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Thrombohaemorrhagic Complications in 101 Cases of Myeloproliferative Disorders: Relationship to Platelet Number and Function*

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Abstract—*A series of* 101 *consecutive patients with chronic myeloproliferative disorders including polycythaemia vera, chronic myelogenous leukaemia, idiopathic myelofibrosis and essential thrombocythaemia have been studied. The aim was to establish the incidence of thrombotic and haemorrhagic complications and the possible role played by platelet number and function. The total incidence of haemostatic complications was* 21% *and the platelet functional tests investigated (platelet aggregation, generation of malondialdehyde, endogenous serotonin, beta-thromboglobulin and platelet coagulant activity) were of little help for predicting these clinical complications.*

INTRODUCTION

THE MYELOPROLIFERATIVE disorders (MD), a group of related diseases of the bone marrow stem cell, including polycythaemia vera (PV), chronic myelogenous leukaemia (CML), essential thrombocythaemia (ET) and idiopathic myelofibrosis, are characterized by a high incidence of haemorrhagic and/or thrombotic complications. The mechanism responsible for these haemostatic disorders, however, has not yet been completely elucidated. Extensive studies of the plasma coagulation system failed to show any consistent pattern of abnormalities which might underlie these phenomena.

Since an elevated platelet count is common in myeloproliferative diseases and defines one of them, essential thrombocythaemia, many investigators have focused their attention on platelets.

A number of qualitative platelet defects have been described in these disorders, including reduced response to various aggregating agents [1–3], reduced platelet coagulant activities [4, 5] defective binding of thrombin to platelets [6], defective lipid peroxidation [7], changes in distribution of platelet membrane glycoproteins [8], deficiency of platelet alpha-adrenergic receptors [9], platelet storage pool deficiency [10], platelet hyperaggregability [11], platelet resistance to prostaglandin D_2 [12], deficiency of platelet lipoxygenase [13] and increased expression of Fc receptors [14]. The association between most of these qualitative platelet defects, platelet count and clinical bleeding or thrombotic sequelae has generally been unsatisfactory. In this study we have evaluated the incidence of haemostatic complications and their possible relationship to platelet number and function in 101 patients with myeloproliferative disorders.

MATERIALS AND METHODS

Patients

One hundred and one patients with myeloproliferative disorders were studied; 48 had PV, 32 CML, 10 IM and 11 ET. Diagnosis was made by standard clinical and laboratory criteria [15–18]. Patients with abnormalities of glucose or lipid metabolism were excluded from the study.

At the time of the study all the patients were either newly diagnosed or in remission; the latter patients had not received any cytotoxic treatment during the previous 4 months. None of the patients reported intake of any drug affecting platelet function during the previous 10 days.

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Each patient was examined on at least two occasions. Results of screening studies of the plasma coagulation system, including partial thromboplastin time, one-stage prothrombin time, thrombin time and fibrinogen levels, were within the normal range in all patients.

All patients with PV had undergone repeated phlebotomies and PCV value was normal at the time of the study. A control group consisted of 51 sex- and age-matched apparently healthy subjects.

Methods

Blood collection from controls and patients and preparation of platelet-rich plasma (PRP) and platelet-poor plasma (PPP) were described previously [19]. When required, PRP was diluted with the subject's own PPP to reach a concentration of 300×10^9 platelets/l (diluted PRP). Washed platelets were prepared by albumin density gradient centrifugation as reported [5].

Platelet counts were made on peripheral blood and PRP by phase-contrast microscopy, using the Unopette diluting system (Becton Dickinson, Novate Milanese, Italy). Thrombocytosis was defined as a platelet count in excess of 500×10^9/l.

Bleeding time was measured using a template-like device kindly supplied by Prof. C. Praga, Milan, Italy. In 30 normal subjects (14 women and 16 men, aged 26–65 yr) it ranged from 3 to 5 min [20].

Platelet aggregation studies were made at 37°C by the photometric technique of Born and Cross [21] using an Elvi 840 Aggregometer (Elvi Logos, Milan, Italy). Spontaneous platelet aggregation (SPA) was recorded by placing 0.25 ml of undiluted or diluted PRP into the cuvette and stirring it at 1000 rev/min for 15 min. SPA was not observed in any PRP sample from normal subjects. Platelet aggregation was also studied by challenging 0.25 ml of diluted PRP with ADP, collagen (both obtained from Mascia-Brunelli, Milan, Italy) and epinephrine (ADR) (Stago, Asnière sur Seine, France). The results were expressed according to the following criteria: (a) normal (N): two aggregation waves with ADP or epinephrine at final concentrations lower than 4 and 5.4 μM respectively; aggregation (at least 50% increase in light transmission) within 10 min with a fixed concentration of collagen (2 μg/ml); (b) abnormal (A): no aggregation detectable within 10 min or absence of the second wave using the highest aggregating concentration mentioned above. All subjects in the control group had 'normal' aggregation patterns.

