

The response to rHuEPO treatment is known to be dependent on adequate iron supplementation.^{4,5} Intravenous iron administration can be associated with acute and long-term complications such as anaphylaxis, infection and oxidative damage. To provide the best risk-benefit ratio, reliable predictors of response to iron supplementation are needed. The %HYPO appears to fulfil this role¹ but is also influenced by both the reticulocyte count^{2,3} and a younger age of the RBC population.³ The specificity and sensitivity of other markers are not sufficient during rHuEPO therapy.⁶ Ferritin and transferrin saturation usually decrease during rHuEPO treatment. However, these parameters have proven poor predictors of response to iron supplementation.¹ Discrepancies between ferritin and TSAT are usual during rHuEPO treatment.⁷ sTfR increased progressively during the study, but this parameter is far more influenced by erythropoietic stimulation than by iron deficiency.⁸ With simultaneous administration of rHuEPO and iron, we observed that MCHr, an accurate early marker of iron-deficient anemia,^{9,10} remained stable and normal, whereas %HYPO increased progressively. This cannot be explained by iron deficient erythropoiesis since MCHr and MCH remained normal throughout the follow-up. In fact, the explanation for this increase in %HYPO is a progressive decrease of erythrocyte Hb concentration due to increasing cell volume, mainly related to the appearance of a younger red cell population, including reticulocytes. This phenomenon was more important during the first 4 weeks of treatment since peak reticulocytosis occurred 1 week after rHuEPO initiation and the ratio between young and older RBC decreases as the anemia is corrected. A further increase in %HYPO was observed at the end of the follow-up. As transferrin saturation was below 20%, this could indicate a possible contribution from functional iron deficiency.

Acute erythropoietic stimulation, induced by rHuEPO, leads to reticulocytosis and renewal of the erythrocyte population leading to biased determination of red cell Hb concentration and percentage of hypochromic red blood cells. The specific determination of RBC parameters of mature erythrocytes, separately from those of reticulocytes, should provide a more accurate assessment of functional iron deficiency under conditions of increased erythropoietic activity.

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Chronic Myeloproliferative Disorders

Instability of PRV-1 mRNA: a factor to be considered in PRV-1 quantification for the diagnosis of polycythemia vera

High expression of PRV-1 mRNA in granulocytes has been proposed as a new diagnostic marker for polycythemia vera. We used real-time reverse transcription polymerase chain reaction (RT-PCR) to measure the levels of PRV-1 mRNA, GAPDH mRNA and 18S rRNA in granulocytes obtained from blood samples processed 2, 24 and 48 hours after collection and observed a significant decrease of PRV-1 levels after 24 and 48 hours. The instability of PRV-1 mRNA may affect the diagnostic value of the PRV-1 test in blood samples stored for extended periods.

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Polycythemia vera (PV) is the most common primary polycythemia¹ and is characterized by clonal expansion of myeloid

cells from an acquired mutation of a single stem cell. The diagnosis of PV is based on the guidelines established by the Polycythemia Vera Study Group,² but atypical cases may not be discriminated readily by these criteria. Recently, it has been reported that levels of PRV-1 (polycythemia rubra vera-1) mRNA are increased in PV granulocytes from peripheral blood, but not in their progenitors.³⁻⁵ While some investigators report a clear distinction between elevated PRV-1 mRNA in granulocytes of PV patients and low PRV-1 levels in normal controls,⁶ we and others have found overlaps between the highest PRV-1 levels in healthy controls and the lowest levels in PV patients.⁷⁻⁹ Further, the expression of PRV-1 mRNA could be modulated by cytokines, i.e. upregulated by granulocyte colony-stimulating factor³ and downregulated by interferon- α treatment.¹⁰ We hypothesized that PRV-1 mRNA in granulocytes might also be less stable than the RNA species used as internal references for PRV-1 quantification by real time RT-PCR and here we report on PRV-1 mRNA instability. We measured the levels of mRNA for PRV-1 in granulocytes from 10 normal controls and 3 PV patients. The study was approved by the Baylor College of Medicine (BCM) Institutional Review Board; all studied subjects participated in the

