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Title: Assessing serum albumin concentration, lymphocyte count and Prognostic Nutritional Index might improve prognostication in patients with myelofibrosis

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Abstract: Background: Primary and secondary myelofibrosis (PMF; SMF) are malignant diseases of the hematopoietic stem-cell characterized by the neoplastic myeloproliferation and strong inflammatory milieu. Prognostic-Nutritional-Index (PNI) integrates information on albumin and absolute-lymphocyte-count (ALC) and reflects inflammatory, nutritional and immune status of a patient. Clinical and prognostic significance of albumin, ALC and PNI in patients with myelofibrosis have not been previously investigated.

Methods: We retrospectively analyzed a cohort of 83 myelofibrosis patients treated in our institution from 2006 to 2017. Albumin, ALC and PNI were assessed in addition to other disease-specific markers.

Results: PMF and SMF patients had significantly lower ALC and PNI and similar albumin compared to controls. Lower albumin was significantly associated with older age and parameters reflecting more aggressive disease biology (anemia, lower platelets, higher LDH, circulatory-blasts, transfusion-dependency, blast-phase-disease), inflammation (higher CRP, constitutional-symptoms) and higher degree of bone-marrow-fibrosis. Lower ALC was significantly associated with lower white-blood-cells (WBC) and lower circulatory-blasts. Low PNI was associated with lower albumin, lower ALC, anemia, lower WBCs, lower serum-iron and lower transferrin-saturation. There was no difference in albumin, ALC and PNI regarding the driver-mutations. In multivariate analysis adjusted for age and gender, low-albumin (HR=4.61, P=0.001), low-ALC (HR=3.54, P=0.004) and DIPSS (HR=2.45, P=0.001) were able to predict inferior survival independently of each other. Accordingly, low-PNI (HR=4.32, P<0.001) predicted poor survival independently of DIPSS (HR=3.31, P<0.001).

Conclusion: Assessing albumin, ALC and PNI might improve prognostication in patients with myelofibrosis and could assist in recognition of patients under increased risk of death.

Keywords: Philadelphia chromosome negative myeloproliferative neoplasm; Primary myelofibrosis; Secondary myelofibrosis; Survival; Nutrition

Introduction:

Philadelphia chromosome negative (Ph-) myeloproliferative neoplasms (MPN) [1] are clonal disorders of the hematopoietic stem cell. Most patients carry a mutation in either of *Janus-kinase-2 (JAK2)*, *calreticulin (CALR)* or *myeloproliferative-leukemia-virus-oncogene (MPL)* genes [2] resulting in a constitutive activation of JAK/signal-transducer-and-activator-of-transcription (STAT) signaling pathway and a highly inflammatory milieu typical for these diseases [3]. Primary myelofibrosis (PMF) exhibits the most aggressive biological behavior among the Ph- MPNs and bears the highest risk of transformation to acute leukemia and death [4, 5]. It is characterized by megakaryocytic proliferation and atypia, progressive bone marrow fibrosis, leukoerythroblastic blood smear and development of hepatosplenomegaly due to induction of extramedullary hematopoiesis. Patients often suffer from debilitating constitutional symptoms and develop varying number and degree of myeloid lineage cytopenias. Patients with two other Ph- MPNs – polycythemia rubra vera (PRV) and essential thrombocythemia (ET) – can also develop bone marrow fibrosis and PMF-related features during their disease course [6], when these conditions are termed secondary myelofibrosis (SMF).

PMF and SMF patients have similar clinical presentation and experience similar clinical course. The risk of death in myelofibrosis patients can be determined using the International Prognostic Scoring System (IPSS) [7] at the time of diagnosis and the Dynamic International Prognostic Scoring System (DIPSS) [8] during the course of the disease. Both prognostic systems assign scores for the patient's age, white blood cell (WBC) count, hemoglobin level, presence of circulatory blasts and the presence of constitutional symptoms.

Serum albumin concentration and absolute lymphocyte count (ALC) are traditional measures of nutritional status that are also influenced by other non-nutritional factors, such as inflammation, stress and specific illness [9-15]. The Prognostic Nutritional Index (PNI), as proposed by Onodera *et al.* [16], integrates information on these two parameters and is calculated as $PNI = \text{serum albumin (g/L)} + [5 \times \text{ALC} (\times 10^9/\text{L})]$. This simple indirect measure of immunocompetence, inflammatory and nutritional status has been shown to be predictive of overall survival and perioperative complications in various malignancies [17-22].

