presence of CD, than in non-anemic subjects. Furthermore, sTfR detects iron deficiency more efficiently in patients with more profound degrees of anemia.

Josefa Piedras, Maria del Carmen Cinta-Severo, Karina Valdéz, Xavier López-Karpovitch

Hematology and Oncology Department, Laboratory of Cell Biology, Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán, Tlalpan, México

Correspondence: Josefa Piedras, Chem D. Hematology and Oncology Department. Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán. Vasco de Quiroga 15, Tlalpan, 14000, México, D.F.
Phone: international +52.5.5731200 Ext 2700. Fax: international +52.5.6551076. E-mail: piedras@quetzal.innsz.mx

Key words: soluble transferrin receptor, iron deficiency, anemic and non-anemic hospitalized patients.

Manuscript processing

This manuscript was peer-reviewed by two external referees and by Professor Mario Cazzola, Editor-in-Chief. The final decision to accept this paper for publication was taken jointly by Professor Cazzola and the Editors. Manuscript received September 24, 2002; accepted February 4, 2003.

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Quantitative real-time polymerase chain reaction shows that treatment with interferon reduces the initially upregulated PRV-1 expression in polycythemia vera patients

We developed a real-time quantitative polymerase chain reaction-based assay for quantification of PRV-1 mRNA. We found that the expression of PRV-1 in granulocytes of patients with polycythemia vera (PV) who were pretreated with phlebotomy or hydroxyurea was significantly higher than that in normal controls. Surprisingly, in PV patients who had received interferon-α (IFN) for five or more months no significant PRV-1 upregulation was found. Observation of four PV patients treated with IFN over six months revealed a uniform time-dependent decrease of initially upregulated PRV-1.

Haematologica 2003;88:349-351
(*http://www.haematologica.org/2003_3/88349.htm)*

Interferon-α (IFN) was reported to induce clinical remissions in patients with polycythemia vera (PV)[1] and even selectively suppress the malignant hematopoesis in PV patients carrying chromosomal markers as demonstrated by cytogenetic analysis.[2,3] Recently, selective expression of PRV-1 was shown by Northern blot in peripheral blood granulocytes of PV patients.[4] We and others have developed real-time quantitative PCR-based assays for PRV-1 mRNA; so far, these assays have only been reported in abstract form.[5-7]

We here report on a group of patients who fulfilled the diagnostic criteria for PV,[8] including endogenous erythroid colony (EEC) growth and who had been pretreated with phlebotomy (PT) or hydroxyurea (HU). This cohort had significantly higher PRV-1 levels than did normal controls ($p<0.01$, Figure 1). Interestingly, a second group of PV patients who met the same diagnostic criteria, including EEC but who had been receiving IFN for five or more months prior to PRV-1 analysis had lower PRV-1 expression ($p<0.05$ vs. PV patients pretreated with PT and HU), which was not significantly different from that in normal controls ($p>0.05$, Figure 1). Although PV-1 was expressed in peripheral blood granulocytes of PV patients, only very low expression was observed in the MNC fraction (mean PRV-1/GAPDH <0.01, data not shown). PRV-1 expression was within the range found in normal controls in patients with chronic myelogenous leukemia (n=2) and in 7 of 8 patients with essential thrombocythemia (ET). The reported data on PRV-1 expression in granulocytes of ET patients are controversial. Teofili *et al.*[9] found PRV-1 expression in all the ET patients they studied (n=37) using qualitative nested RT-PCR. Klippel *et al.*[6] reported PRV-1 overexpression in 50% of ET patients using a quantitative real-time PCR assay. Mutual validation of methods would be helpful to clarify this issue.

Of 33 patients with suspected unclassifiable chronic myeloproliferative disorders (CMPD) not meeting the required diagnostic criteria of the PV study group, 16 were PRV-1 positive (PRV-1/GAPDH ratio >0.09). All seven patients who were later found to have secondary erythrocytosis had normal PRV-1 values. We followed the kinetics of PRV-1 expression in granulocytes of four PV patients with initially high PRV-1 expression who then received 50 μg/week of PEG-Intron™ (pegylated IFN). Before the start of the IFN treatment the PRV-1/GAPDH ratio was 0.849±0.319 (mean±SD). PRV-1 expression was assessed every two months thereafter and was found to decrease uniformly in all four patients. At six months a near six-fold decrease of the mean PRV-1 expression (0.143±0.036) was observed (Figure 2). Apart from the obvious implications of PRV-1 analysis for the diagnosis of PV and differential diagnosis of CMPD our data allow us to hypothesize that PRV-1 expression is a surrogate marker of therapeutic response to IFN in PV. Larg-

