trols (9%). Half of the patients (6 of 12) with atypical MPD demonstrated PRV-1 over-expression without any disease predilection (Table 2). In both PV and ET, multivariate analysis including several variables (age, sex, disease duration, hematocrit, erythropoietin level, platelet count, LAP score) showed a significant correlation between PRV-1 expression and LAP score, only. Among 18 PV patients in whom both tests were performed, 17 had concordant abnormalities. The corresponding numbers for ET were 13 and 12. The current study confirms the suboptimal diagnostic accuracy of the neutrophil PRV-1 assay in terms of distinguishing PV from SP (76% sensitivity and 85% specificity).[3] Our experience is further supported by recent evidence from another large prospective study involving 99 patients with PV that reported similar sensitivity (68%) and specificity (60%) values.[6] Although the small sample size does not allow definitive conclusions, the current study also confirms previous observations regarding the high prevalence of the specific abnormality in PPMM as opposed to in other subtypes of MMM.[3] One of the original observations from the current study is the demonstration of neutrophil PRV-1 over-expression in a substantial proportion (50%) of patients with atypical MPD. This information strongly suggests that altered neutrophil PRV-1 expression is a non-specific marker of clonal myeloproliferation. Furthermore, in the current study, we were able to demonstrate that a PRV-1/GAPDH ratio of < 1.17 (i.e. increased PRV-1 expression) was almost always associated with an increased LAP score (>100) in both PV and ET. Obviously, this raises the issue of added value regarding consideration of the particular assay as a new diagnostic test and warrants careful evaluation in a larger study.

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Chronic Myeloproliferative Disorders

Mutation of the prothrombin gene and thrombotic events in patients with polycythemia vera or essential thrombocythemia: a cohort study

The association between a prothrombin mutation and the risk of thrombosis was analyzed in 214 patients with polycythemia vera or essential thrombocythemia. The rate for venous thrombotic events was 14.7/100 patient-years in patients with the prothrombin mutation compared to 0.8 in patients without the mutation (rate ratio 17.5).

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Thromboembolism remains the major cause of morbidity and mortality in polycythemia vera (PV) and essential thrombocythemia (ET).[1-4] It has been shown that the diagnosis of PV is frequently preceded by thromboembolic events with an increasing incidence during the last 7 years before diagnosis. Once the diagnosis has been established, the incidence of thrombosis has been estimated to range from 4 to 11 events per 100 patient-years.[5] A similar frequency of thromboembolic complications was reported for ET.[6] The *G20210A* mutation in the prothrombin gene is associated with elevated levels of this zymogene and was identified as a congenital risk factor for deep venous thrombosis. Heterozygous carriers of the mutation have a 2- to 9-fold higher risk of deep venous thrombosis than do individuals with a normal genotype.

We performed a retrospective cohort study to assess the association between this prothrombin mutation and the risk of thrombotic events in PV or ET. The primary end-points were the occurrence of a venous thromboembolic event (i) in the seven years preceding the diagnosis of PV or ET and (ii) after diagnosis.

We determined the *G20210A* mutation of the prothrombin gene, factor V Leiden mutation, total homocysteine levels, plasma folate, vitamin B12 levels, protein C activity, free protein S antigen and antithrombin activity as previously described.[7] Poisson regression was used to model the association between the prothrombin mutation and the incidence of venous thrombosis/embolism.

We assessed 214 patients with a chronic myeloproliferative disorder. The clinical characteristics of patients without and with the prothrombin mutation appeared to be comparable (Table 1).

Prothrombin mutation and venous thrombosis seven years before the diagnosis of PV or ET. Within 1,509 total observation years at risk, the incidence rate for a venous thrombotic event was 6.2 per 100 patient-years in patients with the

Table 1. Characteristics of patients with and without the prothrombin mutation.