A subset of myelofibrosis patients presents with a significant weight loss and cachexia which represents an important clinical problem [23]. Similarly, a majority of myelofibrosis patients presents with a lymphocytopenia and a disturbance in lymphocyte subsets [24]. However, potential prognostic implications of low serum albumin, low ALC and PNI have not been previously studied in patients with myelofibrosis.

In this study, we aim to investigate clinical associations of serum albumin concentration, ALC and PNI in patients with myelofibrosis and assess potential prognostic significance of these parameters.

Patients, Materials and Methods:

Patients

A total of 83 patients with myelofibrosis that were treated in our department in the period from 2006 to 2017 were retrospectively analyzed. 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 [6]. A total of 69 (83.1%) patients were evaluated at the time of establishing diagnosis and 14 (16.9%) patients were evaluated at the time of referral to our institution. All patients provided a written informed consent for the molecular analyses. The study was approved by the Institutional Review Board. All procedures were in accordance with the ethical standards of the responsible committee on human experimentation and with the Helsinki Declaration of 1975, as revised in 2008.

Degree of bone marrow fibrosis was determined according to the current European consensus [25]. Serum albumin concentration and ALC were recorded in addition to other demographic, hematological and clinical parameters (age, gender, WBC count, circulatory blasts, hemoglobin level, mean corpuscular volume (MCV), red cell distribution width (RDW), platelet count, C reactive protein (CRP) level, lactate dehydrogenase (LDH) level, serum iron level, total iron binding capacity (TIBC), transferrin saturation, ferritin level, presence of constitutional symptoms, blast phase disease, transfusion dependency, *JAK2*, *CALR* and *MPL* mutational status). Disease was staged according to the DIPSS, spleen and liver size were assessed by palpation. PNI was calculated as serum albumin (g/L) + $[5 \times \text{ALC} (\times 10^9/\text{L})]$. Additionally, albumin, ALC and PNI of PMF and SMF patients were compared to 30 age and gender matched healthy controls.

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 [26], *CALR1* and *MPL* exon 10 mutations were screened by high-resolution melting dye assays [27, 28] and any sample sequence that deviated from normal was Sanger sequenced.

Statistical analyses

The normality of data distribution was tested using the Kolmogorov-Smirnov test. Numerical variables were presented as either median with interquartile range (IQR), or as arithmetic mean \pm standard deviation depending on the normality of distribution. Categorical variables were presented as proportions. The Mann Whitney U test/the T-test, the Kruskal-Wallis test, the χ^2 (Chi squared) test and the Spearman rank correlation were utilized where appropriate. The Jonckheere-Terpstra test for trend was used to test trends of increase in numerical values over DIPSS risk categories. Survival analyses [29] were performed using the methods of Kaplan and Meier, the Cox-Mantel version of the log-rank test [30] and the Cox regression analysis. Receiver operating characteristic (ROC) curve analysis using survival status as a classification variable was performed in order to determine optimal cut-off values for survival analyses. P values <0.05 were considered significant. Associations of different prognostic factors with survival were screened for via a custom made MS Excel workbook [31]. Analyses were performed using MedCalc Statistical Software version 17.6 (MedCalc Software BVBA, Ostend, Belgium).

Results:

Overview of myelofibrosis patients

In our study, we analyzed a total of 83 patients with myelofibrosis, 63 (75.3%) of whom were diagnosed with PMF and 20 (24.7%) with SMF. Mean patient age was 65.6 ± 10.4 years, with 51/83 (61.4%) patients of male gender. Patients' characteristics are listed in Table 1.

Median follow up of our cohort of patients was 51 months. Median overall survival was 67 months and it did not differ between PMF and SMF patients.

Serum albumin concentration

Median serum albumin concentration was 44 g/L, IQR (40 - 46) and it did not significantly differ between PMF, SMF and controls ($P=0.865$) as shown in Figure 1A. Only a small subset of patients, 7/83 (8.4%), presented with hypoalbuminemia, defined as a serum albumin level lower than 35 g/L.

