Qualitative platelet defect and thrombohaemorrhagic complications in a patient with polycythemia vera

Case 10

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Polycythaemia vera, platelet function, thrombosis, antiplatelet agents

Summary
We describe a 67-year-old patient with polycythemia vera and pathological functional platelet studies. He not only suffered a transient ischaemic attack despite taking of antiplatelet agents, but also showed bleeding diathesis with cerebral bleeding and spontaneous subdural hemorrhages. Platelet function studies and clinical findings improved after phlebotomy and cytoreductive treatment with hydroxyurea.

Thrombosis and haemorrhage account predominantly for morbidity and mortality in patients with polycythemia vera. The pathophysiological mechanisms to explain thrombosis and bleeding in patients with myeloproliferative disorders including polycythemia vera were intensively studied. However, up to now no clear correlation of laboratory findings in relation to clinical history of thrombosis and bleeding was demonstrated. In this report the most important pathophysiological mechanisms and therapy with antiplatelet agents are discussed.

Schlüsselwörter
Polycythemia rubra vera, Plättchenfunktionsstörung, Thrombose, Thrombozytenaggregationshemmer

Zusammenfassung


Thrombose und Blutung als Komplikationen einer qualitativen Plättchenfunktionsstörung bei Polycythemia vera

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A 67-year-old patient was referred to our outpatient ward for haematological evaluation after having suffered two transient ischaemic attacks (TIA) and bleeding complications under antiplatelet therapy. His personal history revealed high blood pressure treated with an ACE-inhibitor for many years and bleeding complications after surgical treatment for an intestinal obstruction.

In 1988 the patient developed a TIA with right-sided hemiplegia and aphasia. Antiplatelet therapy was started without any further investigations. The patient reported gastrointestinal intolerance to acetylsalicylic acid. Therefore, the patient was treated with another (unspecified) antiplatelet agent for years. In 1994 the antiplatelet regimen was discontinued and replaced by acetylsalicylic acid without any gastrointestinal complications. Afterwards he suffered from frequent spontaneous suffusions over both hands and some episodes of left-sided slightly reduced facial and digital sensibility. Otherwise he spent the following years in excellent health.

In September 2000 he suffered a second TIA with left-sided hemiplegia. In a computed tomographic scan of the brain several small ischaemic areas in the internal capsule and the pons as well as a small haemorrhage in the right-sided basal ganglia were detected. Color-coded duplex sonography of the cranial arteries showed no evidence of haemodynamically significant stenoses. Acetylsalicylic acid was replaced by clopidogrel (75 mg/d). Thereafter, the patient was referred to our department for evaluation of thrombophilia and bleeding diathesis.

At the time of presentation the patient was in good health and clinical examination was unremarkable except for small suffusions over both hands. The spleen was not palpable. For assessment of platelet function, antiplatelet therapy had been discontinued and replaced by a low molecular weight heparin (LMWH) ten days before presentation. The haemostaseologic tests showed a normal prothrombin time and a slightly prolonged activated partial thromboplastin time (38.7 s), clotting fac-
Platelet defect in polycythaemia vera

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Platelet aggregation studies at the initial evaluation (a, b) and after platelet reduction with hydroxyurea (c, d): increase in light transmission resulting from platelet aggregation with final concentrations of ADP of 6 µmol/l (a, c) and of collagen 1.5 µg/ml (b, d).

Fig. 1: Platelet aggregation studies at the initial evaluation (a, b) and after platelet reduction with hydroxyurea (c, d). Increase in light transmission resulting from platelet aggregation with final concentrations of ADP of 6 µmol/l (a, c) and of collagen 1.5 µg/ml (b, d).

Time: interval between dotted vertical lines 15 s (running from right to left); light transmission: 0% corresponds to the light transmission of patient’s platelet rich plasma (PRP), 100% to that of his platelet poor plasma (PPP). After 1 min of pre-incubation at 37°C of 200 µl PRP, 10 µl of aggregating agent is added (arrows).

Platelet aggregation studies in platelet rich plasma (PRP) showed pathological aggregation after platelet activation with ADP. Three minutes after addition of ADP, aggregation was below 10% with a final concentration of 4 µmol/l ADP; ~10% at 6 µmol/l (Fig. 1a) and was complete with 10 µmol/l of ADP. Activation with 1.5 µg/ml collagen showed no aggregation (Fig. 1b). However, with 3 µg/ml collagen normal aggregation was found. The aggregation curves after activation with arachidonic acid and ristocetin showed no abnormalities.