Generation of malondialdehyde (MDA) induced by stirring PRP at 37°C for 5 min with 25 NIH U/ml thrombin was measured spectrophotometrically according to Villa *et al.* [22]. Normal values were: 0.50–1.35 nmol/3×10^8 platelets.

Endogenous serotonin (5-hydroxytryptamine, 5-HT) was measured by formation of a fluorophore with *o*-phthaldehyde (OPT) in 1 ml of PRP according to the spectrofluorometric method of Drummond and Gordon [23]. Normal values were: 0.23–0.64 nmol/10^8 platelets.

Beta-thromboglobulin (Beta-TG) was measured in PPP by radioimmunoassay with kits from the Radiochemical Centre without stasis into plastic tubes containing 'Edinburgh mixture' (2.7:0.3 ml), consisting of 0.1 ml 10% Na_2-EDTA, 0.1 ml theophylline (5.4 mg/ml) and 0.1 ml prostaglandin E_1 (1 μg/ml). Normal values were: 5–50 ng/ml. The beta-TG ratio in ng/10^8 platelets was determined according to Zahavi *et al.* [24] by the following formula: plasma beta-TG (ng/ml) / platelet concentration in 1 ml of whole blood (in normal controls it was $\leqslant 22$).

The coagulant activity of washed platelets (platelet coagulant activity, PCA) from controls and patients was measured by a one-stage clotting assay as previously described [5]. The results were expressed as percentage of PCA using a standard reference curve [5]. In 35 normal subjects PCA ranged between 60 and 150%.

Platelet factor V and *platelet factor 3 (PF 3) activities* were determined as described previously [5].

RESULTS

Clinical observations

Selected clinical and laboratory data of patients and control group are shown in Table 1. Twenty-one of the 101 patients in this series had clinical signs of disordered haemostasis. Haemorrhagic complications were observed in 11 patients (8 with PV, 1 with CML and 2 with ET). The most frequent symptoms were repeated epistaxis, excessive bleeding after dental extraction and gastrointestinal bleeding. Thrombotic manifestations occurred in 10 patients with PV. All suffered repeated episodes of transient cerebral ischaemia and recurrent painful attacks in toes and fingers; 2 patients also had repeated episodes of venous thrombosis. The symptoms occurred at the time of study or during the previous 2 months. Thrombocytosis was present in all patients with haemostatic disorders. In the group of asymptomatic patients 54 had normal platelet count and 26 had thrombocytosis.

Laboratory results

The patients were divided into four groups on the basis of haemostatic disorder and platelet count: group 1 (patients with bleeding, $n = 11$),

group 2 (patients with thrombotic complications, $n = 10$), group 3 (asymptomatic with thrombocytosis, $n = 26$) and group 4 (asymptomatic without thrombocytosis, $n = 54$). Bleeding time was prolonged (more than 6 min) in 4 (36%) haemorrhagic patients and in 4 (7%) asymptomatic patients with normal platelet counts (Table 2).

Spontaneous platelet aggregation was observed in 5 (45%) patients with bleeding, 6 (60%) with thrombotic complications, 11 (42%) asymptomatic patients with thrombocytosis and 13 (24%) asymptomatic without thrombocytosis (Table 2). Abnormal ADP-induced aggregation was noted in 4 (36%) patients with bleeding, 1 (10%) with thrombotic manifestations, 5 (19%) asymptomatic with high platelet counts and 5 (9%) asymptomatic with normal platelet numbers. Eight patients (73%) in the bleeding group, 5 (50%) in the thrombotic group, 12 asymptomatic patients (46%) with thrombocytosis and 14 asymptomatic with normal platelet counts (26%) had no epinephrine-induced aggregation.

Collagen-induced aggregation was abnormal in 2 (18%) patients with bleeding, 4 (15%) asymptomatic with thrombocytosis and 4 (7%) asymptomatic without thrombocytosis.

MDA generation in response to thrombin was increased (mean and range: 1.80, 1.56–2.05 nmol/3×10^8 platelets) in 7 (70%) patients with thrombotic complications, 1 (4%) asymptomatic patient with a high platelet count and 3 (5.5%) asymptomatic patients with normal platelet numbers (Table 3). Reduced MDA production (mean and range: 0.25, 0–0.43 nmol/3×10^8 platelets) was found in 4 (36%) patients with bleeding, 4 (15%) asymptomatic patients with thrombocytosis and 8 (15%) asymptomatic patients without thrombocytosis (Table 3). Serotonin content of unstimulated platelets was reduced (mean and range: 0.13, 0–0.20 nmol/10^8 platelets) in all but 17 patients.

