

Factor IX activation peptide and soluble selectins in patients with chronic myeloproliferative disorders

We evaluated factor IX activation peptide and soluble adhesion molecules of the selectin class (endothelial, leukocyte and platelet) in a group of patients affected by chronic myeloproliferative disorders (essential thrombocythemia and polycythemia vera) with high platelet counts in a stable condition. Using these markers, we could not demonstrate evidence of hypercoagulability or endothelial damage/dysfunction in such patients.

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Essential thrombocythemia (ET) and polycythemia vera (PV) are chronic myeloproliferative disorders (CMD) characterized by a high incidence of thromboembolic complications. Increased levels of markers of platelet activation and activated coagulation system have been reported in patients affected by such disorders.¹⁻³ Factor IX activation peptide (FIXP) may be a vascular-bed-specific marker for thrombosis in the coronary artery.⁴ Selectins (platelet-P, endothelium-E, leukocyte-L) together with other adhesion molecules play a key role in the pathogenesis of thrombosis, inflammation, immunologic responses, and infections in the vasculature.⁵ They are up-regulated and released into the circulation, where they can be detected in soluble form, in a variety of disease states such as sepsis, acute stroke, and heparin-induced thrombocytopenia.⁶ It has been shown recently that soluble P-selectin, but not soluble E-selectin is increased in patients with essential thrombocythemia.⁷ We studied 26 patients with CMD and high platelet counts (platelets 818.9 ± 226 , range $610-1610 \times 10^9/L$). Nineteen were affected by ET (mean age 53.73, range 22-87 years) and 7 by PV (mean age 69.28, range 51-84 years). All patients, in a stable condition, had been followed by us for at least 3 years. None of them had thrombotic complications within one year prior to the study and during a two year follow-up. Eighteen patients were taking, as antiplatelet agent, 100 mg of aspirin daily alone, five were taking this drug in combination with hydroxyurea, and six took 250 mg of ticlopidine b.i.d. in combination with hydroxyurea (6 patients); 2 young ET subjects of 38 and 40 years with a platelet count of 621 and $627 \times 10^9/L$, respectively, were not taking any treatment. At the time of the study the PV patients had a packed cell volume below 50%. All patients, after blood collection, had a follow-up of two years. As a control group we collected plasma from 31 apparently healthy, non-smoker volunteers who were age- and sex-matched with the patients (platelets 224.69 ± 38.4 , range 172 - 260 $10^9/L$). Patients and controls were divided into 3 groups by age: up to 44 years, from 45 to 59 years and from 60 to 87 years.

FIXP expressed in picomoles/L (pmol/L) was determined in patients and controls using a radioimmunoassay (RIA) with a double-antibody approach developed by us and previously described.⁸ Soluble adhesion molecules of the selectin class (sES endothelial, sPS platelet and sLS leukocyte) with concentrations expressed in nanograms/mL (ng/mL) were determined in 18 patients (5 PV, 13 ET) and 27 control subjects using a commercial enzyme-linked immunosorbent sandwich assay (ELISA) supplied by Beder, Vienna, Austria. The results were expressed as a mean \pm standard deviation (SD) of the mean. The analysis of the differences was performed by Student's t test. A *p* value of <0.05 was taken to be statistically significant.

In healthy subjects FIXP plasma levels increased, although not statistically so, with older age (Table 1). CMD patients showed levels similar to those of controls (Table 1). No statistically significant differences were found in sES and sLS between patients

Table 1.

	Factor IX activation peptide plasma levels pmol/L		
	22-44	45-59	60-87
Age (years)			
Controls	144.5 ± 73.7	134.8 ± 31.1	184.4 ± 76.4
n.	10	13	8
Patients	124.0 ± 60.8	131.8 ± 31.9	142.8 ± 53.1
n.	7	4	15

Table 2.

	n.	Soluble selectins plasma levels ng/mL		
		Endothelial	Leukocyte	Platelet
Controls	27	29.7 ± 17.9	723.7 ± 180.0	130.7 ± 55.2
Patients	18	36.0 ± 22.6	783.9 ± 123	$208.7 \pm 82.4^*$

**p* = 0.012.

and controls. In contrast sPS was significantly higher in CMD. (Table 2) However the concentration of sPS per platelet was significantly reduced in CMP: CMP 0.28 ± 0.11 fg/platelet, controls 0.59 ± 0.24 fg/platelet; *p* < 0.0001.

In patients with stable CMD treated with antiplatelet agents we did not observe increased FIXP levels. Moreover sES and sLS were also normal, in agreement with data from Fijnheer *et al.*⁷ and Musolino *et al.*⁹ Although sPS was increased, its concentration per platelet was significantly lower. This could be due either to reduced synthesis or to reduced release since almost all our patients were taking antiplatelet agents. Falanga *et al.*³ showed activation of the hemostatic system with elevated levels of prothrombin fragment F1+2 and thrombin-antithrombin III complex in patients with ET and PV. Since they found polymorphonuclear leukocyte activation in such patients, they suggested that an increased release of polymorphonuclear proteases may provide a potential mechanism for interference with the hemostatic system. Platelets play a vital role as systemic components of the hemostatic system and they are critical in the activation of the intrinsic pathway. In our patients we could not demonstrate enhanced platelet activation expressed as an increased concentration of sPS per platelet like that found in thrombotic consumptive platelet disorders.⁷ In conclusion we did not find evidence of a hypercoagulable state nor of leukocyte or platelet activation in these patients using FIXP, sLS and sPS as markers. Moreover in agreement with Bellucci *et al.*¹⁰ no systemic dysfunction/damage of endothelial cells seemed to be present in our patients as the levels of sES were normal.⁵ These facts could partially explain why we found a normal concentration of FIXP.

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