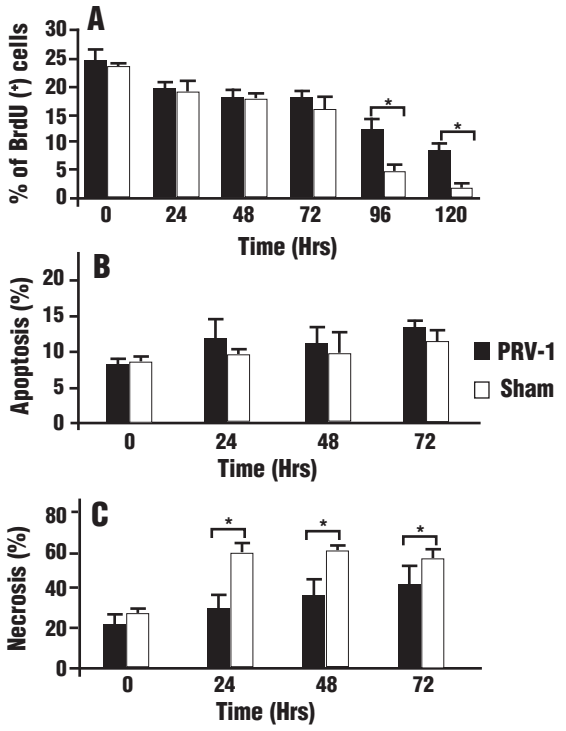

Figure 2. Effect of PRV-1 expression on cell proliferation and cell death. A. *Cell proliferation*: equal numbers of transfected CHO cells (1x10⁴) were seeded in 4-well Laboratory Teck chamber slides (Nalge Nunc International, Rochester, NY, USA). Cells were serum-starved for 0, 24, 48, 72, 96, and 120 hours; pulsed with 50 mol/L BrdU, and then fixed in 70% ethanol solution at -20°C. The Y-axis shows the percentage of BrdU-positive cells [BrdU-positive cells/total cell count) × 100]. BrdU incorporation into the nuclei of cells was evaluated by light microscopy at 20 X magnification and for each time point, a total of 1,000 cells was counted. B-C. *Cell death*: equal numbers of transfected CHO-cells were serum starved for different time intervals. We used flow cytometry to measure the surface binding of annexin V (apoptotic cells) or DNA incorporation of SYTOX green dye together with surface binding of annexin V (necrotic cells). Percentages of apoptotic (B) or necrotic (C) cells are shown on the X-axis. The experiment was repeated three times and the results are summarized as bar graphs *shows $p<0.05$ (Student's t-test).

express PRV-1 mRNA? Is there a difference in the expression of PRV-1 on hematopoietic progenitor cells between normal subjects and patients with myeloproliferative diseases ? Is there a correlation between the level of mRNA and surface expression of PRV-1? There are controversial data about the answer to this last question.[4,7] Besides being a possible diagnostic marker for PV and ET, PRV-1 overexpression may alter cellular function. We found that expression of PRV-1 on CHO cells decreased the dependency of these cells on serum for survival and proliferation. The role of PRV-1 in proliferation of myeloid cells and in the pathogenesis of PV and ET should be studied further in other *in vitro* systems and in animal models.

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Chronic Myeloproliferative Disorders

Neutrophil polycythemia rubra vera-1 expression in classic and atypical myeloproliferative disorders and laboratory correlates

The current study of 153 subjects with both classic and atypical myeloproliferative disorders suggests that neutrophil polycythemia rubra vera-1 (PRV-1) over-expression is a non-specific feature of clonal myeloproliferation that displays significant correlation with leukocyte alkaline phosphatase score. These observations undermine the utility of the PRV-1 assay as a diagnostic test of additional value.

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Previous studies have found a strong association between neutrophil PRV-1 over-expression and polycythemia vera (PV) which is neither invariable (test sensitivity ranging from 69-100%)[1-6] nor exclusive (a substantial minority of patients with either essential throm-

Table 1. Neutrophil PRV-1 transcript levels in typical myeloproliferative disorders, secondary or apparent polycythemia, and controls.