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The haematological findings showed an elevated haemoglobin concentration (177 g/l; reference range: 135-168 g/l) and haematocrit (0.55; reference range: 0.40-0.50), low MCV (79 fl, reference range: 80-98 fl) and MCH (25 pg; reference range: 27-33 pg) as well as a marked elevation of the red blood cell count (10.3 × 10¹²/l; reference range: 4.2-5.7 × 10¹²/l). Leucocytes were within the upper limits (10.3 × 10⁹/l; reference range: 4.2-5.7 × 10⁹/l). Platelets were slightly increased (145 × 10⁹/l; reference range: 140-380 × 10⁹/l). Isotope dilution haematologic tests revealed an elevated red blood cell volume (43 ml/kg; reference range: 20-34 ml/kg), but normal plasma volume (43 ml/kg; reference range: 31-52 ml/kg). Any evidence for secondary erythrocytosis was not detected. Elevated red blood cell volume together with an arterial oxygen saturation >92% and splenomegaly by abdominal ultrasound fulfilled diagnostic criteria for polycythaemia vera (PV) (20).

This diagnosis was additionally supported by the following laboratory findings: spontaneous growth of 182 erythroid colony forming units (CFU-E) in vitro and serum erythropoietin concentration below detection limit.

Therapy

Immediately, therapy with phlebotomy was started. After several phlebotomies, the value for haematocrit fell below 0.47. However, as leucocytes and platelet concentrations were rising, therapy with hydroxyurea was started. With regard to the platelet dysfunction, current antiplatelet therapy was discontinued and LMWH in prophylactic dose administered.

As haematological parameters, particularly the platelet count, returned to normal, platelet function was tested again. PFA-100° occlusion times as well as platelet aggregation tests showed normal results (Fig. 1c, d), thus justifying resumption of antiplatelet therapy with clopidogrel. No further complications occurred up to now.

Discussion

We report the case of a 67-year-old man with polycythaemia vera (PV), transient ischaemic attacks and cerebral haemorrhage. Laboratory investigation for hereditary thrombophilia was unrevealing (as might be expected in arterial thromboembolism), anticardiolipin antibodies or elevated homocysteine levels were not detected, whereas clinical and laboratory findings confirmed the diagnosis of PV according to established criteria (20).
PV is a clonal stem cell disorder characterized by an increase of red blood cell mass, granulocytes and platelets. The incidence rates, although highly variable, were reported as 1.9 per 100,000 persons and year (95% CI 1.4-2.5) in a large retrospective study examining all in- and outpatient records of Olmsted County, Minnesota, over a period of 55 years. Incidence rates increased with advancing age for men up to 23.5 per 100,000 and year. The median age at presentation was 55 to 60 years (2). Clinical features include aquagenic pruritus, skin changes (acne rosacea, plethoric facies), gout, and splenomegaly. Transformation into myelofibrosis with myeloid metaplasia or acute leukemia were described repeatedly (28). Arterial and venous occlusion as well as hemorrhagia are the most common clinical features, occurring in 30-50% of PV patients (22). However, it should be taken into account that only severe thrombohemorrhagic events were included in most recordings, leading to a potential underestimation (16). Thrombotic events, along with secondary malignancies due to aggressive chemotherapy, are the main cause of mortality in PV.

The Gruppo Italiano Studio Policitemia (GISP) performed a retrospective evaluation of more than 1200 PV patients and found an incidence of thrombotic events in PV of 3.4% per year (8). Unfortunately, 60% of all thrombotic events occurred at or prior to diagnosis. Predominant risk factors for thrombotic events are advanced age, a history of recurrent thrombosis and repeated phlebotomies (the latter possibly an epiphenomenon reflecting high myeloproliferation). The risk is highest in the first few months after diagnosis and declines considerably after the first three years of follow up (16, 17, 27).

The thrombotic diathesis mainly presents as microvascular occlusion, but thrombosis of large vessels is not uncommon. Arterial thrombosis is more frequently found than venous occlusion. Typically, erythromelalgia and neurologic symptoms (headache, dizziness, dysarthria, transient hemiparesis) are encountered. Erythromelalgia consists of congestion, redness, and burning pain involving the extremities and typically resolves promptly after administration of acetylsalicylic acid in low dosage. Histopathological studies of the affected area show chronic inflammation and recurrent thrombosis of arterioles. Other sites of microvascular occlusion include retinal or cochlear vessels, Raynaud’s phenomenon can occur as well (14, 16).

