Središnja medicinska knjižnica

Lucijanić M., Prka Ž., Pejša V., Štoos-Veić T., Lucijanić J., Kušec R.
(2018) *Prognostic implications of low transferrin saturation in patients with primary myelofibrosis.* Leukemia Research, 66. pp. 89-95. ISSN 0145-2126

http://medlib.mef.hr/2899

Title: Prognostic implications of low transferrin saturation in patients with primary myelofibrosis

Authors:

Marko Lucijanic [a], Zeljko Prka [b], Vlatko Pejsa [a, b], Tajana Stoos-Veic [c, d], Jelena Lucijanic [e], Rajko Kusec [a, b, f]

Affiliations:

[a] Hematology Department, University Hospital Dubrava, Av. Gojka Suska 6, 10000 Zagreb, Croatia

[b] School of Medicine, University of Zagreb, Salata 3, 10000 Zagreb, Croatia

[c] Department of Clinical Cytology and Cytometry, University Hospital Dubrava, Av. Gojka Suska 6, 10000 Zagreb, Croatia

[d] Faculty of Medicine, University of Osijek, Ul. Josipa Huttlera 4, 31000 Osijek, Croatia

[e] Health Care Center Zagreb-West, Prilaz baruna Filipovica 11, 10000 Zagreb, Croatia

[f] Divison of Molecular Diagnosis and Genetics, Clinical Department of Laboratory Diagnostics, University Hospital Dubrava, Av. Gojka Suska 6, 10000 Zagreb, Croatia

Corresponding author: Marko Lucijanic, MD PhD, Hematology Department, University hospital Dubrava, Av. Gojka Suska 6, 10000 Zagreb. Tel: +38512902444 Email: markolucijanic@yahoo.com

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.

Keywords: Philadelphia chromosome negative myeloproliferative neoplasm; Primary myelofibrosis; Survival; Iron metabolism, Transferrin saturation; Functional iron deficiency

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 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.

4

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 χ2 (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 (Figure 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 (Figure 1C)], transfusion independency [median TSAT 23.2% vs 32.7% for transfusion independent and dependent patients; P=0.003 (Figure 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 x10^9/L vs 1.5 x10^9/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 (Figure 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 -0.34; P=0.006), lower MCV (Rho 0.36; P=0.004), lower ferritin (Rho 0.76; P<0.001), lower albumin (median albumin 41 g/L vs 45 g/L; P=0.015) and lower absolute lymphocyte count (Rho 0.29; P=0.025). There was no clear association with hemoglobin level (Rho -0.18; P=0.149), degree of bone marrow fibrosis (Rho -0.02; P=0.869), DIPSS risk category (Rho 0.1; P=0.449) or other tested variables.

4.3. *Parameters related to iron metabolism and survival*

Although PMF and SMF patients did not differ in overall survival (P=0.362) and TSAT (P=0.672), patients with PMF presenting with low TSAT (20% or lower) experienced statistically significantly shorter overall survival than PMF patients with TSAT >20% [HR=2.43; P=0.017 (Figure 2A)], whereas low TSAT showed no statistically significant association with OS in SMF patients [HR=1.48; P=0.623 (Figure 2B)]. PMF patients with TSAT ≤10% and TSAT between 10 and 20% experienced similar survival (P=0.822), that was worse than in well-saturated patients as said previously. Microcytosis defined as MCV <82 fL showed no association with survival in neither of diseases [HR=1.06; P=0.904 for PMF (Figure 2C) and HR=2.65; P=0.333 for SMF (Figure 2D)], whereas both PMF and SMF patients with ferritin values >686 μg/L [HR=4.49; P=0.003 for PMF (Figure 2E) and HR=6.58; P=0.005 for SMF (Figure 2F)] had significantly inferior overall survival when

compared to non-iron overloaded patients (optimal cut-off value defined using ROC curve analysis). Thus, iron overload bears negative prognostic implications in both diseases, but iron deprivation does not seem to have same detrimental effect on survival in SMF as in PMF. This implies that substantial differences in iron homeostasis between these two diseases could exist.