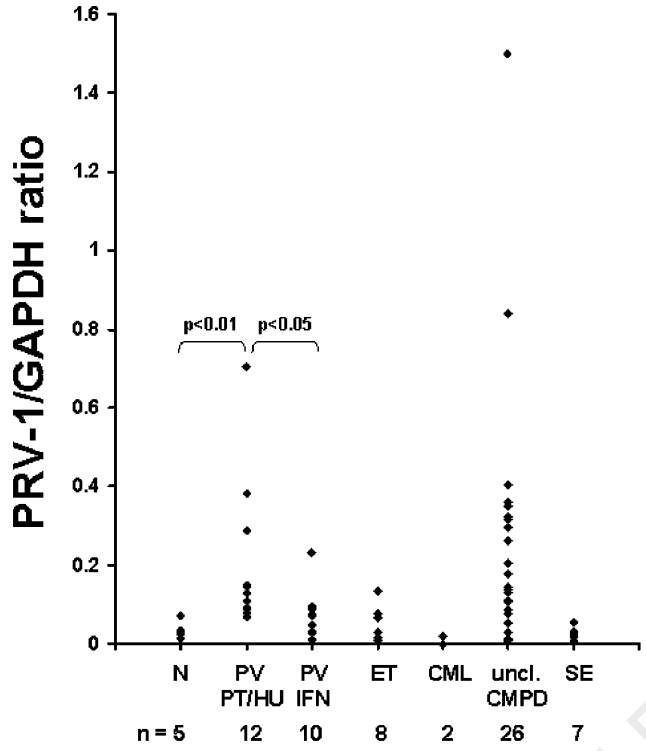

Figure 1. Individual data on normal controls (N), patients with polycythemia vera who fulfilled the diagnostic criteria of the PV study group (PV) including growth of endogenous erythroid colonies (EEC), patients with ET, CML, unclassified CMPD (uncl. CMPD) and secondary erythrocytosis (SE) are given. In the PV group patients receiving phlebotomy or hydroxyurea (PT/HU) and PV patients treated with IFN are given separately. Mononuclear cells (MNCs) and granulocytes were separated by Ficoll centrifugation. Afterwards erythrocytes were eliminated by lysis with NH_4Cl. Total RNA was isolated using Qiagen RNeasy Mini Kit (Qiagen, Hilden, Germany). First-strand cDNA synthesis was primed by random hexamers using TaqMan Reverse Transcription Reagents (Applied Biosystems, Weiterstadt, Germany) with 2 µg total RNA in 100 µL reaction volume. PRV-1 gene expression in relation to the GAPDH housekeeping gene was quantified according to manufacturer's protocol[10] by comparison with standard curves obtained with a control plasmid (10^2-10^6 copies/reaction) that included fragments of both genes. Primers prv1-fp 5'-CGTGGCCCAACCTTCCA-3' and prv1-rp 5'-CGCTTCTCACGCGCAGA-3' amplified a 72-bp fragment that was detected by the TaqMan probe prv1-p 5'-TTCTTGTTGAACCACACCAGACAAATCGG-3'. For detection of the GAPDH gene primers gapdh-fp 5'-TGGAAGATGGTGATGGGATTTC-3' and gapdh-rp 5'-GATTCCACCCATGGCAAATTC-3' and the TaqMan probe gapdh-p 5'-ATGACAAGCTTCCCGTTCTCAGCCTTGA-3' were used (amplicon length 86 bp). Final concentrations were 300 nM for primers and 200 nM for probes. The amplification of PRV-1 and GAPDH of each sample was performed in separate wells of the same 96-well plate on an ABI PRISM 7700 Sequence Detection System (Applied Biosystems). Amplification was carried out using TaqMan Universal PCR Master Mix (Applied Biosystems) in 30 µL total reaction volume containing 3 µL cDNA using standard cycling conditions: 50°C for 2 min, 95°C for 10 min, then 40 cycles at 95°C for 15 sec and 60°C for 60 sec.

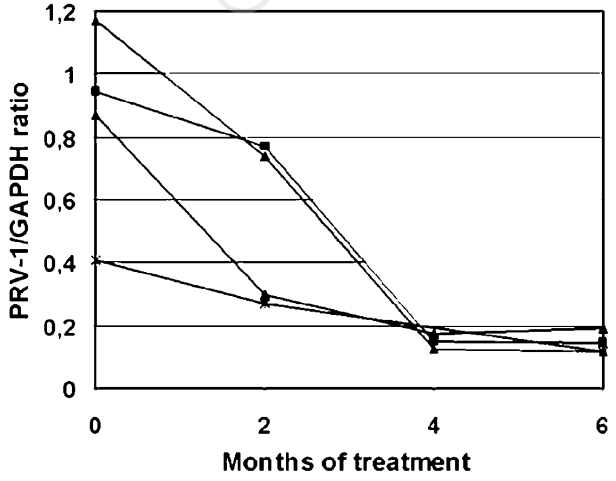

Figure 2. PRV-1/GAPDH ratio in granulocytes of 4 PV patients treated with IFN: the mean PRV-1 expression decreased by nearly six-fold six months after the start of IFN therapy.

er, prospective, controlled studies are warranted to investigate whether PRV-1 downregulation correlates with clinical response. If so, quantification of this surrogate marker would allow more rapid assessment of the potential of new therapeutic strategies for PV.