30 to 40% of large vascular events occur in cerebral arteries, but acute coronary syndromes or peripheral arterial syndromes were also described (16). Venous thrombosis and pulmonary embolism occur less frequently. However, in 10% of the cases an uncommon thrombosis location, e.g. in the mesenteric, splenic, portal, hepatic, or subclavian vein, is encountered (21, 22).

In 1997, De Stefano et al. (6) found that overt PV was present in 10% of patients with Budd Chiari syndrome. Moreover, in almost 80% of so-called idiopathic Budd Chiari syndrome and in about 50% of patients with portal, splenic and/or mesenteric vein thrombosis spontaneous endogenous erythroid colony formation was observed even in the absence of abnormal haematological values (6).

As described above, bleeding problems also occur. But their frequency decreased over the preceding 20 years (16). Mucocutaneous sites of bleeding are predominant, typically noted are easy bruising, epistaxis, and gingival bleeding (33). Haemorrhage may involve the gastro-intestinal tract, particularly in PV patients who receive antiplatelet agents and/or whose platelet count was not controlled (16).

The pathogenesis of thromboses and haemorrhages in myeloproliferative disorders (MPD) including PV is not yet fully understood. High haematocrit and associated pathophysiological changes as well as quantitative and qualitative platelet disorders have been studied, but only few correlations between laboratory findings and clinical manifestations could be established. This suggests that additional factors such as haemodynamic impairment and endothelial dysfunction might play an important role (16). Two major features of PV, elevated haematocrit and altered platelet concentration and function are discussed here with regard to the pathophysiology of thrombosis and haemorrhage.

Elevated haematocrit and associated pathophysiological changes

An elevated haematocrit has been closely related to the incidence of vascular occlusion, the latter being up to six times more common in patients with haematocrit levels >0.50 as compared to those with haematocrit values <0.45 (22). Pearson (22) showed in an in vitro study that high haematocrit levels correlate with high blood viscosity, which returns to reference values after phlebotomy. In addition, high haematocrit and haemoglobin levels cause increased arterial oxygen content. This reduces cerebral blood flow. However, the reduction of blood flow may be caused not only by vasomotor response to increased oxygen transport, but also by particular cell-to-cell interactions encountered in viscous blood. It was demonstrated that with increasing haematocrit, axial migration of red blood cells leads to a progressive displacement of circulating platelets towards the vessel wall. Thus platelet-platelet and platelet-vessel contacts occur more frequently, thus enhancing the possibility of platelet aggregation. Enhanced platelet aggregation is more marked in arterial vessels, because high shear forces seem to activate GPIb binding sites of vWF and therefore increase adhesion of platelets to vWF (22). In addition, Ambrus et al. (1) described decreased red cell membrane deformability in patients with PV leading to an increased susceptibility to microvascular occlusions.

Thus, haematocrit seems to play an important role in the development of thromboembolic events. This hypothesis is supported by the fact that the incidence of thromboembolic events is higher in PV compared to essential thrombocythaemia, where elevated haematocrit values are usually lacking. On the other hand, thromboembolic events are found less frequently in secondary erythrocytosis, although haematocrit levels may be similar to those in PV. This strongly suggests that other factors (e.g. platelet disorders) may contribute to vascular occlusive events in PV (22).
Quantitative and qualitative platelet abnormalities

The role of platelet concentration in the pathogenesis of haemostatic complications in patients with PV still remains uncertain. In contrast to haematocrit levels, no clear overall correlation between platelet concentration and incidence of major thrombotic or haemorrhagic events in PV patients could be demonstrated (3). Nevertheless, microcirculatory disturbances such as erythromelalgia and neurological/visual manifestations are clearly associated with high platelet count. These events become rather uncommon at a platelet count $<400 \times 10^9/l$. On the other hand, erythromelalgia never occurs in secondary thrombocytosis. Generally, thrombosis and haemorrhage are encountered both in the presence and in the absence of a high platelet count (14). These facts strongly suggest that qualitative platelet abnormalities are of importance in the pathogenesis of thrombohaemorrhagic events.

