Myeloproliferative disorders

Thrombotic and bleeding complications in four subpopulations of patients with essential thrombocythemia defined by c-Mpl protein expression and *PRV-1* mRNA levels

C-Mpl and PRV-1 expression was measured in a cohort of 48 patients with essential thrombocythemia (ET). A retrospective analysis was conducted to asses whether the presence of one or both markers correlates with a higher risk of developing thromboembolic complications. In this cohort, PRV-1 overexpression was associated with a significantly increased risk of thrombosis, whereas decreased c-Mpl expression was not. The results of this retrospective analysis must now be corroborated in a large, prospective trial of newly diagnosed patients.

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Thromboembolic events are the major cause of morbidity and mortality in patients with essential thrombocythemia (ET).[1] Indeed, 20-30% of patients will develop a major thrombotic episode during their lifetime. However, the clinical course for individual patients is heterogeneous and may comprise several decades of thrombosis-free survival. Therapeutic decisions are complicated by the fact that to date no laboratory test has been shown to predict the development of thrombotic or vascular complications.[1]

The recent description of cellular and molecular markers in ET raises the possibility of using these biomarkers for risk stratification. Three studies have described an increased risk of thrombotic complications in patients carrying a biomarker. Harrison *et al*. observed a higher risk in patients with monoclonal hematopoiesis than in those with polyclonal hematopoiesis.[2] In addition, sub-

Table 1. Characteristics and clinical and laboratory data of the 48 patients with ET. Patients are stratified according to PRV-1 mRNA levels and c-Mpl protein expression. For laboratory data, both the data at the time of diagnosis and at the time of PRV-1/C-Mpl analysis are given. *values denote mean (range). HU: hydroxyurea, IFN: interferon-α, ^{32}-P: radio phosphorus; ANA: anagrelide, ASA: aspirin.

	Group 0 PRV-1 normal c-Mpl normal	*Group 1 PRV-1 high c-Mpl normal*	*Group 2 PRV-1 normal c-Mpl low*	*Group 3 PRV-1 high c-Mpl low*	*p value#*
Patients	23	8	7	10	
Female/male	17/6	6/2	6/1	8/2	ns
Age	58 (27-86)	57 (50-80)	74 (55-90)	73 (32-82)	ns
Duration of disease (months)	37 (7-201)	36 (24-177)	46 (24-181)	66 (21-267)	ns
Follow-up (months)	37 (7-201)	36 (24-177)	46 (24-181)	66 (21-267)	ns
Cytoreductive treatment					
Drug: number of patients	12 HU: 5 IFN: 2 ^{32}P: 3 ANA: 2	5 HU: 4 ^{32}P: 1	6 HU: 3 IFN: 1 ^{32}P: 2	9 HU: 8 IFN:1	ns
Aspirin Treatment	10	1	3	3	ns
Hb at diagnosis (g/dL)	13.9 (11.2-16.8)	14.1 (12.6-16.0)	12.6 (11.2-15.3)	13.4 (10.6-15.1)	ns
Hb at sample (g/dL)	13.7 (10.9-16.3)	13.5 (11.3-15.4)	12.4 (11.4-13.6)	12.7 (10.8-13.1)	*
Hct at diagnosis (%)	43 (38-52)	44 (42-48)	38 (33-48)	40 (32-47)	ns
Hct at sample (%)	43 (35-49)	43 (39-49)	37 (36-42)	37 (32-40)	°
Plts. at diagnosis (×10^9/L)	624 (410-1850)	774 (504-1115)	1260 (752-1550)	963 (633-1392)	#
Plts. at sample (×10^9/L)	507 (339-946)	442 (232-929)	433 (256-553)	356 (233-776)	ns
WBC at diagnosis (×10^9/L)	8 (5.3 -15.1)	9.6 (5.8-12.5)	9.8 (7.8 – 14.6)	10.7 (4.7 – 14.5)	ns
WBC at sample (×10^9/L)	7.5 (4.0-10.4)	6.6 (4.1-12.2)	5.3 (4.0 – 9.8)	4.6 (3.4 – 13.5)	ns