Higher serum albumin concentration was significantly associated with younger age (Rho= -0.25, P=0.026), higher hemoglobin level (Rho=0.41, P<0.001), lower percentage of circulatory blasts (Rho= -0.33, P=0.002), higher platelet count (Rho=0.26, P=0.021), lower LDH level (Rho= -0.39, P<0.001), lower RDW (Rho=-0.37, P=0.001), lower CRP level (Rho= -0.5, P<0.001), higher serum iron level (Rho=0.38, P=0.003), higher TIBC (Rho=0.28, P=0.029), absence of constitutional symptoms (median 42 g/L vs 44.5 g/L for patients with and without constitutional symptoms, P=0.001), absence of blast phase disease (median 35 g/L vs 44 g/L for patients with and without leukemic transformation of myelofibrosis, P<0.001), transfusion independency (median 40 g/L vs 44 g/L for transfusion dependent and transfusion independent patients, P=0.019) and lower degree of bone marrow fibrosis (Rho= -0.25, P=0.035) as depicted in Figure 2A. There was a clear and significant trend of decrease in serum albumin values over DIPSS risk categories (P<0.001) as shown in Figure 2B. There was no statistically significant correlation between serum albumin concentration and gender, WBC count, ALC, spleen and liver size, MCV, transferrin saturation, ferritin level, *JAK2*, *CALR* or *MPL* mutational status.

Optimal serum albumin cut-off value for survival analyses was determined using the ROC curve analysis and patients were divided into High albumin (>42 g/L) and Low albumin (\leq 42 g/L) groups. Low albumin was univariately associated with shorter overall survival (HR=6.49, P<0.001) as shown in Figure 3A. This association remained significant in the Cox regression model adjusted for age, gender and DIPSS (Low albumin HR=3.84, P<0.001; DIPSS HR=2.51, P<0.001; age and gender not significant).

Absolute lymphocyte count

Median ALC was $1.5 \times 10^9/L$, IQR (1 - 1.9) and it differed significantly between patients and controls (P<0.001). Although there was no significant difference between PMF and SMF patients in regard to ALC, both subgroups had significantly lower values in comparison to controls (P<0.05 for both comparisons) as depicted in Figure 1B. A subset of 16/83 (19.3%) patients presented with ALC values lower than $1 \times 10^9/L$.

Higher ALC was significantly associated with higher WBC count (Rho=0.48, P<0.001) and higher percentage of circulatory blasts (Rho=0.28, P=0.010). We did not observe statistically significant correlation with other tested parameters (age, gender, hemoglobin level, platelet count, LDH level, CRP level, albumin, MCV, RDW, parameters of iron metabolism, spleen or liver size, constitutional symptoms, transfusion dependency, blast phase disease, degree of bone marrow fibrosis, *JAK2*, *CALR* or *MPL* mutational status). In addition, ALC did not correlate with DIPSS.

Optimal ALC cut-off value for survival analyses was determined by the ROC curve analysis and patients were divided into High ALC ($>0.86 \times 10^9/L$) and Low ALC ($\leq 0.86 \times 10^9/L$) groups. Low ALC group presented with significantly shorter overall survival (HR=2.86, P=0.005) as shown in Figure 3B. Low ALC remained significantly associated with poor survival in the multivariate Cox regression model adjusted for age, gender and DIPSS (Low ALC HR=3.65, P=0.002; DIPSS HR=3.66, P<0.001; age and gender not significant). We could also demonstrate that Low ALC, Low albumin and DIPSS can predict inferior overall survival independently of each other in the model including Low ALC, Low albumin and DIPSS, age and male gender as specified in Table 2.

Prognostic Nutritional index (PNI)

We further investigated how PNI, which incorporates information on serum albumin concentration and absolute lymphocyte count, affects survival of patients with myelofibrosis. Median PNI was 50.5, IQR (47 - 55.5) and it did not statistically significantly differ between PMF and SMF patients, but was lower in both groups of patients when compared to healthy controls (P<0.05 for both comparisons). Patients were divided into subgroups based on the PNI score quartiles and we could demonstrate that there is a significant difference in overall survival between patient groups (P<0.001) as shown in Figure 3C. Patients with PNI lower than the first quartile (PNI <47) had significantly inferior overall survival in comparison to all the other subgroups (P<0.008 for all comparisons, considering the Bonferroni correction for multiple comparisons). Patients in other three subgroups based on PNI quartiles had similar disease course and did not significantly differ in overall survival. Having PNI <47 remained statistically significantly associated with shorter overall survival in the Cox regression model adjusted for age, gender and DIPSS (PNI <47 HR=4.32, P<0.001; DIPSS HR=3.31, P<0.001; age and gender not significant).