We further tested association of low TSAT with inferior overall survival in PMF patients in a series of age and gender adjusted multivariate Cox regression models. Due to the implications of iron homeostasis on quality of erythropoiesis, we investigated whether effects of low TSAT on survival could be mediated through consequences of anemia that is a known negative prognostic factor in PMF. Low TSAT and low hemoglobin both retained prognostic significance and predicted inferior survival independently of each other in the Cox regression model containing low TSAT (HR=4.86; P=0.001), hemoglobin <100 g/L, (HR=11.99; P<0.001), age (HR 0.99; P=0.918) and male gender (HR=1.89; P=0.224). This observation is in line with lack of significant association of these two parameters that we observed in our cohort of PMF patients.

We also investigated prognostic properties of low TSAT in comparison to transfusion dependency with which it was significantly associated, and which is a known negative parameter in PMF patients. Both low TSAT and transfusion dependency predicted inferior survival independently of each other in the Cox regression model containing low TSAT (HR=2.87; P=0.024), transfusion dependency (HR=3.09; P=0.039), age (HR=1.03; P=0.303) and male gender (HR=2.03; P=0.190). This is not surprising since low TSAT patients were more likely to be transfusion independent.

We further compared prognostic properties of low TSAT with ferritin values <100 µg/L and observed they were independently associated with inferior survival, but in opposite directions

in the Cox regression model containing low TSAT (HR=3.62; P=0.020), ferritin <100 µg/L (HR=0.21; P=0.019), age (HR=1.0; P=0.932) and male gender (HR=1.25; P=0.702). Therefore, low ferritin levels do not seem to bear same prognostic information as low TSAT, and ferritin might be better at representing negative effects of iron overload that is probably affecting its prognostic properties even at lower cut-off levels. In line with this assumption are the optimal cut-off values that were obtained by ROC curve analysis in our patient cohort for these two parameters: TSAT at ≤20% and ferritin at >686 µg/L were able to best discriminate patients with inferior overall survival in univariate analyses. We further tested how do prognostic properties of TSAT and ferritin compare in a subset of transfusion independent patients and found that low TSAT (HR=4.15, P=0.047) remained significantly associated with inferior survival, while effect of ferritin <100 µg/L was nearly statistically significant (HR=0.22, P=0.061) suggesting that described relationships hold even without influx of external iron through RBC transfusions.

We analyzed whether there is an overlap in prognostic properties of different iron metabolism related parameters. In the Cox regression model including low TSAT (HR=3.48; P=0.038), ferritin >686 µg/L (HR=9.29; P=0.005) and transfusion dependency (HR=1.39; P=0.578), only low TSAT and high ferritin remained statistically significantly associated with shorter survival. After additionally including anemia in the model, now comparing low TSAT (HR=8.16; P=0.003), hemoglobin <100 g/L (HR 21.47; P=0.002), ferritin >686 µg/L (HR=9.32; P=0.008) and transfusion dependency (HR=0.52; P=0.287), we observed that low TSAT remained significantly associated with shorter survival and predicted worse prognosis independently of anemia and high ferritin levels. Univariate effect of transfusion dependency was probably mediated through iron overload and was lost after controlling for other parameters. Same associations with survival were retained after additionally adjusting model for age and gender, Table 2.

In addition, we compared negative prognostic significance of low TSAT to DIPSS prognostic scoring system. Low TSAT predicted shorter survival when compared to each of the components of DIPSS score individually, as well as when analyzed in the Cox regression model containing low TSAT (HR=14.07; P<0.001), age >65 years (HR=0.71; P=0.399), constitutional symptoms (HR=4.33; P=0.037), WBC >25 x109/L (HR=13.51; P<0.001), >1% circulatory blasts (HR=0.01; P=0.917), hemoglobin <100 g/L (HR=20.07; P<0.001) and male gender (HR=1.54; P=0.214). Low TSAT (HR=4.36; P=0.002) remained statistically significant when compared to DIPSS itself (HR=4.74; P<0.001), as well.

5. Discussion:

To the best of our knowledge, our study is first to report that 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.

Our data show that one third of myelofibrosis patients experience low TSAT. These patients are more likely to be transfusion independent, *JAK2* mutated and to have higher platelets, hence usually not considered to be under elevated risk of death. There was similar distribution of TSAT values over different DIPSS risk categories and low TSAT predicted inferior survival independently of DIPSS, further implying that patients with low TSAT remain unrecognized through standard process or risk stratification.

