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The Plasma Levels of Prostanoids and Plasminogen Activator Inhibitor-1 in Primary and Secondary Thrombocytosis

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Summary: An elevated platelet count is a common finding in both hospitalized and ambulatory patients. Thrombosis and bleeding complications are more frequently observed in patients with clonal thrombocytosis than secondary thrombocytosis. The aim of this study was to investigate the behaviors of plasminogen activator inhibitor type 1 (PAI-1), the inhibitor of fibrinolysis; and thromboxane A2 and 6-keto-PGF1 α, the products of endoperoxides, in 16 patients affected with clonal thrombocytemia as compared with 16 patients with reactive thrombocytosis and 15 normal controls. In the clonal thrombocytemia group, plasma levels of PAI-1 antigen and activity were significantly higher than both reactive thrombocytosis and control group. Plasma levels of 6-keto-PGF1α were significantly higher in the clonal thrombocytemia group than the other two groups and also higher in the reactive thrombocytosis group than the control group, which was also significant. This study confirms that arachidonate metabolism is frequently deranged in patients with thrombocytosis and hypofibrinolysis due to increased PAI-1 plasma levels as shown in the clonal thrombocytosis group. This may explain the thrombotic tendency in myeloproliferative disorders.
Key Words: Thrombocytosis—Plasminogen activator inhibitor type 1—Thromboxane A2—6-keto-PGF1α.

Thrombocytosis is caused by three major pathophysiologic mechanisms: reactive or secondary thrombocytosis, familial thrombocytosis, and clonal thrombocytosis, including essential thrombocytemia and related myeloproliferative disorders. Reactive thrombocytosis can be caused by iron deficiency and a variety of inflammatory conditions, infections, malignancy, bleeding or hemolysis, splenectomy, and drugs, in which hemostatic complications are rare. Clonal thrombocytemia is a chronic myeloproliferative disorder characterized by clonal megakaryocyte proliferation and increased platelet production. The clinical course, although often asymptomatic, may be complicated by thrombotic or bleeding events, which represent the major causes of morbidity and mortality (1–3).

The plasminogen activator inhibitor type 1 (PAI-1) is a 50-kDa glycoprotein belonging to the serine protease inhibitors' superfamily (4). As physiologic inhibitor of the fibrinolytic system, it rapidly reacts with both tissue type and the urokinase-type plasminogen activators (5). Other than by hepatocytes and fibroblasts, PAI-1 is synthesized and released by endothelial cells, which represent the source of PAI-1 either in plasma or in the subendothelial matrix. Megakaryocytes were also found to synthesize PAI-1 (6,7), which is then stored within platelet alpha granules (8). Platelets constitute the main reservoir of PAI-1 in blood, whereas plasma PAI-1 represent a minor pool (9). An impairment of the fibrinolytic potential deriving from a pathologic excess of PAI-1 levels may enhance the risk of both arterial and venous thrombosis (10–13).

Modifications of arachadonic acid (AA) metabolism by either the cyclooxygenase or lipoxy-

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genase pathways have been observed frequently in myeloproliferative disorders (14–17). The measurement of thromboxane B2 (TXB2) is considered to be a reliable index of the efficiency of the AA metabolic pathway in human platelets (18,19). On the other hand, 6-keto-PGF1α production expresses the AA metabolic capacity of white blood cells and the exchange of metabolic substrates between activated platelets and leukocytes (18–21).

In this study, we investigated the behaviors of plasminogen activator inhibitor type 1; the inhibitor of fibrinolysis; and also thromboxane A2 and 6-keto-PGF1 α; the stable nonenzymatic metabolites of thromboxane A2 and prostacyclin, respectively, in a group of patients affected with thrombocytemia, as compared with a group of healthy controls.

MATERIALS AND METHODS

Patients

Sixteen patients (nine females and seven males) aged 24–57 years, affected with clonal thrombocytosis (group 1), 16 patients (10 females and six males) aged 22 to 51 years affected with secondary thrombocytosis (group 2), and 15 healthy controls (10 females and five males) aged 18 to 55 years (group 3) with normal platelet counts were investigated. Informed consent was obtained from each subject after explanation of the study protocol. In the first group, seven patients had chronic myelogeneous leukemia, five had polycythemia vera, three had essential thrombocythemia, and 1 had osteomyelofibrosis. In the second group, seven patients had rheumatoid arthritis, eight had iron deficiency anemia, and one had ulcerative colitis.

The following tests were carried out on each subject: hemoglobin, platelet count, PAI-1 antigen and PAI-1 activity, TXA2, 6-keto-PGF1α concentrations. No patient had clinical sign of thrombosis for at least 3 months before the study, and no patient or control had received any drug known to affect platelet and fibrinolytic tests or had been smoking.