Stefan Fruehauf,† Julian Topaly,*† Matthias Villalobos,* Marlon R. Veldwijk,° Stephanie Laufs,° Anthony D. Ho**

*Both authors contributed equally to this work.

*Department of Internal Medicine V, University of Heidelberg, Heidelberg, Germany; °D0200, German Cancer Research Center, Heidelberg, Germany

Acknowledgments: we are grateful to S. Heil, B. Geisler, K. Ristow, and C. Hoppstock for technical assistance. B. Gentner helped in DNA sequencing. A. Benner provided valuable advice on the statistical analysis of the data.

Correspondence: Dr. Stefan Fruehauf , MD, Department of Internal Medicine V, University of Heidelberg, Hospitalstrasse 3, D-69115, Heidelberg, Germany. Phone: international +49.6221562781. Fax: international +49.6221562781. E-mail: stefan_fruehauf@med.uni-heidelberg.de

Manuscript processing

This manuscript was peer-reviewed by two external referees and by Professor Mario Cazzola, Editor-in-Chief. The final decision to accept this paper for publication was taken jointly by Professor Cazzola and the Editors. Manuscript received October 15, 2002; accepted February 5, 2003.

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Acute myeloid leukemia with recurring chromosome abnormalities as defined by the WHO-classification: incidence of subgroups, additional genetic abnormalities, FAB subtypes and age distribution in an unselected series of 1,897 patients with acute myeloid leukemia

The classification of acute myeloid leukemia (AML) has been based on cytomorphology and cytochemistry since the introduction of the FAB-classification in 1976.[1] In 1999 the WHO proposed a classification for tumors of hematopoietic and lymphoid tissues.[2,3]

Haematologica 2003;88:351-352
(*http://www.haematologica.org/2003_3/88351.htm*)

The classification incorporated morphologic, immunophenotypic, genetic and clinical features in order to define biologically homogenous entities which have clinical relevance. Thus, the WHO classification of AML encompasses four major categories: (i) AML with recurring genetic abnormalities, (ii) AML with multilineage dysplasia, (iii) AML, therapy-related and (iv) AML not otherwise categorized.

The first category includes the following subcategories: a) AML with t(8;21)(q22;q22);AML1/ETO, b) AML with abnormal bone marrow eosinophils inv(16)(p13q22) or t(16;16)(p13;q22);CBFB/MYH11, c) acute promyelocytic leukemia (AML with t(15;17)(q22;q12); PML-RARα and variants and d) AML with 11q23/MLL abnormalities. The aim of the current study was to characterize this category further using data from an unselected series of 1,897 patients with ALL, cytogenetically analyzed at diagnosis at our institution between 1996 and 2001. Molecular studies, using fluorescence *in situ* hybridization (FISH) and/or reverse transcriptase polymerase chain reaction (RT-PCR) were also performed, especially in cases with 11q23 abnormalities.

While published data on frequencies of chromosome aberrations are mostly derived from clinical trials which are often restricted to patients with *de novo* AML and those in a certain age range, our cohort included 1,632 cases of *de novo* AML, 148 cases of AML after an antecedent hematologic disorder and 117 therapy-related AML (cases with balanced translocations were included in the analysis of the International Workshop on t-AML).[4] The median age of the patients was 61 years (range 16-88). In total, 87 cases with t(8;21) (4.6%), 99 with t(15;17) (5.2%) (no alternative translocations involving RARα but not PML were included in this series), 87 with inv(16)/t(16;16) (4.6%), and 53 with 11q23/MLL rearrangement (2.8%) were observed. These cytogenetic subgroups were observed in 17.6% of *de novo* AML and in 31.6% of t-AML, but in none of 148 cases of AML occurring after an antecedent hematologic disorder. The incidences of MLL abnormalities, and of inv(16) were significantly higher in t-AML than in *de novo* AML (8.5% vs. 2.6%, p=0.0005; 11.1% vs. 4.5%, p=0.0016), respectively (Table 1).

All 87 cases with inv(16)/t(16;16) showed an AML M4eo FAB subtype. Seventy of the cases with t(15;17) had AML M3 while in 29 patients an AML M3v was diagnosed. In patients with t(8;21) 67 had AML M2, 5 had M1 and one had AML M4 (no data on FAB subtype was available for 14 patients). In AML with 11q23/MLL rearrangement AML M5a, M5b and M4 were the most common morphologies (present in 38.5%, 21.2% and 21.2%, respectively) but M0, M1 and M2 cases were also observed (in 1.9%, 7.7% and 9.6%, respectively). Therefore, AML M4eo with inv(16)/t(16;16)-CBFB-MYH11 is the only subtype showing a 100% correlation between genetics and a unique cytomorphologic picture. In AML with t(15;17)-PML-RARα, two distinct cytomorphologic subtypes