	*Wild type (n=205)**	*Prothrombin mutation (n=9)**	*p value*
Age (years)	58 (48 to 68)	56 (26 to 67)	0.45
Female	106 (52%)	6 (67%)	0.30
Diagnosis			
Polycythemia vera	124 (61%)	7 (78%)	0.25
Essential thrombocythemia	81 (40%)	2 (22%)	
Observation (months from diagnosis)	86.8 (1 to 335.1)	93.9 (11.3 to 180.3)	0.79
Factor V Leiden mutation	13/204 (6%)	2/9 (22%)	0.125
Hematocrit at diagnosis (%)	47 (43 to 53) (n=184)	48 (29 to 61) (n=9)	0.70
Platelet count ($\times 10^9$/L)	613 (319 to 898)	519 (235 to 1,143)	0.76
Homocysteine levels at diagnosis (μmol/L)	12.4 (9.5 to 16.3) (n=123)	11.0 (6.2 to 16.8) (n=4)	0.51
Folic acid levels at diagnosis (nmol/L)	13.9 (11.1 to 17.8) (n=144)	11.9 (9.3 to 20.9) (n=5)	0.78
B12 levels at diagnosis (pmol/L)	281 (179 to 438) (n=144)	437 (180 to 780) (n=5)	0.19
AT III levels at diagnosis (%)	93 (85 to 100) (n=158)	99 (86 to 104) (n=5)	0.38
Protein C levels at diagnosis (%)	87 (74 to 101) (n=158)	83 (62 to 106) (n=5)	0.51
Protein S levels at diagnosis (%)	78 (63 to 94) (n=158)	50 (47 to 93) (n=5)	0.08

*Continuous data are given as the median and interquartile range (in the wild type group) and the median and range in patients with the prothrombin mutation; *number of patients if not indicated otherwise.*

prothrombin mutation (95% confidence interval 2.0 to 19.4) compared to 0.8 per 100 patient-years in patients without the mutation (0.4 to 1.4) (Table 2) (rate ratio 7.8, 2.2 to 27.9). Sex, type of chronic myeloproliferative disorder and presence of the factor V Leiden mutation did not confound the association between the risk factor and the primary outcome. There was also no significant interaction between these covariates and the prothrombin mutation.

Prothrombin mutation and venous thrombosis after the diagnosis of PV or ET. After diagnosis of PV or ET – with 1,678 patient-years at risk – the rate of venous thrombotic events increased greatly among patients with the prothrombin mutation but remained stable in patients without the mutation: if the mutation was present the rate was 14.7 per 100 patient-years (5.5 to 39.2) whereas if it was absent the rate was 0.8 (0.5 to 1.4) (Table 2). The unadjusted rate ratio was 17.5 (5.7 to 53.6). Sex, presence of factor V Leiden mutation, and platelet count at diagnosis did not confound the association between the risk factor and the outcome,

Table 2. Number of events and observation time.

	Number of events	*Observation time until first event* (years)*	*Rate (per 100 patient-years)*
Venous thrombotic/thromboembolic event; in the seven years prior to diagnosis			
Wild type	11	13.7	0.8
Prothrombin mutation	3	0.5	6.2
Venous thrombotic/thromboembolic event; after diagnosis			
Wild type	13	15.5	0.8
Prothrombin mutation	4	0.3	14.7

**Patients were excluded if the myeloproliferative disorder was diagnosed because of venous or arterial thrombosis; these patients do not contribute to the observation time.*

nor was there evidence of interaction between sex or factor V Leiden mutation and the risk factor. Controlling for increasing age over time increased the rate ratio to 29.6 (8.6 to 101.4). The location of the venous thrombotic/thromboembolic event was not equally distributed when comparing patients with and without the mutation: in patients with the mutation thrombosis occurred more often in less usual locations such as splanchnic veins and upper extremity veins (2/7 vs. 3/33 in patients with and without the mutation, respectively, p=0.045). The pathophysiological mechanisms linking venous thromboembolic events to the prothrombin mutation are still not completely resolved but high plasma prothrombin levels may lead to an imbalance between the procoagulant, anticoagulant and the fibrinolytic systems.[8] This mutation may be linked to an increased risk of thrombosis, particularly in sites with low venous blood flow. Activated platelets or other hemostatic defects in PV or ET in coincidence with elevated prothrombin plasma levels in carriers of the mutation might even increase the thrombogenic effect.

