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Research paper

Prognostic implications of low transferrin saturation in patients with primary myelofibrosis

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ABSTRACT

Objectives: Transferrin saturation (TSAT) 20% or less is considered to represent functional iron deficiency in the context of malignant disease, phenomenon mediated through inflammatory changes of iron homeostasis. We aimed to investigate clinical and prognostic significance of low TSAT in patients with primary (PMF) and secondary myelofibrosis (SMF), malignant diseases characterized by strong inflammatory milieu.
Methods: We retrospectively analyzed 87 patients with myelofibrosis and compared TSAT with disease specific parameters.
Results: One-third of patients had TSAT ≤20%. Lower TSAT was significantly associated with *Janus-kinase-2* (*JAK2*) mutation (P = 0.007), transfusion independency (P = 0.003), higher platelets (P = 0.004), lower mean-corpuscular-volume (P < 0.001), lower ferritin (P < 0.001), higher absolute-neutrophil-count (P = 0.027), lower absolute-lymphocyte-count (P = 0.041) and lower albumin (P = 0.018). PMF patients presenting with low TSAT (≤20%) experienced significantly shorter overall-survival (OS) (HR = 2.43; P = 0.017), whereas TSAT did not affect OS of SMF patients (HR = 1.48; P = 0.623). Low TSAT remained significantly associated with inferior OS in PMF in a series of multivariate Cox regression models comparing its properties to anemia, transfusion dependency, ferritin and Dynamic-International-Prognostic-System (DIPSS).
Conclusions: Low TSAT has detrimental effect on survival of PMF patients. This effect is independent of anemia and of ferritin levels that seem to be better at representing iron overload in PMF patients.

1. Introduction

Philadelphia chromosome negative myeloproliferative neoplasms (Ph- MPNs) [1] are malignant diseases developing from clonally transformed hematopoietic stem cell [2]. They have classically been divided into primary myelofibrosis (PMF), polycythemia vera (PV) and essential thrombocytosis (ET). A majority of Ph- MPN patients bear a mutation in either of *Janus-kinase-2* (*JAK2*), *Calreticulin* (*CALR*) or *Myeloproliferative-leukemia-virus-oncogene* (*MPL*) genes [3] resulting in constitutive activation of JAK/signal-transducer-and-activator-of-transcription (STAT) signaling pathway, strong myeloproliferation and high inflammatory atmosphere characteristic for these diseases [4]. PMF is the most aggressive among Ph- MPNs and bears the highest risk of transformation to acute leukemia [5] and death [6]. It is characterized by development of bone marrow fibrosis, varying number and degree of myeloid lineage cytopenias, prominent constitutional symptoms, and progressive hepatosplenomegaly due to activation of extramedullary hematopoiesis. PV and ET can develop substantial degree of bone marrow fibrosis and PMF-related features during disease course when these conditions are termed secondary myelofibrosis (SMF) [7]. Although similar in clinical presentation and prognosis, PMF and post-PV/post-ET SMF still harbor different molecular backgrounds that resemble diseases of origin [8]. The risk of death in myelofibrosis patients can be assessed using the International Prognostic Scoring System (IPSS) at the time of diagnosis [9], or the Dynamic International Prognostic Scoring System (DIPSS) during course of the disease [10]. Both prognostic systems assign points for older age, higher white blood cell (WBC) count, lower hemoglobin level, presence of circulatory blasts

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and presence of constitutional symptoms; higher score indicates worse prognosis. Transfusion dependency, thrombocytopenia and unfavorable karyotype are additionally included as negative prognostic factors into DIPSS-plus prognostic model [11].

Regulation of iron metabolism is disturbed in Ph- MPN patients, due to dysregulated inflammatory cytokine expression, iron overload introduced through red blood cell (RBC) transfusions (in myelofibrosis) and iron depletion due to phlebotomies (in PV). Hepcidin, a central regulator of iron metabolism, is overproduced in PMF patients and both high ferritin and high hepcidin levels were shown to independently predict inferior survival in this disease [12]. In contrast, PV and ET patients were reported to have similar hepcidin levels as healthy controls [13]. Hepcidin can be upregulated by excess iron, lower erythropoietic activity and variety of inflammatory and microbial stimuli [14]. It binds to iron exporting channel ferroportin, which leads to its internalization and degradation, and thereby prevents iron from being released from enterocytes and macrophages. This in turn blocks dietary iron absorption and release of iron from its stores, as well as reduces bioavailability of iron through lowering transferrin saturation (TSAT) [15]. In the context of chronic inflammation/malignant disease in general, TSAT of 20% or less is considered to represent functional iron deficiency, despite normal or elevated ferritin values [16]. This phenomenon of iron deprivation results in iron-restricted erythropoiesis and usually manifests with development or worsening of pre-existing anemia. Low TSAT, microcytosis and anemia are relatively frequent findings among myelofibrosis patients [17,18]. Clinical and prognostic significance of low TSAT have not been investigated in this population so far.

2. Theory

We hypothesized that low TSAT could potentiate development of anemia in myelofibrosis. Therefore, we aimed to investigate TSAT in a population of PMF and SMF patients, to assess its clinical associations and potential prognostic value.