*#p values were calculated using the extended Fisher's exact test (categorical data) or the Kruskal-Wallis test (continuous variables). The p value gives the statistical significance for comparison of all four groups among each other. Ns : not significant. An asterisk that at least one comparison between two groups showed a statistically significant difference. When a statistically significant result was obtained with the Kruskal-Wallis test, we used the Dunn's test (reported below) to assess which groups were significantly different in those cases: ***Hb at sample (g/dL):** PRV-1 normal, c-Mpl normal vs PRV-1 normal, c-Mpl low (group0 vs group2) p=0.0269; PRV-1 normal, c-Mpl normal vs PRV-1 high, c-Mpl low (group0 vs group3) p=0.0047; PRV-1 high, c-Mpl normal vs PRV-1 normal, c-Mpl low (group1 vs group2) p=0.0383; PRV-1 high, c-Mpl normal vs PRV-1 high, c-Mpl low, (group1 vs group3) p=0.0122; **°Hct at sample (%):** PRV-1 normal, c-Mpl normal vs PRV-1 normal, c-Mpl low, (group 0 vs group2) p=0.0153; PRV-1 normal, c-Mpl normal vs PRV-1 high, c-Mpl low, (group0 vs group3) p=0.0024; PRV-1 high, c-Mpl normal vs PRV-1 normal, c-Mpl low p=0.0218; (group1 vs group2); PRV-1 high c-Mpl normal vs PRV-1 high c-Mpl low p=0.0068; (group1 vs group3). **#Plts. at diagnosis (×10^9/L):** PRV-1 normal, c-Mpl normal vs PRV-1 normal, c-Mpl low (group0 vs group2) p=0.0102; PRV-1 high, c-Mpl normal vs PRV-1 normal, c-Mpl low (group1 vs group2) p=0.0369.*

Table 2. Occurrence of vascular and bleeding complications in the 48 ET patients. A multivariant logistic regression was carried out and adjusted for the use of aspirin (ASA) to determine the odds ratio, 95% confidence interval and the statistical significance of marker status and the occurrence of complications.

	Group 0 PRV-1 normal c-Mpl normal	*Group 1 PRV-1 high c-Mpl normal*	*Group 2 PRV-1 normal c-Mpl low*	*Group 3 PRV-1 high c-Mpl low*
Patients	23	8	7	10
Patients with complications	2 (8.7 %)	4 (50 %)	1 (14.3 %)	2 (20 %)
Patients receiving ASA at complication	2/2 (100%)	1/4 (25%)	0	0
Odds ratio		12.52	1.76	2.83
95 % CI	–	1.47-106.35	0.13 - 23.12	0.33-24.32
p value	–	0.0206	0.6667	0.3435

The following complications occurred (the platelet count at the time of complication, cytoreductive therapy and aspirin use are given in parentheses). Among the 32 patients with PRV-1 normal, c-Mpl normal, one had a stroke, (80×10^9/L: 32P, ASA) and another had a cerebrovascular occlusion (709×10^9/L) at diagnosis. In the group of 8 patients with PRV-1 high, C-Mpl normal, there were 4 complications: amaurosis fugax (951×10^9/L: HU), mesenteric venous thrombosis (515×10^9/L: at diagnosis), microvascular complications (633×10^9/L: no treatment), and transient ischemic attack and myocardial infarction (1115×10^9/L: HU, ASA). Among the 7 patients with PRV-1 normal, c-Mpl low there was one episode of gastrointestinal bleeding (600×10^9/L: ^{32}P). Finally, among the 10 patients with PRV-1 high, c-Mpl low, one presented with deep vein thrombosis (855×10^9/L) at diagnosis and another with microvascular complications (650×10^9/L) again at diagnosis.

normal erythropoietin concentrations, weak or absent c-Mpl staining in bone marrow megakaryocytes and PRV-1 overexpression have been suggested to be independent risk factors for thrombotic complications.[3-6] Clonality, decreased c-Mpl expression and PRV-1 overexpression are present only in a subset of ET patients (30–60%), raising the question of whether they are acquired concurrently or separately in individual patients. We and others have recently demonstrated a close concordance between PRV-1 overexpression and endogenous erythroid colony (EEC) formation.[6,7] In contrast, decreased expression of c-Mpl appears to be acquired independently (Table 2).[7] Hence, quantification of c-Mpl and PRV-1 expression can be used to distinguish four subclasses of ET patients (PRV-1 normal, c-Mpl normal; PRV-1 high, c-Mpl normal; PRV-1 normal, c-Mpl low; PRV-1 high, c-Mpl low). We determined whether any of these subgroups is at increased risk of developing thromboembolic complications.

Peripheral blood samples were obtained from 48 patients with ET (Table 1), diagnosed according to the updated PVSG criteria. Platelets and granulocytes were isolated and c-Mpl and PRV-1 expression quantified as previously described.[8,9]

Of the 48 ET patients studied, 17 (35%) displayed decreased c-Mpl expression and 18 (37%) overexpressed PRV-1 (Table 2). As previously reported, the two markers appeared to be acquired independently, as they were present concurrently in only 10 patients (21%, Table 2).[7]

The following end-points were defined as constituting thromboembolic complications: venous or arterial thrombosis, cerebrovascular events, pulmonary embolism, myocardial infarction, microvascular complications or stroke. One gastro-intestinal bleeding episode was noted.

In our cohort of 48 patients, 8 individuals suffered one thromboembolic incident, one patient suffered recurrent complications, which were counted as one incident. The patients' characteristics and laboratory data at diagnosis were not significantly different between the four groups (Table 1). Hence, by the clinical criteria currently used to assess thrombotic risk (age, platelet number and previous history of thrombosis), none of the subgroups appeared to cluster patients at higher risk.