Patients with the PNI lower than the first quartile expectedly had lower serum albumin concentration (median 38.5 g/L vs 45 g/L, P<0.001) and lower ALC (median $0.93 \times 10^9/L$ vs $1.5 \times 10^9/L$, P<0.001), but also had lower hemoglobin level (100.5 ± 16.7 vs 117.1 ± 26.8 g/L, P=0.018), lower WBC count (median 6.9 vs $11.4 \times 10^9/L$, P=0.044), lower serum iron level (median 5.3 vs 13.2, P=0.006) and lower transferrin saturation (median 8% vs 27%, P=0.020) compared to those with the higher PNI, but did not significantly differ in other disease-specific parameters.

Discussion:

To the best of our knowledge, this study is first to investigate clinical correlations and prognostic significance of serum albumin, ALC and PNI in patients with myelofibrosis and to report their DIPSS-independent prognostic properties.

Although being traditionally considered as measures of nutritional status [9-12], serum albumin concentration and ALC are strongly affected by a variety of other factors, most notably inflammation [13, 32]. As we have shown in our current study, lower serum albumin level was associated with parameters reflecting more aggressive disease biology (higher LDH level, higher percentage of circulatory blasts, blast phase disease, transfusion dependency), higher inflammatory milieu (higher CRP level, presence of constitutional symptoms) and also with parameters that could reflect nutritional status of PMF/SMF patients (lower serum iron level, anemia). Lower serum albumin was associated with an increasing degree of bone marrow fibrosis suggesting that these two phenomena might be regulated by a similar cytokine profile. Associations of low serum albumin with a large number of aforementioned negative prognostic parameters were reflected in inferior overall survival in our cohort of patients. Treatment of myelofibrosis patients with JAK1/2 inhibitor ruxolitinib typically results in a reduction of inflammatory cytokines, improvement of constitutional symptoms and a reduction in spleen size, but also in an improvement in body weight and serum albumin concentration [33]. Survival advantage seen in a subset of ruxolitinib-treated patients might be at least in part mediated through the improvement in nutritional status. However, role of albumin as prognostic marker in this setting has not yet been evaluated.

Our finding of a decreased ALC in myelofibrosis patients in comparison to healthy controls is in line with the previous observation of lymphocytopenia as a characteristic feature of the disease [24]. Remarkably, ALC did not show statistically significant associations with most parameters of disease severity, except positive correlations with WBC count and circulatory blasts which are considered to be negative prognostic markers [7]. Quite oppositely, higher ALC in our cohort of patients seems to bear positive prognostic implications, and its effect on improved survival could be mediated by decreased susceptibility to infections as observed in other clinical situations [34]. Recent large retrospective study that investigated factors associated with infections in patients with myelofibrosis unfortunately did not investigate lymphocyte count as a possible factor [35], leaving this issue unresolved. In addition, neither serum albumin, nor ALC were associated with mutations in *JAK2*, *CALR* and *MPL* genes suggesting that their changes do not reflect driver mutation-specific phenomena.

Our most striking observation was that low serum albumin, low ALC and DIPSS all seem to provide additional prognostic information and could predict inferior overall survival independently of each other (Table 2). This

suggests that all three parameters reflect different pathophysiological processes and assessing all three of them might improve prognostication of patients with myelofibrosis. Due to their independent associations with survival in these patients, it is reasonable to combine information on serum albumin and ALC into a composite index abbreviated PNI as previously proposed by Onodera *et al.* [16]. PNI is considered to reflect inflammatory, nutritional and immune status of an oncological patient and was shown to be of prognostic value in a variety of malignant neoplastic diseases [17-22]. As we have observed in our cohort of myelofibrosis patients, a quarter of patients presenting with the lowest PNI values (<47) had lower WBC count, lower hemoglobin level, lower serum iron level and lower transferrin saturation in addition to lower serum albumin concentration and lower ALC which all might have been affected by the nutritional status of a patient. A quarter of patients presenting with the lowest PNI values experienced unfavorable disease course with the significantly shorter overall survival in comparison to other three sub-cohorts (Figure 3C). Interestingly, all patients with $\text{PNI} \geq 47$ had similar prognosis and their survival was not significantly affected by the assignment to the particular PNI subgroup. Therefore, only reaching below critical PNI threshold seems to be negatively affecting survival through a process that might be independent of disease-specific mechanisms measured by the currently established DIPSS prognostic system.