TSAT and ferritin levels were strongly correlated and patients with low TSAT had reduced iron stores (median ferritin 49.5 µg/L vs 309 µg/L). Ferritin is of limited value in estimating iron deficiency status in PMF as it forms a part of inflammatory response and can be artificially elevated. As our data suggest, lower ferritin is protective in PMF, even at low cut-off points and might be more sensitive to detect adverse effects of iron overload, than iron

11

deficiency. This effect of ferritin on survival is overlapping with transfusion dependency, but it is possible, although not clear at the moment, that adverse effect of higher ferritin holds even in transfusion independent patients. Our study was underpowered to properly investigate this issue. Evaluation of bone marrow iron stores is also of limited usefulness as it was shown that up to 75% of PMF patients have diminished or absent iron deposits but this finding was not associated with decreased ferritin levels [25]. This phenomenon could be due to extramedullary hematopoiesis in the spleen that dominates iron uptake, and thus restrains iron supply to the bone marrow [26]. Due to aforementioned problems, some authors proposed low TSAT as a parameter of choice to evaluate iron deficiency in PMF [17]. Low TSAT could be a consequence of absolute or functional iron deficiency, but this is challenging to discriminate. Either way, low TSAT patients surely suffer from reduced iron bioavailability. Functional iron deficiency in anemia of chronic disease develops due to elevated hepcidin that impairs iron utilization from its stores [16]. Abnormally high levels of hepcidin were reported in a population of PMF patients [12] and are probably contributing to low TSAT. Aforementioned study did not assess correlation between hepcidin and TSAT, but reported correlation between high hepcidin and high ferritin levels which could predict inferior survival independently of each other. In contrast to PMF, hepcidin levels in PV and ET were reported to be similar to healthy controls [13] illustrating that significant differences in iron homeostasis between different Ph- MPNs exist. Our study suggests that patients with SMF, although experiencing similar levels of TSAT, do not have same adverse effect of low TSAT on survival as PMF patients. Our SMF cohort consisted mostly of post-PV SMF patients. Therapeutic phlebotomies are the mainstay of PV treatment and they result in hematocrit reduction and depletion of iron stores [27]. Phlebotomies improve survival in PV, but it is not clear how do they reflect on the biology of leukemic stem cell. It could be however, that positive iron deprivation associated effects are retained even after disease transformation into

myelofibrosis. One should be aware that regulation of iron stores, especially in the context of inflammation, is incompletely understood process and discordant results regarding prognostic value of reduced/increased iron availability are present in the literature. Increased TSAT was reported to predict higher mortality in critically ill patients [28] and to be associated with higher risk of cancer death [29]. On the opposite, low TSAT was reported to be associated with increased cardiovascular and all cause mortality [30], as well as with more advanced disease features and poor performance status in cancer patients [31]. To add to the confusion, one study reported that both low and high TSAT were associated with increased total and cardiovascular mortality, hazards for low TSAT being more pronounced [32]. These conflicting data probably reflect changes in iron regulation specific to different clinical settings and different studied populations, and emphasize the fact that there are two edges of the "iron sword".

We hypothesized that low TSAT could potentiate development of anemia in myelofibrosis. Interestingly, low TSAT was observed regardless of anemia severity and was not correlated with hemoglobin level as expected. It should be noted that factors like transfusion dependency and *JAK2* mutation might disguise relationship between TSAT and hemoglobin. RBC transfusions are given in severe anemia and result in an increase of TSAT due to the introduction of excess iron. *JAK2* mutated patients have stronger proliferative potential of disease with significantly higher hemoglobin and absolute neutrophil count (data not shown), but also lower TSAT. This might be due to increased iron utilization associated with increased erythropoiesis or due to changes in inflammatory profile associated with *JAK2* mutation. Nevertheless, TSAT affected quality of erythropoiesis, as observed by decrease in MCV associated with lower TSAT values. Two author groups previously investigated properties of microcytosis in a population of PMF patients [17, 18]. They found that although microcytosis was relatively frequent (28% and 24% of untreated patients), it had no

association with overall survival. It was also reported that microcytosis showed significant association with low TSAT, both univariately and after adjustment for other correlated factors, but had no association with anemia [17]. Our results are in line with these findings. Oppositely to microcytosis, higher heterogeneity of erythrocyte size (i.e. anisocytosis, measured as RDW) seems to be associated with anemia and predictive of inferior survival, but not correlated with iron metabolism parameters in PMF [33]. These findings indicate that MCV and RDW, although useful for recognition of iron deficiency anemia in general population, might be suboptimal for this purpose in PMF. Patients with low TSAT were also more likely to have lower absolute lymphocyte count and lower albumin concentration. These could indicate worse nutritional status or higher inflammatory atmosphere and were shown to be predictive of inferior survival in myelofibrosis independently of each other and DIPSS [34]. Thus, numerous pathophysiologic processes modulate TSAT and are reflected in its reduction.