Functional platelet abnormalities probably occur due to dysmegakaryopoiesis (23). Morphologically, platelets often show a highly variable volume, ranging from microplatelets to giant platelets. Megakaryocytes growing in clusters in the bone marrow show increased size and ploidy (32). Cytoplasmic granules are often reduced or absent. Both, deficiency of $\alpha$-granules (containing proteins relevant for platelet interaction, such as fibrinogen, vWF, thrombospondin, and fibronectin, proteins relevant for coagulation and fibrinolysis regulation, such as factor V, $\alpha_2$-antiplasmin, plasminogen activator inhibitor 1, and other proteins, such as $\beta$-thromboglobulin and platelet factor 4) and dense bodies ($\delta$-granules, containing among others calcium ions, ADP, ATP, serotonin) are described in patients with MPD and may exert a considerable impact on reduced haemostatic effectiveness of platelets because aggregate formation or stabilization is impaired (11, 16, 19).

Abnormalities of arachidonic acid metabolism are also known to occur in patients with MPD (24). In platelets, arachidonic acid is converted enzymatically by cyclooxygenase (via the cyclic endoperoxides PGG$_2$ and PGH$_2$) to thromboxane $A_2$ (TxA$_2$) and by lipoxygenase to 12- or 15-hydroperoxycicosatetraenoic acid (HPETE), respectively. TxA$_2$ acts as a powerful vasoconstrictor and platelet agonist. Although HPETE has no known direct effect on platelet function, it was reported that it potentiates platelet response to subthreshold concentrations of arachidonic acid at physiological concentrations (4).

Other platelet abnormalities in MPD consist of glycoprotein (GP) alterations leading to changes of the binding characteristics of the associated receptors. Reduction of vWF-receptor (GPIb/IX/V) (12), fibrinogen receptor (GPIIb/IIIa) (16), and collagen receptor (GPIa/IIa) (9) were described. Fibrinogen binding sites were reported as being heterogeneous, some exhibiting an enhanced affinity to fibrinogen, some moderate fibrinogen binding (16, 18). Besides cases with reduced GPIb/IX/V, other cases with plasma deficiency of vWF (acquired von Willebrand syndrome) were observed in patients with high platelet concentration in myeloproliferative disease (see case 6).

Other membrane abnormalities including partial loss of $\alpha$-adrenergic and PGD$_2$ receptors leading to a defective platelet aggregation and reduced platelet procoagulant activity also were described as well as abnormal platelet signal transduction in PV. However, the functional role of these defects has still to be examined (32).

Several tests regarding the mentioned abnormalities in PV patients were studied in vitro and in vivo. In vitro, reduced platelet aggregation after activation with epinephrine is considered as characteristic for MPD. However, spontaneous platelet aggregation was found as well. The coexistence of these two opposite phenomena may be responsible for simultaneous bleeding and thrombotic disorders. Abnormal platelet aggregation in response to collagen and ADP, as observed in our patient, was described formerly (32). Typically, no second wave of aggregation induced by epinephrine or ADP is detected at all, while the primary aggregation response is diminished. Le Blanc et al. (18) reported on deficient platelet aggregation because of reduced GPIIb/IIIa levels.

Prolonged bleeding time, increased plasma levels of $\beta$-thromboglobulin, P-selectin and platelet factor 4 in addition to increased urinary excretion of TxA$_2$ metabolites are considered typical in PV and essential thrombocythaemia. However, these markers must be judged with caution when used to predict haemostatic function in vivo (16, 32). Although prolonged bleeding time is considered to reflect a disturbed primary haemostasis, it is of limited value in predicting haemorrhagic risk (16). The role of the PFA-100$^\text{®}$ in assessing the haemorrhagic risk in MPD is not defined yet.

High concentration of platelet activation markers may be caused by platelet activation during blood sampling and processing, rather than representing an in vivo existing abnormality. In order to overcome these problems, TxA$_2$ metabolites were determined in urine. Urinary excretion was found to be significantly increased in PV patients, thus reflecting platelet activation in vivo. Furthermore, urinary excretion of TxA$_2$ metabolites was successfully suppressed by low doses of acetylsalicylic acid (13, 17). This suggests an important role of acetylsalicylic acid in the treatment of thrombotic complications in PV and essential thrombocythaemia. On the other hand, in patients with an acquired functional platelet defect as in our case, cytoreduction may normalize platelet function (Fig. 1).