Main limitations of our work are single institution experience, retrospective study design, small number of patients and heterogenous patient population. Retrospective study design prevents us from assessing anthropometric measures of nutritional status such as body mass index, waist circumference or triceps skin fold, as well as correlating them to parameters of interest. In addition, study was conducted over an 11-year period during which patients were exposed to different disease-oriented and other supportive therapies that might modulate effects of measured parameters. A subset of patients that were treated upfront with ruxolitinib (at the time of diagnosis) was relatively small and most of intermediate-2 and high risk patients received the drug later during course of their disease (when it became available), and not synchronous with the study baseline. Short follow up / small number of events and consequent statistical power limitations prevent us from properly investigating whether studied phenomena persist in a subcohort of ruxolitinib treated patients. Nevertheless, our study identifies serum albumin, ALC and PNI as useful and easy-to-obtain parameters that bear valuable clinical information in patients with myelofibrosis. Hence, future studies investigating these parameters in larger prospective cohorts of patients are warranted, especially from the point of view of new and emerging therapies with “cachexia modulatory” properties.

In conclusion, serum albumin, ALC and DIPSS predict survival independently of each other. PNI integrates information on serum albumin and ALC and similarly provides DIPSS-independent prognostic information.

Assessing these parameters could improve prognostication of patients with myelofibrosis and help in recognition of patients under the increased risk of death, therefore enabling timely supportive interventions.

Ethical approval: The study was approved by the Institutional Review Board.

Conflict of interest: All authors report no conflicts of interest.

Informed consent: All subjects in whom molecular studies were performed provided written informed consent.

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Table 1: Patients' characteristics. Abbreviations: PMF – primary myelofibrosis; SMF – secondary myelofibrosis; *JAK2* – Janus kinase 2; *CALR* – calreticulin; *MPL* – myeloproliferative leukemia virus oncogene; WBC – white blood cells; IQR – interquartile range; RDW – red cell distribution width; LDH – lactate dehydrogenase; CRP – C reactive protein, ALC – absolute lymphocyte count, PNI – Prognostic Nutritional Index.

Number of patients	83
Diagnosis	PMF 63/83 (75.9%) SMF 20/83 (24.1%)
Age (years)	65.6 ±10.4
Gender	Male 51/83 (61.4%) Female 32/83 (38.6%)
Bone marrow fibrosis	Grade 0-I 33/83 (39.8%) Grade II-III 50/83 (60.2%)
<i>JAK2</i> mutated	52/80 (65%)
<i>CALR</i> mutated	8/63 (12.7%)
<i>MPL</i> mutated	2/63 (3.2%)
Constitutional symptoms	30/82 (36.6%)
Massive splenomegaly	19/80 (23.8%)
Blast phase disease	8/83 (9.6%)
WBC (x10 ⁹ /L)	11 IQR (6.9 - 17.4)
>1% circulatory blasts	30/82 (36.6%)
Hemoglobin level (g/L)	113.6 ±25.9
Platelets (x10 ⁹ /L)	336 IQR (178 - 574)
RDW (%)	19.6 IQR (18.1 - 21.1)
LDH (U/L)	539 IQR (326 - 766.5)
CRP (mg/L)	5.3 IQR (2 - 15.1)
Albumin (g/L)	44 IQR (40 - 46)
ALC (x10 ⁹ /L)	1.5 IQR (1 - 1.9)
PNI	50.5 IQR (47 - 55.5)

Table 2: Age and gender adjusted Cox regression analysis model demonstrating independent prognostic properties of low albumin, low absolute lymphocyte count (ALC), and Dynamic International Prognostic Scoring System (DIPSS) in patients with myelofibrosis. Abbreviations: HR – hazard ratio, C.I. – confidence interval.

Variable	HR	95% C.I. for HR	P value
Low albumin	4.61	[1.96 - 10.85]	0.001*
Low ALC	3.54	[1.5 - 8.37]	0.004*
DIPSS	2.45	[1.42 - 4.22]	0.001*
Age	1.02	[0.98 - 1.07]	0.338
Male gender	0.79	[0.36 - 1.71]	0.548

* statistically significant result (P<0.05)

Figure 1: A) There was no statistically significant difference in serum albumin concentration between patients with primary myelofibrosis (PMF), secondary myelofibrosis (SMF) and healthy controls. **B)** Patients with PMF and SMF had significantly lower absolute lymphocyte count (ALC) in comparison to controls, whereas there was no statistically significant difference between PMF and SMF.