Considering information presented above, challenging questions about possible therapeutic options emerge. First, would PMF patients with low TSAT benefit from iron supplementation? Due to dangers associated with iron overload [35] (which are evident from our data as well), it is hard to identify who would be candidates for such therapy, but these would probably need to be transfusion independent patients. Intravenous iron might overcome hepcidin mediated iron restriction and improves erythropoiesis in a context of anemia in different malignancies [16]. TSAT had no association with hemoglobin level (it was predictive of inferior survival on different levels of anemia), but was associated with microcytosis indicating that link with quality of erythropoiesis in PMF exists. Therefore, low TSAT, but non transfusion dependent patients with stable disease seem to be the population of interest. Second, would PMF patients with low TSAT benefit from therapies that increase iron utilization? Compounds that inhibit expression or effects of hepcidin seem to be

promising in the context of anemia associated with chronic disease [36]. As mentioned previously, same concerns with possible development of iron overload in context of myelofibrosis and such drugs would exist. Ruxolitinib, JAK inhibitor that already found its place in treatment of myelofibrosis, was reported to improve iron metabolism indices in a population of PV patients [37]. This analysis was exploratory, only descriptive statistics were used and it is yet unclear if these improvements are associated with clinical benefit in PV. It would be interesting to see if similar associations are present in patients with myelofibrosis and how do potential changes in iron indices translate into clinical outcomes.

Main limitations of our study are retrospective study design, small number of patients, single center experience and unavailable data on hepcidin. We were unable to assess various mechanisms that potentially modulate TSAT on different biological levels (especially the role of pro-inflammatory environment in the bone marrow and the spleen). We also acknowledge that our study could be underpowered to detect statistical significance of some associations that we did investigate. Nevertheless, our findings implicate there is a substantial subset of PMF patients that might experience shorter survival associated with low TSAT. It would be of interest to further investigate relationship between hepcidin expression, ferritin and TSAT and potential overlap of their prognostic properties in populations of transfusion dependent and independent myelofibrosis patients.

6. Conclusion

One third of myelofibrosis patients experience low TSAT. Both iron deprivation (low TSAT), anemia and iron overload (high ferritin) seem to be independently hazardous for PMF patients, whereas low TSAT seems not to affect survival in SMF. It remains a challenging

question whether there is a subset of PMF patients that would benefit from iron supplementation therapy.

Conflict of interest: none

7. References:

[1] D.A. Arber, A. Orazi, R. Hasserjian, J. Thiele, M.J. Borowitz, M.M. Le Beau, C.D. Bloomfield, M. Cazzola, J.W. Vardiman, The 2016 revision to the World Health Organization classification of myeloid neoplasms and acute leukemia, Blood 127(20) (2016) 2391-405.

[2] M. Buschle, J.W. Janssen, H. Drexler, J. Lyons, B. Anger, C.R. Bartram, Evidence for pluripotent stem cell origin of idiopathic myelofibrosis: clonal analysis of a case characterized by a N-ras gene mutation, Leukemia 2(10) (1988) 658-60.

[3] T. Sliwa, C. Beham-Schmid, S. Burgstaller, V. Buxhofer-Ausch, G. Gastl, K. Geissler, M. Krauth, P. Krippl, A. Lang, A. Petzer, S. Wohrer, A. Wolfler, H. Gisslinger, Austrian recommendations for the management of primary myelofibrosis, post-polycythemia vera myelofibrosis and post-essential thrombocythemia myelofibrosis: an expert statement, Wiener klinische Wochenschrift 129(9-10) (2017) 293-302.

