Soluble transferrin receptor in iron-deficient patients with and without anemia

Soluble transferrin receptor (TfR) identified iron deficiency in 86.8% of patients with Hb level <10 g/dL, in 68.8% of those with Hb between 10-13 g/dL, and in 52.4% of non-anemic iron-deficient patients. The diagnostic efficiency of soluble TfR was lower (28.6%) in non-anemic patients with chronic disease (CD) and similar in anemic patients regardless the presence of CD.

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The uptake of di-ferric transferrin is mediated by cellular transferrin receptor (TfR). The density of surface TfR is proportional to cellular iron requirement. The TfR shed from cells by proteolytic cleavage, a truncated form of the membrane-associated TfR, circulates in the blood as soluble transferrin receptor (sTfR).[1] Laboratory measurements of iron status have elicited two stages of iron deficiency: storage iron depletion and iron deficiency anemia. The greatest difficulty is in assessing subjects with absent iron stores but who have not yet developed overt anemia. Significant elevation of sTfR in patients with iron deficiency anemia has been a consistent finding reported by several authors.[2,3] However, evidence that sTfR can also identify iron depletion has been obtained in healthy subjects[4,5] but not in diseased populations.

The aim of the present study was to compare the ability of sTfR to identify iron deficiency in non-anemic and anemic patients with and without chronic disease (CD) attending an Internal Medicine Hospital in Mexico City.

From August 29 to November 16, 2001, serum ferritin (SF) levels were measured in 604 samples from inpatients and outpatients. One hundred and thirty-six patients were identified as iron-depleted (SF concentration <20 mg/L). Serum iron (SI), total iron binding capacity, and blood cell count were measured in iron-depleted patients. sTfR was measured in duplicate serum samples by a sandwich enzyme-linked immunosorbent assay (R&D Systems, Minneapolis, MN, USA). Based on our sTfR reference values and also those reported by Sandoval *et al.*[6] a value of 25.8 nmol/L was set as the upper reference limit. Iron-deficiency anemia was considered to be present when, besides of hypoferritinemia, transferrin saturation index (TS) was <15% and Hb concentration was <14.5 g/dL in males or <13.0 g/dL in females (reference values at 2240 meters above sea level).[7] Patients with low SF and TS values and normal Hb levels were considered to have non-anemic iron-deficiency. Of 136 iron-depleted patients, 10 were eliminated because their TS >15% and 3 had received blood transfusion. Of the 123 patients, 102 were classified as anemic and 21 as non-anemic. In the anemic group, 33 patients had CD (infectious, 4; inflammation, 17; malignant, 8; and miscellaneous, 4) while 7 of the non-anemic group did so (infectious, 3; malignant, 3; and miscellaneous, 1).

When both anemic and non-anemic patients were further analyzed according to the absence or presence of CD (Table 1), SI was found to be significantly lower in anemic patients without CD and sTfR was significantly higher in non-anemic subjects without CD. Eighty anemic patients (78.4%) and 11 non-anemic subjects (52.4%) had sTfR values above the cut-off level. In the anemic group the proportion of cases with elevated sTfR was similar in those with and without CD, whereas the proportion of cases with sTfR >25.8 nmol/L was lower in non-anemic subjects with CD (28.6%) than in those without CD (64.3%). In the anemic group, 38 patients had severe to moderate anemia (Hb<10.0 g/dL) and 64 cases had mild anemia (Hb between 10.0-13.0 g/dL). It was found that the percentage of cases with high sTfR levels increased to 86.8% in subjects with severe anemia while 68.8% of those with mild anemia showed elevated sTfR values.

Table 1. Mean+standard deviation of hematologic parameters in iron-deficient anemic and non-anemic patients with and without chronic disease (CD).

	Hb *g/dL*	*sTfR*[a] *nmol/L*	*SI*[b] *µg/dL*	*TIBC*[c] *µg/dL*	*% cases sTfR >25.8nmol/L*
Anemic (n=102)	10.4+1.68	44.6+25.45	25.1+12.47	397.0+77.11	78.4
with CD (n=33)	10.4+1.56	44.1+21.93	28.5+14.12[d]	405.3+83.65	81.8
without CD (n=69)	10.4+1.75	44.9+27.12	23.5+11.35	393.0+74.57	76.8
Non-anemic (n=21)	14.2+0.95	27.4+9.67	42.2+12.82	404.3+64.03	52.3
with CD (n=7)	14.0+1.00	21.4+5.34[d]	42.2+13.97	411.6+50.95	28.6
without CD (n=14)	14.3+0.93	30.5+10.07	42.2+12.95	401.1+70.41	64.3

a: soluble transferrin receptor; b: serum iron; c: total iron binding capacity; d: p value <0.05 (Wilcoxon-Mann-Whitney test) comparing 'with CD' vs 'without CD'.

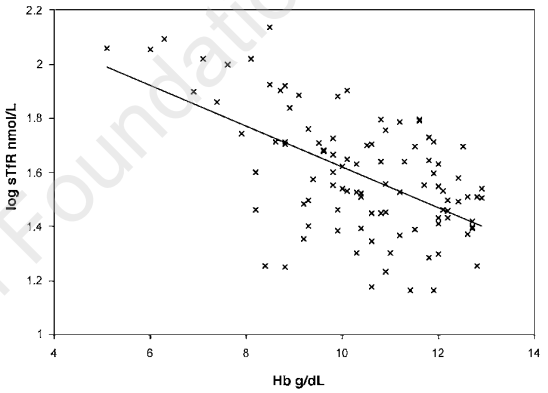

Figure 1. Correlation between Hb levels and logarithm of soluble transferrin receptor (sTfR) values in iron-deficient anemic patients.

A significant inverse linear correlation ($r = -0.5696$; $p<0.001$) between Hb concentration and logarithm of sTfR was obtained in the anemic group (Figure 1). In contrast, this correlation (r = -0.144) did not reach statistical significance in non-anemic patients.

In the current study the diagnostic efficiency of sTfR in detecting iron deficiency in anemic patients was 78.4%, a result similar to the 77.9% reported by Harthoornn-Lasthuizen.[8] As previously reported,[9] we found that the diagnostic efficiency of sTfR was similar in anemic patients with and without CD. However, in non-anemic subjects with CD sTfR detected only 28.6% of iron-deficient cases. Of interest was our finding that the diagnostic efficiency of sTfR increased to 86.8% in patients with more profound degrees of anemia (Hb<10 g/dL). These results are in line with data published by Ferguson *et al.*[10] The evidence that sTfR can also detect iron deficiency without anemia in healthy subjects submitted to repeated phlebotomies[4] and in iron-deficient non-anemic females receiving iron[5] are not in line with our data since sTfR detected iron deficiency in only 52.4% of non-anemic patients. Summarizing, measurement of sTfR is more efficient at identifying iron deficiency in anemic patients, regardless of the

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

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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.

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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-