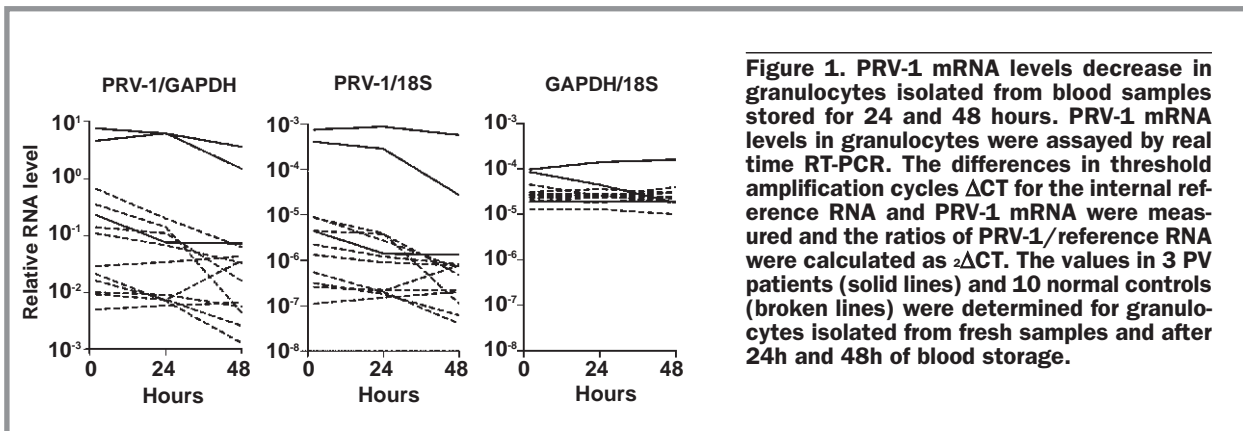


Figure 1. PRV-1 mRNA levels decrease in granulocytes isolated from blood samples stored for 24 and 48 hours. PRV-1 mRNA levels in granulocytes were assayed by real time RT-PCR. The differences in threshold amplification cycles Δ CT for the internal reference RNA and PRV-1 mRNA were measured and the ratios of PRV-1/reference RNA were calculated as $2^{\Delta\Delta\text{CT}}$. The values in 3 PV patients (solid lines) and 10 normal controls (broken lines) were determined for granulocytes isolated from fresh samples and after 24h and 48h of blood storage.