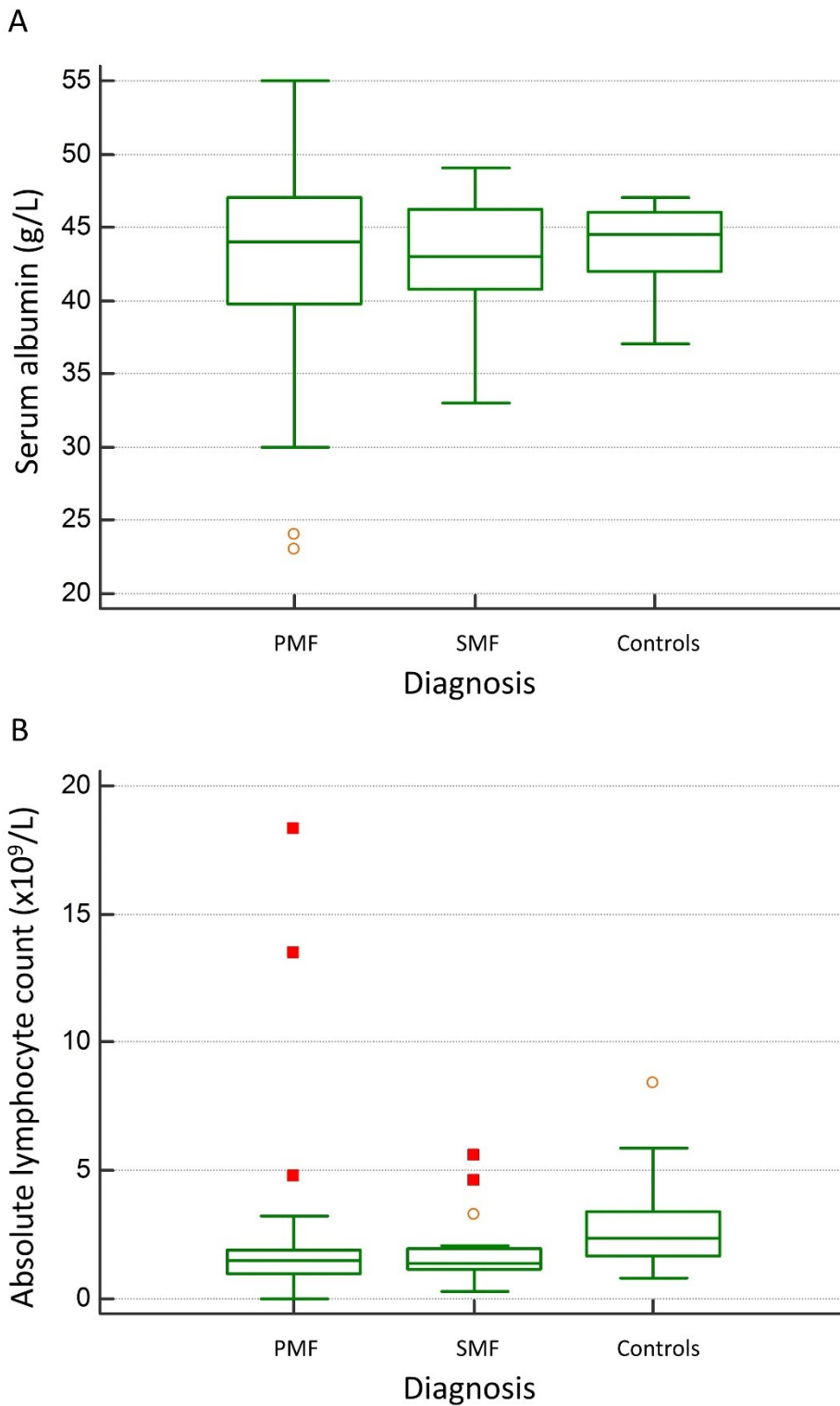
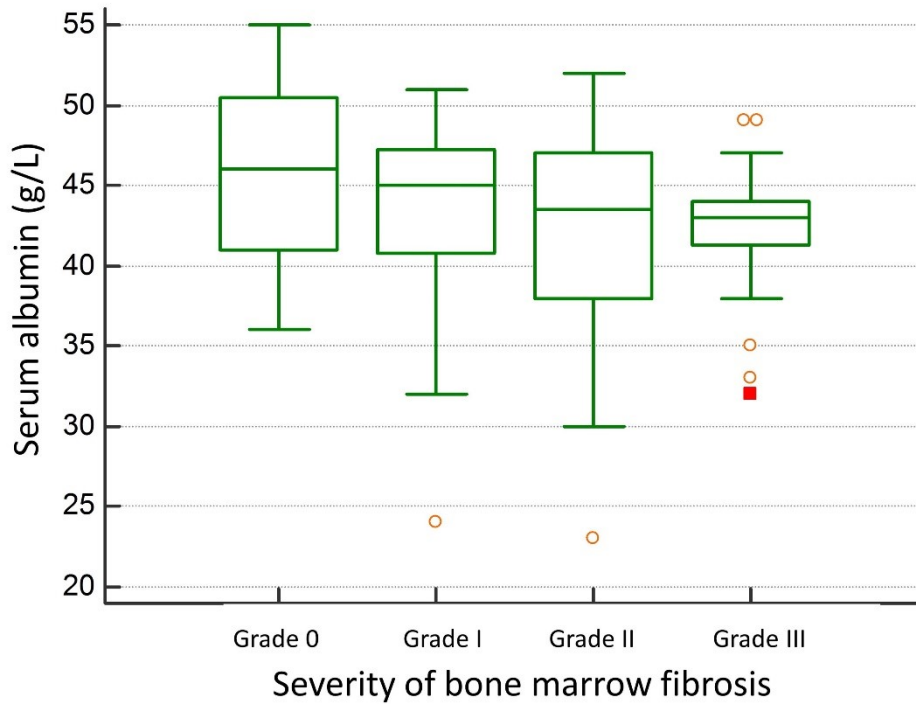