Although the prothrombin mutation in the general population has been shown to be associated with only a moderate risk of venous thrombosis, its contemporary occurrence with other risk factors or another procoagulant state can increase the thrombotic risk: users of oral contraceptives had a markedly increased risk of sinus vein thrombosis.[8,9] In addition a high frequency of carriers of the prothrombin mutation was observed among patients with idiopathic portal vein thrombosis.[10] Although the present data show that the risk of a thromboembolic event attributable to the prothrombin mutation in PV or ET is excessive, the question of whether all patients with PV or ET should be tested for the prothrombin mutation can only be answered by large prospective trials.

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Chronic Lymphoproliferative Disorders

A pilot study of low-dose subcutaneous alemtuzumab therapy for patients with hemotherapy-refractory chronic lymphocytic leukemia

Subcutaneous low-dose alemtuzumab (10 mg t.i.w. for 18 weeks) induced a 50% response rate, including 25% complete response, in 16 patients with refractory chronic lymphocytic leukemia (CLL) patients. The responses were substantial even in patients with unfavorable cytogenetics, fludarabine/rituximab refractoriness, Rai stage IV, previous infections, and age over 65 years. Subcutaneous low-dose alemtuzumab is effective in poor prognosis B-CLL, and has a particularly favourable toxicity profile.

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Patients with chronic lymphocytic leukemia (CLL) who are resistant/refractory to alkylating agents and/or fludarabine[1-3] have a poor prognosis, with a median survival duration of only 10 months.[4]

In this subset of high-risk patients, alemtuzumab has been shown to induce a significant overall response rate of 33%.[2] However, treatment with alemtuzumab according to the conventional administration schedule (30 mg three times a week intravenously) is associated with a consistent number of reactions and significant infectious morbidity.[5,6] It has recently been reported that both the percentage and severity of *first dose* reactions can be dramatically reduced by the *subcutaneous* administration of alemtuzumab 30 mg three times weekly for 18 weeks.[7]

On the basis of these data we decided to assess the efficacy and safety of prolonged treatment with subcutaneous low-dose alemtuzumab (10 mg three times a week for 18 weeks) in a cohort of 16 heavily pre-treated B-CLL patients.

Sixteen patients were enrolled. The patients had received a median of three prior lines of therapy, all were refractory to alkylating agents, fourteen were refractory to fludarabine, and two were not allowed a purine analog-containing therapy due to previous Coombs'-positive anemia. Half of the patients were also refractory to rituximab-containing regimens. Half of the patients had had infections during the six months preceding the start of alemtuzumab therapy. Two patients with hepatitis B virus (HBV) reactivation were on lamivudine therapy, which was continued during treatment with alemtuzumab (Table 1).

All patients received at least four weeks of alemtuzumab therapy; twelve patients completed all 18 weeks of treatment. The reasons for treatment withdrawal during weeks 4-14 were the achievement of a complete response (3 patients), and infection (1 patient).

The overall response, according to NCIWG criteria,[8] of the patients enrolled in this pilot study was 50%, including 25% complete responses. No progressive disease was observed during treatment. An objective response was documented in 50% of the patients refractory to both alkylators and fludarabine. Three of the eight patients resistant to rituximab responded to alemtuzumab. Overall response was 43.3% in the patients with an abnormal karyotype and 37.5% in patients with unfavorable cytogenetic alterations. The two patients with p53 gene deletions [del(17)(p13.1)] both showed a partial response after alemtuzumab therapy. The therapy was exceptionally well tolerated by the older patients, with remarkable responses in terms of percentage and quality (Table 2). A higher proportion of patients achieved responses in blood (93.7%) and bone marrow (62.5%), as compared with lymph nodes and spleen (50% and 42.9%, respectively). The time to achieve a 1-log depletion of peripheral blood lymphocytes (PBL) was more rapid in responders than in non-responders (3 weeks versus 8 weeks), and was particularly quick in complete responders (2.5 weeks). This observation, simply made by assessing PBL counts using a hematologic analyzer, is in line with the results of the elegant study by Rawstron *et al.*[9]

Four patients died of progressive disease or infectious complications after a median follow-up of nine months, all of whom were non-responders. The median survival of our non-responding patients is therefore comparable with that observed in CLL patients failing purine analog therapy. In contrast, the patients who achieve a response after low-dose alemtuzumab are all alive after a median