[4] H.C. Hasselbalch, Perspectives on chronic inflammation in essential thrombocythemia, polycythemia vera, and myelofibrosis: is chronic inflammation a trigger and driver of clonal evolution and development of accelerated atherosclerosis and second cancer?, Blood 119(14) (2012) 3219-25.

[5] M. Yogarajah, A. Tefferi, Leukemic Transformation in Myeloproliferative Neoplasms: A Literature Review on Risk, Characteristics, and Outcome, Mayo Clinic proceedings 92(7) (2017) 1118-1128.

[6] M. Hultcrantz, S.Y. Kristinsson, T.M. Andersson, O. Landgren, S. Eloranta, A.R. Derolf, P.W. Dickman, M. Bjorkholm, Patterns of survival among patients with myeloproliferative

neoplasms diagnosed in Sweden from 1973 to 2008: a population-based study, Journal of clinical oncology : official journal of the American Society of Clinical Oncology 30(24) (2012) 2995-3001.

[7] G. Barosi, R.A. Mesa, J. Thiele, F. Cervantes, P.J. Campbell, S. Verstovsek, B. Dupriez, R.L. Levine, F. Passamonti, J. Gotlib, J.T. Reilly, A.M. Vannucchi, C.A. Hanson, L.A. Solberg, A. Orazi, A. Tefferi, R. International Working Group for Myelofibrosis, Treatment, Proposed criteria for the diagnosis of post-polycythemia vera and post-essential thrombocythemia myelofibrosis: a consensus statement from the International Working Group for Myelofibrosis Research and Treatment, Leukemia 22(2) (2008) 437-8.

[8] M. Brecqueville, J. Rey, R. Devillier, A. Guille, R. Gillet, J. Adelaide, V. Gelsi-Boyer, C. Arnoulet, M. Chaffanet, M.J. Mozziconacci, N. Vey, D. Birnbaum, A. Murati, Array comparative genomic hybridization and sequencing of 23 genes in 80 patients with myelofibrosis at chronic or acute phase, Haematologica 99(1) (2014) 37-45.

[9] F. Cervantes, B. Dupriez, A. Pereira, F. Passamonti, J.T. Reilly, E. Morra, A.M. Vannucchi, R.A. Mesa, J.L. Demory, G. Barosi, E. Rumi, A. Tefferi, New prognostic scoring system for primary myelofibrosis based on a study of the International Working Group for Myelofibrosis Research and Treatment, Blood 113(13) (2009) 2895-901.

[10] F. Passamonti, F. Cervantes, A.M. Vannucchi, E. Morra, E. Rumi, A. Pereira, P. Guglielmelli, E. Pungolino, M. Caramella, M. Maffioli, C. Pascutto, M. Lazzarino, M. Cazzola, A. Tefferi, A dynamic prognostic model to predict survival in primary myelofibrosis: a study by the IWG-MRT (International Working Group for Myeloproliferative Neoplasms Research and Treatment), Blood 115(9) (2010) 1703-8.

[11] N. Gangat, D. Caramazza, R. Vaidya, G. George, K. Begna, S. Schwager, D. Van Dyke, C. Hanson, W. Wu, A. Pardanani, F. Cervantes, F. Passamonti, A. Tefferi, DIPSS plus: a refined Dynamic International Prognostic Scoring System for primary myelofibrosis that incorporates prognostic information from karyotype, platelet count, and transfusion status, Journal of clinical oncology : official journal of the American Society of Clinical Oncology 29(4) (2011) 392-7.

[12] A. Pardanani, C. Finke, R.A. Abdelrahman, T.L. Lasho, A. Tefferi, Associations and prognostic interactions between circulating levels of hepcidin, ferritin and inflammatory cytokines in primary myelofibrosis, American journal of hematology 88(4) (2013) 312-6.

[13] P. Tarkun, O. Mehtap, E.B. Atesoglu, A. Geduk, M.M. Musul, A. Hacihanefioglu, Serum hepcidin and growth differentiation factor-15 (GDF-15) levels in polycythemia vera and essential thrombocythemia, European journal of haematology 91(3) (2013) 228-35.

[14] E. Nemeth, Targeting the hepcidin-ferroportin axis in the diagnosis and treatment of anemias, Advances in hematology 2010 (2010) 750643.

[15] L.T. Goodnough, E. Nemeth, T. Ganz, Detection, evaluation, and management of iron-restricted erythropoiesis, Blood 116(23) (2010) 4754-61.

