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Circulating YKL-40 in Philadelphia-negative myeloproliferative neoplasms

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Original article

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Abstract

OBJECTIVES: Philadelphia-negative chronic myeloproliferative neoplasms (MPNs), essential thrombocythemia (ET), polycythemia vera (PV) and myelofibrosis (MF), are characterized by clonal myeloproliferation and a strong inflammatory atmosphere. YKL-40, expressed in granulocytes, macrophages, megakaryocytes and malignant cells, is an acute phase reactant with an important role in tissue remodeling and atherosclerotic inflammation. The aim of this study was to investigate serum YKL-40 levels in MPNs and to assess its clinical correlations. METHODS: ELISA test was used to measure serum YKL-40 levels in 111 MPN patients and 32 healthy controls. RESULTS: Serum YKL-40 levels were higher in ET, post-ET MF, PV, post-PV MF and primary MF patients, when compared to healthy controls ($p<0.001$). Higher serum YKL-40 levels were associated with parameters indicative of the increased inflammatory state (higher C-reactive protein, poor performance status, presence of constitutional symptoms and cardiovascular risk factors). Additionally, higher serum YKL-40 levels in MF patients were associated with blast phase disease, lower hemoglobin and higher Dynamic International Prognostic Scoring System score. In the multivariate Cox regression models, higher serum YKL-40 levels in ET and PV patients were associated with an increased risk of thrombosis (HR 4.64, $p=0.031$) and impaired survival in MF patients (HR 4.31, $p=0.038$). CONCLUSION: These results indicate that higher circulating YKL-40 levels in MPNs might have a pathophysiological role in disease progression and thrombosis development. Assessing circulating YKL-40 could help in identification of ET and PV patients at a high risk of future cardiovascular events and has a good potential for improving prognostication of MF patients.

Keywords: YKL-40; myeloproliferative disorder; myelofibrosis; essential thrombocythemia; polycythemia vera

Introduction

Philadelphia-chromosome-negative chronic myeloproliferative neoplasms (MPNs), essential thrombocythemia (ET), polycythemia vera (PV) and myelofibrosis (MF), share similar clinical and biological characteristics; overproduction of erythroid, megakaryocytic and granulocytic cells, bone marrow (BM) fibrosis, frequent splenomegaly and an increased risk of thrombosis. The majority of MPN patients bear mutations in the Janus kinase 2 (JAK2) [1,2] or calreticulin (CALR) [3,4] genes that constitutively activate the JAK-STAT signaling pathway. Dysregulation of this signaling pathway promotes aberrant synthesis of inflammatory cytokines and chemokines, triggering persistent, systemic inflammatory response marked by frequent debilitating symptoms associated with the disease and elevated levels of various circulating proinflammatory cytokines [5]. Moreover, this chronic inflammatory state has been shown to impair overall survival (OS) and promote cardiovascular (CV) complications in MPNs [6-9].

Life expectancy in all three MPNs is worse than that of the age- and sex-matched general population, mainly due to thrombosis and fibrotic transformation [10,11]. MPNs are burdened with CV complications and the main therapeutic objective is to prevent thrombotic complications, as no therapy can prolong survival. Thus, current risk stratification in ET and PV is designed to estimate the likelihood of recurrent thrombosis. Patient age ≥60 and history of previous thrombosis have been shown to be the most reliable risk factors for future arterial or venous events. Additionally, in ET and MF patients, there is an increased risk of thrombosis in JAK2 mutated patients in comparison to non-mutated patients [12]. Therefore, even in the absence of arterial or venous thrombosis, PV patients aged ≥60 are usually classified as high-risk for thrombosis development. In ET, there are 4 risk categories that consider patient age, history of thrombosis and presence of JAK2 mutation. Risk-adapted therapy includes acetylsalicylic acid therapy for all PV and low- to high-risk ET patients, whereas cytoreductive

therapy (hydroxycarbamide) is usually recommended only in high-risk ET and PV patients [11].

Transformation to secondary myelofibrosis (SMF) is a potentially fatal disease complication in MPNs. MF exhibits the most aggressive biological behavior among MPNs and patients often suffer from debilitating constitutional symptoms, develop varying numbers of myeloid lineage cytopenias, and are at a high risk of transformation to acute leukemia. Primary myelofibrosis (PMF) and SMF follow similar clinical course [10,11,13]. The risk of death in MF can be estimated anytime during the follow-up using the Dynamic International Prognostic Scoring System (DIPSS) [14], which assigns scores for patient age, total leukocytes, hemoglobin level, presence of circulatory blasts and constitutional symptoms. Although treatment with ruxolitinib in MF patients has been shown to improve constitutional symptom control, splenomegaly and quality of life, allogeneic stem cell transplantation remains the only curative option for these patients [11,15].

Glycoprotein YKL-40 (chitinase-3-like protein 1) is an acute phase reactant expressed in various cell types including granulocytes [16], macrophages [17], bone marrow megakaryocytes [18], as well as in malignant cells [19]. In normal BM, YKL-40 is stored in the granules of myelocytes and metamyelocytes [20]. Mainly due to its role in inflammation and extracellular matrix remodeling, YKL-40 has been investigated as a potential biomarker of several autoimmune [21] and malignant [19] conditions, as well as those that include fibroblast activation [18,22,23]. There is also accumulating evidence that YKL-40 might promote atherosclerosis, as it is involved in endothelial dysfunction by promoting chemotaxis, cell attachment and migration, reorganization and tissue remodeling, in response to endothelial damage. In addition, YKL-40 overexpression was demonstrated in macrophages and smooth muscle cells in atherosclerotic plaque formations [24,25]. Furthermore, several studies

demonstrated that elevated serum YKL-40 levels were associated with diabetes [26,27], coronary artery disease [28,29] and ischemic cerebrovascular disease [30,31].