	PV n=49	*ET n=23*	*AMM n=11*	*PPMM n=4*	*PTMM n=3*	*SP n=40*	*Controls n=11*
PRV-1 /GAPDH (median)	1.01	1.24	1.26	1.04	1.25	1.25	1.25
PRV-1 /GAPDH (range)	0.83-1.28	0.9-1.44	1.08-1.43	0.93-1.11	1.23-1.27	0.99-1.41	1.14-1.43
% with increased *PRV-1* expression *	76%	18%	17%	100%	0%	15%	9%

*PV: polycythemia vera; ET: essential thrombocythemia; AMM: agnogenic myeloid metaplasia; PPMM, post-polycythemic myeloid metaplasia; PTMM: post-thrombocythemic myeloid metaplasia; SP, secondary or apparent polycythemia; NA, not applicable. *neutrophil PRV-1 expression was considered increased when the neutrophil PRV-1/GAPDH ratio was below 1.17.[10]*

bocythemia (ET) or myelofibrosis with myeloid metaplasia (MMM) also display the specific abnormality).[2-4,6,7] Nevertheless, neutrophil PRV-1 over-expression in myeloproliferative diseases (MPD) other than PV has been interpreted by some to represent a biological link with PV suggesting either an inevitable progression to PV, in the case of ET,[8] or a marker of antecedent PV, in the case of MMM.[3] In order to address the issue further, we prospectively studied neutrophil PRV-1 expression patterns in a large cohort of patients with typical MPD (PV, ET, MMM) as well as in a smaller cohort with atypical MPD including hypereosinophilic syndrome (HES) and systemic mastocytosis (SM). In addition, we explored for laboratory correlates of neutrophil PRV-1 expression in both PV and ET.

A total of 153 subjects were accrued to the study between April 2003 and July 2004. The diagnoses of PV, ET, MMM, HES, SM, and chronic myelomonocytic leukemia (CMML) were made according to the World Health Organization (WHO) diagnostic criteria.[9] SP represented both secondary polycythemia (a co-morbidity known to be associated with secondary polycythemia was identified) and apparent polycythemia (the diagnosis of either PV or secondary polycythemia could not be made and the stability of hematocrit values was documented by serial measurements). Neutrophil PRV-1 transcript level was quantitatively measured by reverse transcriptase polymerase chain reaction (RT-PCR) and interpreted according to previously published methods.[3,10]

Among the 153 study patients, 90 had *classic* MPD including 49 with PV, 23 with ET, and 18 with MMM (Table 1). It should be emphasized that cases with PV included both newly diagnosed cases and patients with a previously established diagnosis. The 18 MMM patients included 11 with agnogenic myeloid metaplasia (AMM), 4 with post polycythemic myeloid metaplasia (PPMM) and 3 with post-thrombocythemic myeloid metaplasia (PTMM). Twelve patients had *atypical* MPD (Table 2). Forty patients had either secondary or apparent polycythemia (SP) and 11 were healthy volunteers (Table 1). Among the 40 patients with SP, 18 had secondary polycythemia (3 with high-oxygen-affinity hemoglobin variants, 3 with a history of exogenous testosterone administration, 8 with either sleep apnea or tobacco use, 1 with atrial septal defect, 1 with renal cell carcinoma, 1 with an erythropoietin receptor mutation, and 1 with renal artery stenosis) and 22 had apparent polycythemia. The highest prevalence of PRV-1 over-expression was documented in

Table 2. Neutrophil *PRV-1* transcript levels in 12 patients with atypical myeloproliferative disorders.

Patient	*Diagnosis*	*Age*	*Sex*	*Neutrophil PRV-1 /GAPDH ratio*	*Neutrophil PRV-1 expression**
1	CMML associated with myelofibrosis	63	F	1.05	Increased
2	*FIP1L1-PDGFRA*⁺ eosinophilic disorder	34	M	1.39	Normal
3	*FIP1L1-PDGFRA*⁺ eosinophilic disorder	52	M	1.19	Normal
4	*FIP1L1-PDGFRA*⁺ eosinophilic disorder	49	M	1.10	Increased
5	HES with cardiac involvement	68	F	1.15	Increased
6	HES with cardiac involvement	57	M	1.13	Increased
7	HES with sinus involvement	53	F	1.29	Normal
8	Aggressive SM associated with CMML	71	M	1.08	Increased
9	Aggressive SM associated with myelofibrosis	63	M	1.09	Increased
10	Aggressive SM with circulating mast cells	74	M	1.29	Normal
11	Indolent SM	39	M	1.20	Normal
12	Indolent SM	34	F	1.18	Normal

*PRV-1: polycythemia rubra vera-1; CMML: chronic myelomonocytic leukemia; HES: hypereosinophilic syndrome; SM: systemic mastocytosis; GAPDH: glyceraldehyde-3-phosphate dehydrogenase. *Neutrophil PRV-1 expression was considered increased when the neutrophil PRV-1/GAPDH ratio was bellow 1.17.[10]*

PV (76%) and PPMM (100%), which was significantly different from the levels seen in both the other classic MPD and SP (Table 1; $p < 0.0001$). On the other hand, the incidence of PRV-1 over-expression was similar among SP (15%), ET (18%), AMM (17%), PTMM (0%), and con-

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