3. Patients and methods

3.1. Patients

We retrospectively analyzed a total of 87 patients with myelofibrosis, 64 patients with PMF and 23 patients with SMF (15 post-PV, 9 post-ET), that were treated and followed in our institution in period from 2004 to 2017. All patients fulfilled the World Health Organization (WHO) 2016 criteria for the diagnosis of PMF [1] and the International Working Group for Myelofibrosis Research and Treatment (IWG-MRT) criteria for the diagnosis of SMF [7]. A total of 72/87 (82.8%) patients were evaluated at the time of diagnosis and 15/87 (17.2%) were evaluated at the time of referral to our institution. None of the patients received iron supplementation regardless of iron metabolism parameters. All patients provided a written informed consent for the molecular analyses. The study was approved by the Institutional Review Board.

3.2. Methods

Patients were staged according to the DIPSS [10]. Bone marrow fibrosis was graded according to the current European consensus [19]. Liver and spleen size were assessed by palpation. TSAT, ferritin and mean corpuscular volume (MCV) were assessed in addition to other demographic, hematological and clinical parameters (age, gender, WBC count, differential blood count, circulatory blasts, hemoglobin level, red cell distribution width (RDW), platelet count, C reactive protein (CRP) level, lactate dehydrogenase (LDH) level, presence of constitutional symptoms, blast phase disease, transfusion dependency, *JAK2*, *CALR* and *MPL* mutational status).

3.3. Molecular analyses

For molecular analyses, DNA was isolated from full blood by QIAamp DNA Blood Mini Kit (Qiagen, ID 51104). *JAK2* V617F was assessed by allele-specific PCR as described previously [20], *CALR1* and *MPL* exon 10 mutations were screened by high–resolution melting dye assays [21,22] and any sample sequence that deviated from normal was Sanger sequenced.

3.4. Statistical analyses

The normality of data distribution was tested using the Shapiro-Wilk test. Numerical variables were presented as median and interquartile range (IQR), or as arithmetic mean ± standard deviation depending on the normality of distribution. Categorical variables were presented as proportions. The Mann Whitney *U* test/the *t*-test, the $\chi 2$ (Chi squared) test and the Spearman rank correlation were used where appropriate. Survival analyses were performed using the methods of Kaplan and Meier, the Cox-Mantel version of the log-rank test [23] and the Cox regression analysis. ROC curve analysis with survival status as a classification variable was used for finding an optimal cut-off value for numerical variables for the purpose of survival analyses. P values < 0.05 were considered statistically significant. Associations of different prognostic factors with survival were screened for using a custom made MS Excel workbook [24]. Analyses were performed using MedCalc Statistical Software version 17.6 (MedCalc Software BVBA, Ostend, Belgium).

4. Results

4.1. Transferrin saturation in myelofibrosis patients

There were total of 87 patients with myelofibrosis, 64 with PMF and 23 with SMF. Median age was 67 years, IQR (59–73), a majority [54/87 (62.1%)] of patients were males. Patients' characteristics are shown in Table 1.

Median TSAT was 24.5%, IQR (13.8%–33.8%) and it did not significantly differ between PMF and SMF patients [median 25.3% vs 23.5%; P = 0.672 (Fig. 1A)]. A total of 28/87 (32.2%) of all patients and 24/72 (33.3%) of newly diagnosed patients presented with TSAT 20% or lower which did not differ between PMF and SMF patients as well (P = 0.834), thereby showing that functional iron deficiency is present in a substantial proportion (one third) of myelofibrosis patients.

4.2. Clinical associations

In a cohort of all 87 myelofibrosis patients, lower TSAT as a continuous variable was statistically significantly associated with *JAK2* mutation [median TSAT 22.2% vs 32.0% for *JAK2* mutated and wild type patients; P = 0.007 (Fig. 1C)], transfusion independency [median TSAT 23.2% vs 32.7% for transfusion independent and dependent patients; P = 0.003 (Fig. 1D)], higher platelets (Rho −0.31; P = 0.004), lower MCV (Rho 0.4; P < 0.001), lower ferritin (Rho 0.72; P < 0.001) and higher absolute neutrophil count (Rho −0.25; P = 0.027). Patients with low TSAT as a categorical variable additionally had significantly lower albumin (median albumin 42 g/L vs 45 g/L; P = 0.018) and lower absolute lymphocyte count (median ALC 1.15×10^9/L vs 1.5×10^9/L; P = 0.041). There was no statistically significant association of TSAT with hemoglobin level (Rho −0.19; P = 0.083), degree of bone marrow fibrosis (Rho 0.03; P = 0.818), DIPSS risk category [Rho 0.16; P = 0.144 (Fig. 1B)] or other measured variables.

In a sub-cohort of 64 PMF patients, lower TSAT remained similarly significantly associated with *JAK2* mutation (median TSAT 23.0% vs 32.5% for *JAK2* mutated and wild type patients; P = 0.015), transfusion independency (median TSAT 23.0% vs 33.0% for transfusion independent and dependent patients P = 0.003), higher platelets (Rho