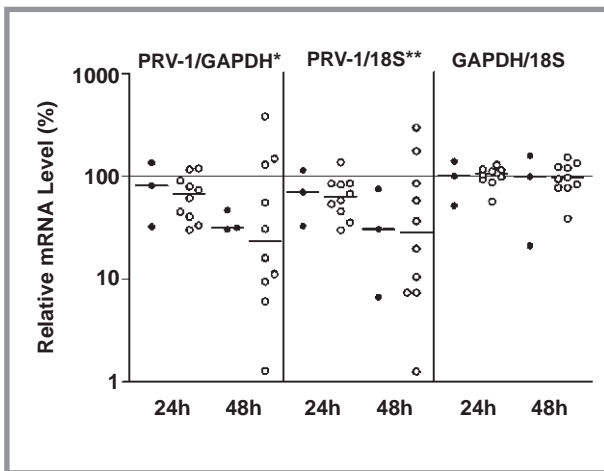


Figure 2. PRV-1 mRNA in granulocytes is less stable than reference RNAs. Relative amounts of PRV-1 mRNA were measured by the real time RT-PCR in RNA from granulocytes isolated immediately, 24 and 48 hours after collection of peripheral blood from 3 PV patients (closed circles) and 10 normal controls (open circles). GAPDH mRNA and 18S rRNA were used as internal reference standards. Relative mRNA levels were expressed as PRV-1/GAPDH, PRV-1/18S and GAPDH/18S ratios, calculated as $2^{\Delta\Delta\text{CT}}$. The values in RNA from granulocytes isolated 2 hours after blood collection (fresh samples) were set as 100% for each subject. The values found in RNA from granulocytes isolated 24 and 48 hours after blood collection were expressed as the percentage of the fresh sample levels. Lines indicate the medians. PRV-1/GAPDH and PRV-1/18S ratios were significantly decreased after the blood samples had been stored for 24 and 48 hours ($*p=0.023$, $p=0.004$) when compared with Friedman's non-parametric test (GraphPad Prism 3.0 Software, San Diego, CA, USA).**

ing control reactions without reverse transcriptase, as described previously.⁷ The PRV-1 mRNA levels in granulocytes isolated from fresh blood samples were within the normal range (as previously established)⁷ in all 10 controls and also in one PV patient on interferon- α treatment. The two remaining PV patients had markedly elevated PRV-1 mRNA, although, the PRV-1 levels decreased in most stored blood samples (Figure 1). When the initial values of PRV-1 mRNA in each subject were normalized as 100% and the PRV-1 levels were expressed relative to GAPDH reference mRNA, we found a median decrease of the PRV-1 level to 73% (range 30-136%) of the initial value after 24 hours and to 31% (range 1-385%) after 48 hours of blood storage. When the PRV-1 mRNA levels were expressed relative to 18S rRNA, the decrease was comparable; i.e. to 68% (range 30-137%) after 24 hours and 31% (range 1-300%) after 48 hours of storage. The decrease of PRV-1 transcript was statistically significant and similar in both normal controls and in PV subjects. In contrast, the stability of the reference RNA standards was identical; when the ratio of GAPDH mRNA to 18S rRNA was set as 100% on day 0; its median was 104% (range 52-140%) after 24 hours, and 94% (range 21-160%) after 48 hours of blood storage. These data are summarized in Figure 2. Our findings suggest that PRV-1 mRNA is significantly less stable than the RNA species used commonly as reference standards. Blood samples stored on ice showed the same kinetics of PRV-1 mRNA decrease (*data not shown*). Consequently, the PRV-1 values in patients with moderately increased levels of PRV-1 mRNA could be negatively affected by the delay between the blood collection and RNA isolation. We conclude that the PRV-1 quantification should be performed in RNA isolated from granulocytes shortly after blood collection. Procedures enabling RNA stabilization in whole blood samples and subsequent isolation of granulocytes would be a useful alternative; however the applicability of this maneuver would first need to be experimentally established.

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studies after signing informed consent. We kept aliquots of each ACD-anticoagulated blood sample at room temperature and isolated RNA from granulocytes as described elsewhere⁷ at three time intervals: (a) within 2 hours after blood collection (initial value), (b) after 24 hours and (c) after 48 hours of storage. Real time RT-PCR with TaqMan[®] probes was used to quantify PRV-1 mRNA in an ABI Prism 7000 Sequence Detection System (Applied Biosystems, Foster City, CA, USA). GAPDH mRNA and 18S ribosomal RNA were employed as internal reference controls. All values were measured within the linear range of the assay. The possibility of genomic DNA interference was ruled out by perform-

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Malignant Lymphomas

Efficacy of a modified Stanford V regimen in patients with advanced Hodgkin's lymphoma

We report treatment results obtained with a modified Stanford V regimen in 32 patients with advanced Hodgkin's lymphoma (stage II bulky disease, III, IV). Treatment results were not superior to those achieved with conventional treatment (ABVD) in terms of complete remission and survival rates (progression-free survival and overall survival at 3 years: 66% and 91%, respectively).