Figure 2: **A)** Serum albumin was negatively correlated with the degree of bone marrow fibrosis. **B)** Serum albumin showed a significant trend of decrease with a higher Dynamic International Prognostic Scoring System (DIPSS) risk category. Abbreviations: Int. – intermediate.

A



B

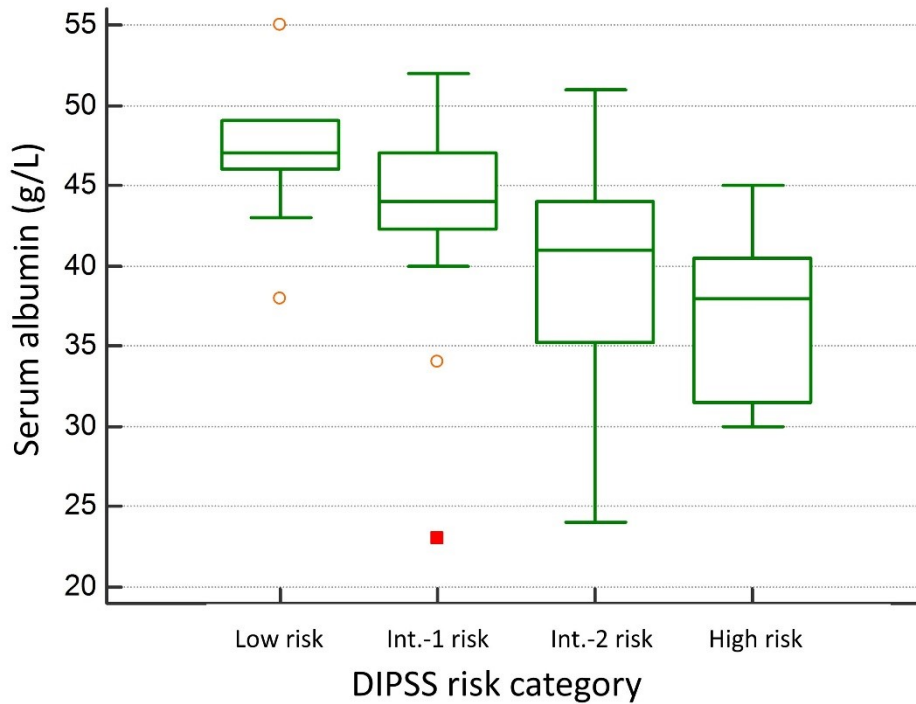


Figure 3: Overall survival (OS) of patients with myelofibrosis stratified by **A)** serum albumin concentration, **B)** absolute lymphocyte count (ALC) and **C)** Prognostic Nutritional Index (PNI). Abbreviations: HR – hazard ratio, qrt. – quartile.