[16] F.A. Naoum, Iron deficiency in cancer patients, Revista brasileira de hematologia e hemoterapia 38(4) (2016) 325-330.

[17] P. Strati, N. Pemmaraju, Z. Estrov, M. Cardenas-Turanzas, S. Pierce, K.J. Newberry, N. Daver, J. Cortes, H. Kantarjian, S. Verstovsek, Clinical significance of microcytosis in patients with primary myelofibrosis, Leukemia research 38(10) (2014) 1212-6.

[18] A. Tefferi, D. Dingli, C.Y. Li, R.A. Mesa, Microcytosis in agnogenic myeloid metaplasia: prevalence and clinical correlates, Leukemia research 30(6) (2006) 677-80.

[19] J. Thiele, H.M. Kvasnicka, F. Facchetti, V. Franco, J. van der Walt, A. Orazi, European consensus on grading bone marrow fibrosis and assessment of cellularity, Haematologica 90(8) (2005) 1128-32.

[20] E.J. Baxter, L.M. Scott, P.J. Campbell, C. East, N. Fourouclas, S. Swanton, G.S. Vassiliou, A.J. Bench, E.M. Boyd, N. Curtin, M.A. Scott, W.N. Erber, A.R. Green, P. Cancer Genome, Acquired mutation of the tyrosine kinase JAK2 in human myeloproliferative disorders, Lancet 365(9464) (2005) 1054-61.

[21] C. Bilbao-Sieyro, G. Santana, M. Moreno, L. Torres, G. Santana-Lopez, C. Rodriguez-Medina, M. Perera, B. Bellosillo, S. de la Iglesia, T. Molero, M.T. Gomez-Casares, High resolution melting analysis: a rapid and accurate method to detect CALR mutations, PloS one 9(7) (2014) e103511.

[22] A. Pardanani, P. Guglielmelli, T.L. Lasho, A. Pancrazzi, C.M. Finke, A.M. Vannucchi, A. Tefferi, Primary myelofibrosis with or without mutant MPL: comparison of survival and clinical features involving 603 patients, Leukemia 25(12) (2011) 1834-9.

[23] M. Lucijanic, M. Skelin, T. Lucijanic, Survival analysis, more than meets the eye, Biochemia medica 27(1) (2017) 14-18.

[24] M. Lucijanic, Survival analysis in clinical practice: analyze your own data using an Excel workbook, Croatian medical journal 57(1) (2016) 77-9.

[25] F. Cervantes, A. Lopez-Guillermo, C. Piera, A. Pereira, J.L. Aguilar, J. Ordi, C. Rozman, [Initial iron deposits in idiopathic myelofibrosis. Analysis of 20 patients], Sangre 38(4) (1993) 279-82.

[26] A. Ferrant, J. Rodhain, F. Cauwe, M. Cogneau, C. Beckers, J.L. Michaux, R. Verwilghen, G. Sokal, Assessment of bone marrow and splenic erythropoiesis in myelofibrosis, Scandinavian journal of haematology 29(5) (1982) 373-80.

[27] T.B. Assi, E. Baz, Current applications of therapeutic phlebotomy, Blood transfusion = Trasfusione del sangue 12 Suppl 1 (2014) s75-83.

[28] F. Tacke, R. Nuraldeen, A. Koch, K. Strathmann, G. Hutschenreuter, C. Trautwein, P. Strnad, Iron Parameters Determine the Prognosis of Critically Ill Patients, Critical care medicine 44(6) (2016) 1049-58.

[29] A.C. Chua, M.W. Knuiman, D. Trinder, M.L. Divitini, J.K. Olynyk, Higher concentrations of serum iron and transferrin saturation but not serum ferritin are associated with cancer outcomes, The American journal of clinical nutrition 104(3) (2016) 736-42.

[30] K.-S. Kim, H.-G. Son, N.-S. Hong, D.-H. Lee, Associations of Serum Ferritin and Transferrin % Saturation With All-cause, Cancer, and Cardiovascular Disease Mortality: Third National Health and Nutrition Examination Survey Follow-up Study, Journal of Preventive Medicine and Public Health 45(3) (2012) 196-203.

