polycythemia vera is usually normal, the circulating platelets may in fact be functionally less active. Further study of the fate of the more rapidly turning over platelet may be helpful in clarifying this.

The fact that therapy, irrespective of type, improved platelet aggregation in 10 of 13 patients suggests that restoration toward a "more normal state" brings forth a significant change such that they respond in the tests in a more normal fashion.

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