Samples

Blood was collected from an antecubital vein without stasis between 8 and 9 AM, after an overnight fast and supine rest. Nine milliliters of each sample was transferred to polpropylene tubes containing 1 mL of 0.109 *M* trisodium citrate. After centrifugation at 3000g for 15 minutes, at 10°C to 18°C, the supernatant plasma were stored at –30°C and tested within 1 month.

PAI-1 Ag was carried out by an enzyme-linked immunosorbent assay (ELISA) method (TintElize PAI-1, Biopool, Sweden) according to the manufacturer's instructions (22). PAI-1 activity was assayed using a chromogenic substrate (Biopool, Sweden) as described earlier (23). Thromboxane B2 and PGF1α concentrations were determined by an ELISA kit (Dosage enzymoimmunologique des eicosanoides, Laboratoire des Stallergenes, Cedex, France). Platelet count was obtained with phase contrast microscopy (blood normal range, $150–450 \times 10^9$/L).

Statistical Analysis

Results were expressed in median, interquartile range. Nonparametric and Mann-Whitney U-tests were used for statistical analysis. A p value less than 0.05 was assumed as significant.

RESULTS

Our results are summarized in Tables 1 to 3. Mean platelet count was significantly higher in both clonal ($610–916 \times 10^9$/L; 690×10^9/L) and secondary thrombocythemia ($612–800 \times 10^9$/L; 682×10^9/L) than counts in normal controls ($149–410 \times 10^9$/L; 235×10^9/L) (Tables 1 and 2), but there was no significant difference between the two groups. Hemoglobin levels in the second group (8.1–12.5; 10.15 gr/dL) were significantly lower than both controls (12.3–16; 14 gr/dL) and the first group (11.5–18; 13.78 gr/dL).

PAI-1 antigen level was 44.47 ng/mL (24–47 ng/mL) in the secondary thrombocythemia group while it was 86.8 ng/mL (33–172 ng/mL) in the clonal thrombocythemia group and 39 ng/mL (22–59 ng/mL) in the control group. PAI-1 antigen levels were significantly higher in the first group than the second and control groups, which were significant ($p<0.001$ and $p<0.001$, respectively).

PAI-1 activity was 12.79 IU/mL (7.8–26 IU/mL) in the first group, 8.52 IU/mL (4.7–13.8 IU/mL) in the second group, and 8.1 (5.6–11.2 IU/mL) in the control group. It was significantly higher in the first group than the second and third groups ($p<0.015$ and $p<0.0005$, respectively).

6-keto-PGF1α levels were significantly higher in the first (6.3–89.5 pg/mL; 44.02 pg/mL) and second groups (57.5–216 pg/mL; 127.75 pg/mL) than the controls (1.5–32.3 pg/mL; 10.53

TABLE 1. Results (Minimum-Maximum Values) of Patients with Clonal Thrombocytosis (Group 1) and Normal Subjects

Characteristic	Group 1	Control	p
Age (yr)	41 (24–57)	31 (18–55)	0.005
Haemoglobin (g/dL)	13.78 (11.5–18)	14 (12.3–16)	>0.05
Platelet count/lt	690 × 10 (610–916)	235 × 10 (149–410)	0.001
PAI-1 Ag (ng/mL)	86.8 (33–172)	39 (22–59)	0.001
PAI-1 activity (IU/mL)	12.79 (7.8–26)	8.1 (5.6–11.2)	0.0005
6-ketoPGF1 (pg/mL)	44.02 (6.3–89.5)	10.53 (1.5–32.3)	0.001
TXA2 (pg/mL)	49.08 (4.5–116)	68.9 (12–133)	>0.05

TABLE 2. Results (Minimum-Maximum Values) of Patients with Secondary Thrombocytosis (Group 2) and Normal Subjects

Characteristic	Group 2	Control	p
Age (yr)	36 (22–51)	31 (18–55)	>0.05
Hemoglobin (g/dL)	10.15 (8.1–12.5)	14 (12.3–16)	0.001
Platelet count/lt	682×10^9 (612–800)	235×10^9 (149–410)	0.001
PAI-1 Ag (ng/mL)	44.47 (24–71)	39 (22–59)	>0.05
PAI-1 activity (IU/mL)	8.52 (4.7–13.8)	8.1 (5.6–11.2)	>0.05
6-keto-PGF1α (pg/mL)	127.75 (57.5–216)	10.53 (1.5–32.3)	0.001
TXA2 (pg/mL)	16.07 (1.4–67)	68.9 (12–133)	0.001