[31] H. Ludwig, E. Muldur, G. Endler, W. Hubl, Prevalence of iron deficiency across different tumors and its association with poor performance status, disease status and anemia, Annals of oncology : official journal of the European Society for Medical Oncology / ESMO 24(7) (2013) 1886-92.

[32] A.G. Stack, A.I. Mutwali, H.T. Nguyen, C.J. Cronin, L.F. Casserly, J. Ferguson, Transferrin saturation ratio and risk of total and cardiovascular mortality in the general population, QJM: An International Journal of Medicine 107(8) (2014) 623-633.

[33] M. Lucijanic, V. Pejsa, O. Jaksic, Z. Mitrovic, C. Tomasovic-Loncaric, T. Stoos-Veic, Z. Prka, M. Pirsic, V. Haris, T. Vasilj, R. Kusec, The Degree of Anisocytosis Predicts Survival in Patients with Primary Myelofibrosis, Acta haematologica 136(2) (2016) 98-100.

[34] M. Lucijanic, I. Veletic, D. Rahelic, V. Pejsa, D. Cicic, M. Skelin, A. Livun, K.M. Tupek, T. Stoos-Veic, T. Lucijanic, A. Maglicic, R. Kusec, Assessing serum albumin concentration, lymphocyte count and Prognostic Nutritional Index might improve prognostication in patients with myelofibrosis, Wiener klinische Wochenschrift (2018).

[35] N. Carreau, D. Tremblay, M. Savona, M. Kremyanskaya, J. Mascarenhas, Ironing out the details of iron overload in myelofibrosis: Lessons from myelodysplastic syndromes, Blood reviews 30(5) (2016) 349-56.

[36] J.M. Lopez-Gomez, S. Abad, A. Vega, New expectations in the treatment of anemia in chronic kidney disease, Nefrologia : publicacion oficial de la Sociedad Espanola Nefrologia 36(3) (2016) 232-6.

[37] S. Verstovsek, C.N. Harrison, J.J. Kiladjian, C. Miller, A.B. Naim, D.C. Paranagama, D. Habr, A.M. Vannucchi, Markers of iron deficiency in patients with polycythemia vera receiving ruxolitinib or best available therapy, Leukemia research 56 (2017) 52-59.

Table 1: Patients' characteristics (all myelofibrosis patients). * statistically significant at P<0.05. ** statistically significant at P<0.05 when TSAT is used as a continuous variable.

	All patients	Transferrin sat. ≤20%	Transferrin sat. >20%	P value
Number of patients	87	28 (32.2%)	59 (67.8%)	-
Age (years)	67 IQR (59 - 73)	66.5 IQR (62.8 - 73.3)	67 IQR (57.5 - 73)	0.418
Male gender	54/87 (62.1%)	14/28 (50%)	40/59 (67.8%)	0.110
Transferrin saturation (%)	**24.5 IQR (13.8 - 33-8)**	**7.6% IQR (5.5% - 12.8%)**	**32 IQR (24.4 – 37.3)**	**<0.001 ***
Hemoglobin level (g/L)	109 IQR (94.3 - 137.3)	108.5 IQR (96 - 146.8)	109 IQR (88.3 - 131.5)	0.191
MCV (fL)	**87.3 IQR (80.8 - 93.3)**	**81.7 IQR (76.4 - 87.8)**	**89.5 IQR (84.8 - 94.8)**	**0.003 ***
Microcytosis (MCV <82 fL)	**23/84 (27.4%)**	**15/28 (53.6%)**	**8/56 (14.3%)**	**<0.001 ***
RDW (%)	19.4 IQR (17.5 - 20.7)	19.6 IQR (18.6 - 21.4)	19.3 IQR (17 - 20.3)	0.099
WBC (x10^9/L)	10.9 IQR (6.8 - 16.3)	13.6 IQR (8 - 19.3)	9.8 IQR (6.4 - 15.6)	0.120
>1% circulatory blasts	31/87 (35.6%)	9/28 (32.1%)	22/59 (37.3%)	0.640
Platelets	**351 IQR (174.8**	**411 IQR (258.3**	**333 IQR (166 -**	**0.192 ***