Plasma beta-TG levels were increased (mean and range: 147, 54–260 ng/ml) in all but 16 patients. However, beta-TG ratio was abnormal (mean and range: 55, 24–118 ng/ml) in only 31

Table 1. Clinical and laboratory data

Group*	No. of patients	Age (yr)	Sex M	Sex F	Platelet count ($\times 10^9$/l)	Patients with: bleeding	Patients with: thrombosis
PV	48	60 (26–84)	30	18	663 (140–1500)	8	10
CML	32	53 (13–74)	14	18	609 (150–2000)	1	0
IM	10	61 (45–76)	5	5	290 (60–750)	0	0
ET	11	48 (28–81)	4	7	911 (700–1500)	2	0
HS	51	51 (26–75)	19	16	280 (210–400)		

*PV = polycythaemia vera; CML = chronic myelogenous leukaemia; IM = idiopathic myelofibrosis; ET = essential thrombocythaemia; HS = healthy subjects.

Table 2. Bleeding time and platelet aggregation studies

Group*	No. of patients	Platelet number ($\times 10^9$/l)	Prolonged bleeding time	SPA†	Abnormal aggregation ADP	ADR	Coll.
1	11	815 (700–1000)	4	5	4	8	2
2	10	733 (600–1000)	0	6	1	5	0
3	26	918 (600–2000)	0	11	5	12	4
4	54	242 (60–400)	4	13	5	14	

*1 = patients with bleeding; 2 = patients with thrombotic complications; 3 = asymptomatic with thrombocytosis; 4 = asymptomatic without thrombocytosis.
†SPA = spontaneous platelet aggregation.

Table 3. MDA, 5-HT, beta-TG and PCA

Group*	No. of patients	MDA Increased	MDA Reduced	5-HT Reduced	Beta-TG plasma level ratio Increased	Beta-TG Increased	PCA Increased	PCA Reduced
1	11	0	4	10	8	3	0	11
2	10	7	0	10	9	2	2	1
3	26	1	4	22	26	6	1	8
4	54	3	8	42	42	20	1	7

*1 = patients with bleeding; 2 = patients with thrombotic complications; 3 = asymptomatic with thrombocytosis; 4 = asymptomatic without thrombocytosis.

(31%) patients (2 in the thrombotic group, 3 in the bleeding group, 6 in the asymptomatic group with thrombocytosis and 20 in the asymptomatic group with normal platelet counts).

Platelet coagulant activity (Table 3) was below the normal range (mean and range: 23, 5–41%) in all patients with bleeding complications. In the thrombotic group reduced PCA (38%) was found in 1 patient and increased PCA (185% and 200%) in 2 patients. Eight (31%) asymptomatic patients with thrombocytosis and 7 (13%) asymptomatic patients without thrombocytosis had PCA levels below the normal range (mean and range: 39, 29–50%), whereas 1 patient in each of these groups had increased levels (350% and 285%).

No differences in PF3 nor in factor V activity were found between the patients and normal washed platelets (data not shown).

When platelet counts were examined within the PV group, it appeared that only 3 out of 30 asymptomatic patients had thrombocytosis (Table 4).

DISCUSSION

We have studied a large series of patients with MD in order to evaluate the incidence of thrombotic and haemorrhagic complications and the possible role played by platelets in the pathogenesis of these haemostatic disorders.

Data from various series indicate that in PV thromboembolic and haemorrhagic complications occur in 26–63% and 16–35% of patients respectively [25]. These figures are comparable with the frequency of 21 and 17% observed in our 48 newly diagnosed PV patients. The fact that only one (3%) out of 32 patients with CML had a bleeding tendency and none of them had thrombotic complications would suggest that disordered haemostasis is rare in CML. Mason *et al.* [26] found that 4 out of 111 patients with CML experienced haemostatic disorders. Two out of 11 patients with ET had haemorrhagic problems and no haemostatic disorders were encountered in the 10 patients with IM. The total incidence of haemostatic complications in our series was 21%.

Platelets have been incriminated in the thrombohaemorrhagic complications of MD with respect to both increased numbers and abnormal function. An analysis of the literature indicates that there is no simple relationship between an elevated platelet count and the development of either thromboembolic or haemorrhagic complications. Our data provide further support to this concept. Thus patients with CML, despite high incidence of thrombocytosis (34%), had almost no haemostatic disorders. Likewise, haemorrhagic problems occurred only in 2 out of 10 patients with ET.

The fact that all patients suffering from thrombotic or haemorrhagic complications had thrombocytosis, however, suggests that the latter should be considered at least a risk factor for haemostatic disorders, particularly in PV, where their incidence is high. Dawson and Ogston [27] and the Polycythemia Vera Study Group [28] also concluded that high platelet counts contributed to the increased incidence of thrombotic and haemorrhagic events in PV and recommended that treatment of this disease be directed toward reduction of both platelet count and whole blood viscosity.