Treatment

Phlebotomy and cytoreduction

Phlebotomy for maintaining haematocrit $<0.45$ is well established for lowering the blood viscosity. If patients are at high risk for thrombosis, cytoreductive therapy should be added and maintenance of platelet count $<400 \times 10^9/l$ is recommended (3, 27). Hydroxyurea was proven to be highly effective in preventing thrombosis in patients at high risk with essential thrombocythaemia (5). For other cytoreductive agents, such as $\alpha$-interferon and anagrelide, no data exist concerning their effects on thrombohaemorrhagic diathesis. Unfortunately, for hydroxyurea an association with elevated risk of leukaemic transformation in the following time is suspected, but no
formal proof is given (27, 32). Despite this uncertainty, hydroxyurea is used in most patients with high risk PV.

As described, high platelet counts are associated with microcirculatory disturbances such as erythromelalgia. Thus, these disorders constitute a clear indication for cytoreductive therapy and antiplatelet agents. The latter seem to be indicated also in patients with relatively low platelet counts and microvascular occlusions (17). Typically, erythromelalgia seems to be highly sensitive to acetylsalicylic acid, but not to antiplatelet agents that do not inhibit cyclooxygenase.

**Acetylsalicylic acid**

Acetylsalicylic acid causes irreversible inhibition of cyclooxygenases, thus suppressing the increased biosynthesis of TxA₂ seen in PV patients (13). Additionally, short platelet survival and increased turnover due to enhanced microcirculatory platelet aggregation were corrected by acetylsalicylic acid (29). These facts suggest a potential beneficial role of acetylsalicylic acid in the treatment of MPD. However, in 1986 Tartaglia et al. (26) reported that it was both ineffective and dangerous for patients with PV, but the increased gastrointestinal bleeding risk was probably due to the high dosage of acetylsalicylic acid (900 mg/d) and the study size was too small to test any thrombosis risk reduction by it (30). According to the results concerning efficacy and safety of low dose acetylsalicylic acid in secondary prevention of ischaemic heart and brain disease, the GISP performed a pilot study to assess its effectiveness and safety for PV patients (7). Chronic administration of low-dose acetylsalicylic acid in PV patients seemed to be effective in inhibiting platelet activity, even in those with markedly increased platelets (7). No major bleeding was observed. For the confirmation of these preliminary results the large, randomized, placebo controlled trial ECLAP (European Collaboration on Low dose Aspirin in Polycythemia vera) was started in 1997 (15). Its results are still unpublished.

A highly feared side effect of acetylsalicylic acid is haemorrhage. Combination of uncontrolled platelet counts together with administration of acetylsalicylic acid constitutes a major risk factor for bleeding in PV patients (see case 6). This was described especially for platelet concentrations >1000 x 10⁹/l (30, 33). The gastrointestinal tract is the most vulnerable site of haemorrhage for any patient taking acetylsalicylic acid. This of course applies to patients with PV, too.

As major bleeding is observed predominantly with high doses, it was proposed to start with acetylsalicylic acid with a dose of 300 mg/d in acute thrombotic situations, followed by prompt reduction to 75-100 mg/d for secondary prophylaxis when symptoms are resolved (33). It must be emphasized that until the results of ECLAP will be available, application of acetylsalicylic acid for the primary prophylaxis of thromboembolic events in PV is not supported by strong evidence and should be pondered thoroughly (27). Our patient demonstrates very well the dilemma in treating PV:

- Despite the intake of acetylsalicylic acid he developed a second TIA.
- On the other hand – probably due to uncontrolled PV combined with antiplatelet therapy – he suffered a cerebral haemorrhage.

**Clopidogrel**

Clopidogrel is a new orally administered antiplatelet agent and like ticlopidine a thienopyridine derivative. It acts by blocking the P2Y₁₂-adenosine diphosphate (ADP) receptor on the platelets’ surface. Consequently, platelet activation and fibrinogen binding to GPIIb/IIIa is inhibited (25). Compared to acetylsalicylic acid for the prevention of vascular ischaemic attacks, clopidogrel has been proven to provide slightly more protection for patients at high risk, but the extent of the additional benefit is uncertain (10). Despite appearing as safe as acetylsalicylic acid, clopidogrel should not be the first choice in antiplatelet therapy. It may replace acetylsalicylic acid in such a selected patient at high risk as our, who suffered already several TIA while taking acetylsalicylic acid.

Because of their different mechanisms of action, an issue is the question whether combination of clopidogrel and acetylsalicylic acid is both safe and more effective than either drug alone in patients at high risk for vascular disease (10). If this combination should turn out as safe and effective, it should also be considered as an important therapeutic option to be studied in PV patients at high risk for ischaemic events.

**References**


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