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Cure rates for patients with advanced Hodgkin's lymphoma range between 60 and 70% when treated with conventional chemotherapy regimens. The combination of doxorubicin, bleomycin, vinblastine, and dacarbazine (ABVD) emerged as the standard therapy because of its low toxicity profile and equivalent efficacy when compared to other regimens.¹ The Stanford V program is an abbreviated intensified chemotherapy regimen which has been proposed as a favorable alternative, with 5-year freedom-from-progression rates reported to be between 85% and 89% and an overall survival (OS) of 96%.²⁻⁴

We treated 32 patients with previously untreated, histologically proven, locally extended or advanced (stage II with bulky disease of more than 5 cm, stage III or IV disease) Hodgkin's lymphoma with a modified Stanford V regimen, in which mechlorethamine was replaced by cyclophosphamide because of the latter's lower leukemogenic risk. Chemotherapy was given weekly for 12 weeks as follows: vinblastine 6 mg/m² and doxorubicin 25 mg/m² in weeks 1, 3, 5, 7, 9 and 11; vincristine 1.4 mg/m² (maximum dose 2 mg) and bleomycin 5 U/m² in weeks 2, 4, 6, 8, 10, and 12; cyclophosphamide 650 mg/m² in weeks 1, 5, and 9; and etoposide 120 mg/m² on weeks 3, 7, and 11; prednisone 40 mg/m² every other day from week 1 until week 10, and tapered during weeks 11 and 12. Local radiotherapy with 36 Gy was scheduled for patients with initial bulky disease > 5 cm or sites of partial remission. As a control group we selected a historical group of 64 patients matched for age, sex, histotype, stage and presence of bulky disease who had been treated with standard ABVD administered every 28 days. Patients received a mean of 7.2±1.6 cycles. The patients' characteristics are listed in Table 1.

Therapy according to the modified Stanford V regimen

was well tolerated, without major toxicities or dose reductions: dose intensity was 0.93±0.098 (mean±standard deviation) of the planned dose. At a median follow-up of 37 months one second malignancy occurred (thyroid cancer).

Treatment failures in the Stanford V patient group were mostly due to disease progression during and early after completion of chemotherapy, while the 3-year disease-free survival for patients obtaining complete remission was 85±8% for the Stanford V group in comparison to 90±4.7% for the ABVD group ($p=0.447$) (Table 2). There was a trend towards a better overall survival for patients treated with ABVD rather than the Stanford V regimen (96% and 91% at 3 years, respectively; $p=0.07$) (Figure 1). The estimated probability of freedom from treatment failure (FFTF) at 3 years was 66% for patients treated according to the Stanford V regimen and 76% for conventionally treated patients ($p=0.11$) (Figure 1). Prognostic factors predicting a poor outcome in patients treated with the Stanford V regimen were the presence of bulky disease ($p=0.046$) and histological grade 2 nodular sclerosis type of lymphoma ($p=0.012$).

Our data compare unfavorably with those reported by the Stanford group.^{2,3} One possible explanation for this difference may be the modification of the chemotherapy regimen we had introduced by substituting mechlorethamine with cyclophosphamide. Efficacy of the MOPPEBVCAD regimen in patients with advanced Hodgkin's lymphoma was reported to be reduced when the alkylating agents lomustine and melphalan were replaced by cyclophosphamide and etoposide, indicating that substitutions may not be equivalent.⁵ However, the most striking difference is the rate of consolidation radiotherapy. At Stanford, 86% of patients received radiotherapy.^{2,3} In our treatment program, radiotherapy was planned only to sites of initial bulky disease > 5 cm and to sites of partial remission. As a consequence, only 56% of our patients were scheduled to receive radiotherapy, and 44% of the patients were actually irradiated.

Our data are in line with two other reports comparing abbreviated regimens with conventional treatments. In an Italian multi-center randomized study the failure-free survival rate at 3 years for patients in the Stanford V group was 53.4% compared to 81.4% for the ABVD group.⁶ Along the same line, the British National Lymphoma Investigation (BNLI) study group reported better treatment results for patients treated with 6 monthly cycles of a hybrid regimen, ChIVPPP/EVA than for those treated with 11 weekly cycles of VAPEC-B, a regimen with remarkable similarity to the Stanford V program.⁷ In both studies, the proportion of patients receiving consolidation radiotherapy was lower