Since a number of qualitative platelet abnormalities have been reported in MD which might contribute to the bleeding or thrombotic diathesis, we investigated various platelet function parameters to assess whether these could be useful in identifying subgroups of patients with thrombocytosis who will develop haemostatic disorders.

In agreement with most reports, there was no single relationship between bleeding time and haemostatic disorders in our patients. Indeed, only 8 out of 101 patients (4 with bleeding and 4 asymptomatic with normal platelet counts) had prolonged bleeding time. Similarly, our platelet aggregation studies indicated that these tests could not distinguish patients with bleeding complications from those with thrombotic manifestations or no symptoms. Spontaneous platelet aggregation, proposed as a sign of platelet

Table 4. Clinical and laboratory data within the PV group

Group*	No. of patients	Age (yr)	Sex M	Sex F	Platelet count (× 10^9/l)
1	8	59 (28–84)	5	3	816 (700–1000)
2	10	62 (40–74)	5	5	733 (600–1000)
3	3	73 (69–79)	1	2	926 (630–1500)
4	27	56 (26–84)	19	8	231 (140–370)

*1 = patients with bleeding; 2 = patients with thrombotic complications; 3 = asymptomatic with thrombocytosis; 4 = asymptomatic without thrombocytosis.

hyperactivity associated with thrombosis [11], was observed in 6 out of 10 patients with thrombotic complications, but also in 5 out of 11 patients with a bleeding tendency and in a high percentage of asymptomatic patients with or without thrombocytosis.

In agreement with other reports [1–3], the most consistent defect of platelet aggregation observed in our patients was the lack of response to epinephrine whereas abnormal ADP-induced aggregation was found less frequently and defective collagen-induced aggregation was rare. There is a disagreement in the literature as to whether abnormalities of platelet aggregation are related to the clinical tendency for haemorrhage and/or thrombosis in patients with MD. Some studies have suggested a correlation between defective aggregation (especially in response to epinephrine) and clinical bleeding [1, 2, 29] and/or thrombosis [30, 31], but others have not [25, 32]. In this study, although abnormal platelet aggregation in response to epinephrine and ADP was observed with a greater incidence in patients with bleeding, it was also seen in a high percentage of asymptomatic patients, particularly in those with high platelet counts. The marked reduction of total platelet serotonin content we found in most patients is in agreement with previous observations [3] and consistent with the concept of acquired 'storage pool deficiency' in MD [10]. Since this defect was present in all but 17 patients, it can be regarded as a marker of MD, entirely unrelated to the haemostatic complications. The storage pool deficiency could be due either to the production of abnormal platelets by malignant megakaryocytes or to platelet injury in the circulation resulting in degranulation. In this study we found that 84% of the patients had elevated plasma levels of beta-TG, a protein released by the platelets during their activation. However, when beta-TG was expressed as 'ratio' (i.e. in relation to the number of circulating platelets), it was high in only 31% of the patients. Although we did not measure the platelet content of beta-TG, our data would suggest an *in vivo* platelet activation at least in some patients. In any case, beta-TG alteration was totally unrelated to the haemostatic complications.

It is generally accepted that platelet prostaglandin metabolism plays a major role in haemostasis and thrombosis. Increased prostaglandin synthesis has been reported in patients with overt thrombosis [33] or with increased thrombotic risk [34, 35], and decreased prostaglandin synthesis has been observed in patients with acquired bleeding tendency [36].

In this study increased MDA production in response to thrombin was found in 7 out of 10 patients with thrombotic complications but only in 4 out of 80 asymptomatic patients, and in none of the haemorrhagic patients. This suggests that an enhanced platelet lipid peroxidation may contribute to the pathogenesis of thrombotic complications. Keenan *et al.* [7] reported decreased platelet lipid peroxidation in some patients with MD, a defect which correlated with bleeding problems. Our data do not support this concept. Indeed, reduced MDA production occurred in 36% of the patients with haemorrhagic complications as well as in 15% of asymptomatic patients with or without thrombocytosis.

As far as PCA is concerned, our findings provide evidence of close association between reduced PCA and bleeding tendency. In fact, reduced PCA was found in all patients with haemorrhagic complications but only in one-fifth of those without. No definite conclusion could be inferred from our data about the behaviour of PCA in thrombotic patients.

In conclusion, our study in a large series of patients with MD indicates that the platelet function parameters investigated seem to be of little help for predicting the haemostatic complications. Indeed, no clear-cut relationship was found between abnormalities of the different tests and the thrombotic and/or haemorrhagic manifestations. As a matter of fact, it has been reported by several investigators that chemotherapy results in normalization of the platelet count and disappearance of clinical symptoms while not normalizing the platelet function parameters [1, 10, 25, 31, 37].

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