(x10⁹/L)	- 575.5)	- 688.5)	559.5)	
Ferritin (µg/L)	**187 IQR (63 - 421)**	**49.5 IQR (16.2 - 143)**	**309 IQR (186 - 628.5)**	**<0.001 ***
LDH (U/L)	539.5 IQR (351.5 - 780)	636 IQR (393 - 859)	513 IQR (348 - 758)	0.363
CRP (mg/L)	4.7 IQR (1.3 - 13.6)	4.9 IQR (2 - 16)	4 IQR (1.3 - 11.1)	0.534
Albumin (g/L)	**44 IQR (40 - 46)**	**42 IQR (40 - 44)**	**45 IQR (42 - 47)**	**0.018 ***
Advanced bone marrow fibrosis	50/87 (57.5%)	16/28 (57.1%)	34/59 (57.6%)	0.966
JAK2 **mutated**	**50/83 (60.2%)**	**21/27 (77.8%)**	**29/56 (51.8%)**	**0.023 ***
CALR mutated	8/60 (13.3%)	1/22 (4.5%)	7/38 (18.4%)	0.238
MPL mutated	1/60 (1.7%)	1/22 (4.5%)	0/38 (0%)	0.367
Constitutional symptoms	30/86 (34.9%)	9/28 (32.1%)	21/58 (36.2%)	0.711
Massive splenomegaly	26/82 (31.7%)	11/27 (40.7%)	15/55 (27.3%)	0.218
Blast phase disease	8/87 (9.2%)	2/28 (7.1%)	6/59 (10.2%)	1.000
Transfusion dependency	**22/87 (25.3%)**	**3/28 (10.7%)**	**19/59 (32.2%)**	**0.031 ***

Table 2: Cox regression model investigating prognostic properties of different parameters related to iron metabolism, adjusted for age and gender. Abbreviations: TSAT – transferrin saturation. * statistically significant at P<0.05.

Variable	Hazard ratio	95% confidence interval	P value
TSAT ≤20%	**HR=15.71**	**[2.69 - 91.66]**	**0.002 ***
Hemoglobin <100 g/L	**HR=37.15**	**[4.5 - 306.5]**	**0.001 ***
Ferritin >686 µg/L	**HR=10.39**	**[1.44 - 75.02]**	**0.020 ***
Transfusion dependency	HR=0.96	[0.21 - 4.29]	0.956
Age	HR=1.01	[0.92 - 1.11]	0.842
Male gender	HR=4.84	[0.82 - 28.42]	0.081

Figure 1: A) Transferrin saturation (TSAT) did not differ between primary (PMF) and secondary myelofibrosis (SMF) patients and **B)** did not differ between the Dynamic International Prognostic Scoring System (DIPSS) risk categories (1 – low risk; 2 – intermediate-1 risk; 3 – intermediate-2 risk; 4 – high risk). **C)** *Janus-kinase-2* (*JAK2*) V617F mutated patients had significantly lower TSAT. **D)** Transfusion dependent patients had significantly higher TSAT.
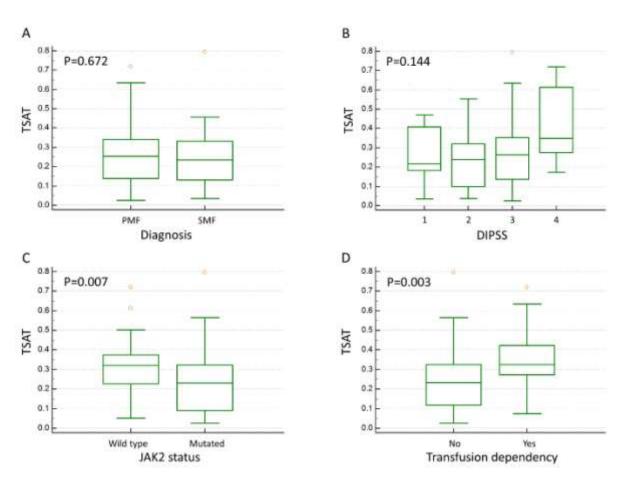
Figure 2: A) Low transferrin saturation (TSAT) was associated with inferior overall survival (OS) in primary myelofibrosis (PMF) patients, **B)** whereas there was no statistically significant association with survival in patients with secondary myelofibrosis (SMF). **C)** Mean corpuscular volume (MCV) had no impact on survival of patients with PMF and **D)** SMF. **E)** High ferritin was associated with inferior survival in both PMF and **F)** SMF patients.