TABLE 3. Results (Minimum-Maximum Values) of Patients with Clonal Thrombocytosis (Group 1) and Secondary Thrombocytosis (Group 2)

Characteristic	Group 1	Group 2	p
Age (yr)	41 (24–57)	36 (22–51)	>0.005
Hemoglobin (g/dL)	13.78 (11.5–18)	10.15 (8.1–12.5)	0.001
Platelet count/lt	690 × 10 (610–916)	682 × 10 (612–800)	>0.05
PAI-1 Ag (ng/mL)	86.8 (33–172)	44.47 (24–71)	0.001
PAI-1 activity (IU/mL)	12.79 (7.8–26)	8.52 (4.7–13.8)	0.015
6-ketoPGF1 (pg/mL)	44.02 (6.3–89.5)	127.75 (57.5–216)	0.001
TXA2 (pg/mL)	49.08 (4.5–116)	16.07 (1.4–67)	0.006

pg/mL) ($p<0.001$). The second group had a higher level of 6-keto-PGF1α than the first group, which was also significant ($p=001$).

TXA2 levels were 49.08 pg/mL (4.5–116 pg/mL) in the first group, 16.07 pg/mL (1.4–67 pg/mL) in the second group, and 68.9 pg/mL (12–133 pg/mL) in the control group. The first group had a significantly higher level of TXA2 than the second group ($p=0.006$). The second group had a lower level of TXA2 than the control group, which was also significant ($p=0.001$).

DISCUSSION

Bleeding and thrombosis are common complications in myeloproliferative disorders and represent the main causes of morbidity and mortality in the course of clonal thrombocytosis (1–3,24,25). The most commonly accepted causes contributing to thrombotic manifestations are increases in blood viscosity and thrombocytosis (1). Thromboses may develop in different sites of venous and arterial systems , including cerebral arteries and sinus veins, portal system veins, coronary arteries, and lower limb vessels (1,26,27).

Many attempts have been made to use clinical laboratory tests to predict which patients with thrombocytosis are prone to the risk of thrombosis; however, most studies have failed to identify these patients by the use of platelet function tests (28,29). Platelet abnormalities that could explain thrombotic complications, such as increased beta-thromboglobulin and platelet factor 4 levels (29–31), and platelet activation (32) have been described. It is possible that the fibrinolytic system may be activated or, better still, inhibited. Defective fibrinolysis is nearly always characterized by elevated PAI-1 levels (10,33,34), which are often involved in the pathogenesis of both arterial and venous thrombosis.

There are conflicting results regarding PAI-1 levels in patients with clonal thrombocytemia (31,35–37). In this study, we observed a significant increase of PAI-1 concentration in the plasma of patients with clonal thrombocytemia than in secondary thrombocytosis and control groups. PAI-1 activity was also higher in the clonal thrombocyemia group than the other groups. Hypofibrinolysis due to increased PAI-1 plasma levels in the clonal thrombocytosis group can explain the thrombotic tendency in this group of patients than that in patients with secondary thrombocytosis.

Platelet–vessel wall interactions also take part in the regulation of haemostasis and thrombosis with TXB2-dependent platelet activation representing a transduction mechanism linking various risk factors to the enhanced risk of vascular occlusive complications (38). An elevation of TXB2 generation in patients with thrombocytosis of myeloproliferative disorders, distinct from that observed in patients with secondary thrombocytosis and normal controls, has been reported (39,40–42). In our study, TXA2 levels were found to be significantly reduced in patients with secondary thrombocytemia, that suggest an impairment in platelet AA metabolism in this condition. In contrast, patients with clonal thrombocytemia showed a significant increase in TXA2 concentration that could reflect a defect in lipooxygenase activity in these patients (43). The role of the increased thromboxane formation in the frequency of thrombotic complications in patients with clonal thrombocytemia remains to be established; large series and large follow-up studies are required to address this problem properly.

Cortelazzo and colleagues reported no significant difference in venous PGI2 production between patients with myeloproliferative disorders and controls (44). In our study, patients with clonal thrombocytemia showed lower 6-keto-PGF1α levels, the stable breakdown product of PGI2, than patients with secondary thrombocytosis.

In conclusion, our study confirms that arachidonate metabolism is frequently deranged in patients with thrombocytosis. The abnormal pattern of thromboxane generation can be a useful laboratory method in the evaluation of patients with primary thrombocytosis. Hypofibrinolysis due to increased PAI-1 plasma levels in the clonal thrombocytosis group can also be considered a factor to explain the thrombotic tendency in myeloproliferative disorders.

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