A logistic regression, adjusted for the use of aspirin, determined that patients with elevated PRV-1 but normal c-Mpl expression had a 12-fold higher odds ratio of thromboembolic complications than did patients who expressed neither of the markers (Table 2). This appeared to be independent of cytoreductive therapy and aspirin use. While there were significant differences in hemoglobin, hematocrit and platelets between several groups at sample collection, the patients with high PRV-1 levels and normal c-Mpl expression, who showed the 12-fold increased risk of thrombosis compared to the control group (PRV-1 normal, c-Mpl normal), were not different from the control group for any of these parameters (Table 1, bottom).

Contrary to a previous report, decreased c-Mpl expression in our cohort was not associated with an increased risk of thrombosis.[4] However, in Teofili's study[4] c-Mpl expression was measured in megakaryocytes, not platelets. It is possible that our limited sample size masks smaller effects. Likewise, our small number of patients limits analysis of the interaction between PRV-1 overexpression and decreased c-Mpl expression. It does, however, appear that patients bearing both markers were at a lower risk of complications than patients overexpressing PRV-1 but with normal levels of c-Mpl (Table 2). Interestingly, c-Mpl expression has been shown to diminish during the progression of polycythemia vera, while the risk for thromboembolic complications is highest in the first two years following diagnosis.[10] One may speculate that decreased c-Mpl expression counteracts the deleterious effect of PRV-1 overexpression in ET patients.

These data extend two recently published studies which demonstrated an increased risk of thromboembolic complications in PRV-1-positive ET patients.[5,6] Here we

show that among PRV-1-positive ET patients, those with normal c-Mpl levels appear to be at highest risk of thromboembolic events. The results of this retrospective analysis must now be corroborated in a large prospective trial of newly diagnosed patients such as that being implemented by the MPD Research Consortium

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Acute Myeloid Leukemia

Mutations of *PTPN11* are rare in adult myeloid malignancies

The *PTPN11* gene encodes the phospho-tyrosyine phosphatase protein SHP-2. Constitutional mutations of this gene are involved in Noonan's syndrome, a developmental disorder in which children have a predisposition to develop a myeloid disorder called juvenile myelomonocytic leukemia. Recently, studies have shown that somatic mutations of *PTPN11* can be found in children with myeloid malignancies. We evaluated the incidence of acquired mutation of *PTPN11* in 76 adults with acute or chronic myeloid malignancies and summarized our results together with others published recently.

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The *PTPN11* gene (protein-tyrosine phosphatase, nonreceptor-type, 11), localized in 12q24, encodes a protein with tyrosine phosphatase activity called SHP-2 (Src homology 2 domain-containing phosphotyrosine phosphatase 2). The SHP-2 protein has 3 functional domains: the PTPase catalytic domain and two SH2 domains (src homology 2), one in the C-terminal part (C-SH2) and the other in the N-terminal part (N-SH2) of the protein. In its inactive form, the PTPase catalytic domain is masked by N-SH2; binding of phospho-tyrosyl peptides on N-SH2 induces a conformational change uncovering the catalytic site. SHP-2 is implicated in signal transduction pathways, particularly those induced by growth factors; activation of SHP-2 generally leads to activation of the RAS/RAF/ERK pathway.[1] The SHP-2 protein is strongly expressed in blood cells and is implicated in the response to KIT-ligand, interleukin-3, granulocyte-monocyte colony-stimulating factor and erythropoietin. The *PTPN11* alterations so far reported in human diseases were exclusively missense mutations. In Noonan's syndrome, *PTPN11* carries constitutional mutations preferentially localized in exon 3, the coding sequence for the domain of N-SH2 implicated in the inhibition of the PTPase catalytic site.[2] Children with this syndrome have a developmental disorder as well as a predisposition to develop juvenile myelomonocytic leukemia (JMML). Furthermore, in children, somatic mutations of *PTPN11* have been reported in 34% of non-syndromic JMML, 18.5% of refractory anemia with excess blasts (RAEB) in transformation, 4% of acute myeloid leukemia (AML), and 6.5% of acute lymphocytic leukemia (Table 1); 98% of those mutations were localized in exon 3.[3-7] In this study, we performed mutational analysis of exon 3 of the *PTPN11* gene to assess the incidence of mutations in adult myeloid disorders. We tested 84 patients: 38 had chronic myelomonocytic leukemia (CMML), 18 RAEB, 4 RAEB in transformation, 4 AML with monosomy 7, 12 AML post-myelodysplastic syndrome and, as a control, 8 non-syndromic JMML. After DNA extraction from bone marrow or blood samples, exon 3 of *PTPN11* was amplified as previously described.[3] Amplification products were purified and sequenced. As expected, 3 of the 8 cases of JMML tested carried mutations (G60R, D61V and G503A). Among the 76 remaining patients, only one had a *PTPN11* mutation (missense mutation: D61N). This patient had AML with monosomy