We identified two prior studies investigating circulating YKL-40 in MPN patients. One study has reported increased YKL-40 levels in PMF patients in comparison to controls, without further assessing its clinical correlations [32]. Another study investigated serum YKL-40 levels in ET and PV patients during vorinostat treatment [33]. This particular study has reported increased baseline serum YKL-40 levels in PV patients when compared to ET and healthy controls. Furthermore, baseline serum YKL-40 levels in PV patients were shown to correlate with parameters indicative of increased myeloproliferation and inflammatory state, e.g., leukocyte count, platelets, JAK2 allelic burden, lactate dehydrogenase (LDH) and serum C-reactive protein (CRP) levels. In addition, patients who experienced response to vorinostat therapy exhibited a significantly greater reduction of YKL-levels than non-responders. These observations implicated that YKL-40 could be a novel circulating biomarker of disease burden and progression in MPNs.

The aim of this study was to investigate circulating YKL-40 in MPNs and to assess its clinical correlations.

Patients and Methods

Consecutive patients with ET, PV and PMF diagnosed according to the World Health Organization (WHO) 2016 criteria [34] and post-ET MF and post-PV MF diagnosed according to the International Working Group for Myelofibrosis Research and Treatment (IWG-MRT) 2008 criteria [35] were enrolled in the study between July 2014 and February 2016. Healthy blood donors served as controls. Excluded from participation were pregnant women, subjects younger than 18 years of age, subjects with acute infections, known autoimmune disorders or concomitant solid tumors.

This was a case-controlled study. Data on patient diagnosis, age, sex, disease duration, JAK2 or CALR mutational status, Eastern Cooperative Oncology Group (ECOG) performance status [36], DIPSS [14], presence of constitutional symptoms (fever ≥37.5 °C, night sweats or weight loss >10% in the preceding 6 months), presence of at least one of the CV risk factors (arterial hypertension, diabetes or hyperlipidemia), history of thrombosis (arterial or venous), transfusion dependency and treatment modalities (hydroxycarbamide or ruxolitinib therapy) were collected at the time of study enrollment. Spleen length was measured in centimeters from the left costal margin. BM fibrosis was graded according to the current European consensus [37].

Laboratory assays

Blood samples for determination of blood counts, LDH and CRP were obtained at the time of study enrollment. Samples for serum YKL-40 were left to clot, and serum was separated from cellular fragments by centrifugation (3500 rpm for 10 minutes). All serum samples were stored at -80 ºC until analysis. Commercially available quantitative sandwich enzyme linked immunosorbent assay (ELISA) kit was used to measure serum YKL-40 levels (R&D Systems Europe, Abingdon, UK).

Ethics

The study was performed in accordance with the Declaration of Helsinki and approved by the institutional Ethics Committee. Prior to enrollment, all participants signed their informed consent**.**

Statistical analysis

All statistical analyses were performed with MedCalc Statistical Software® (version 19.0.3, Ostend, Belgium). Distribution of data was checked using Kolmogorov-Smirnov test. Categorical variables were expressed as absolute and relative frequencies and compared using χ^2-test or Fisher exact test, as appropriate. Quantitative variables were expressed as medians

and interquartile ranges. Differences between two independent samples were assessed with Mann-Whitney U test. Multiple comparisons were done by Kruskal-Wallis test. The Jonckheere-Terpstra trend test was used to test trends of increase in serum YKL-40 levels across DIPSS categories. Spearman correlation coefficients were calculated to assess correlations between serum YKL-40 and different continuous variables. Receiver operating characteristic (ROC) curve analysis was used for sensitivity and specificity testing. Survival analyses were performed using Kaplan and Meier methods, log-rank test and the Cox regression analysis. The following end-points were analyzed: OS and thrombosis-free survival (TFS), measured from the time of blood sampling, with failures defined as death from any cause and an ascertained thrombotic (arterial or venous) episode, respectively. Data on surviving or thrombosis-free surviving patients were checked on the day of the last follow-up visit. Arterial thrombosis was defined as myocardial infarction, transitory cerebral ischemic attack, acute cerebral ischemic stroke or acute peripheral arterial occlusion. Venous thrombosis was defined as peripheral vein thrombosis, pulmonary embolism or splanchnic vein thrombosis. In all analyses, the level of statistical significance was set at $p<0.050$.

Results

We included 42 ET, 33 PV, 17 PMF and 19 SMF patients. Among the 19 SMF patients, there were 11 post-ET MF and 8 post-PV MF patients. Thirty-two healthy blood donors served as controls. There were 45 (40.5%) newly diagnosed patients; the remaining patients were diagnosed earlier. There was no statistically significant difference between patients and healthy controls according to sex ($p=0.195$) or age ($p=0.099$).

Serum YKL-40 levels significantly differed between MPN patients (median 1238.9 pg/mL, range 57.8-4000) and controls (median 466 pg/mL, range 117.3-801.7; $p<0.001$). Serum YKL-40 levels were significantly higher in ET (median 1053.2 pg/mL, range 728.9-4000), post-ET

MF (1432.3 pg/mL, range 371.4-4000), PV (1489.9 pg/mL, range 324.7-4000), post-PV MF (median 2048.35 pg/mL, range 57.8-4000) and PMF patients (median 1079.1 pg/mL, range 220.1-4000), when compared to healthy controls (overall $p<0.001$; $p<0.050$ for all individual analyses) (Figure 1). The differences in serum YKL-40 levels between ET, post-ET MF, PV, post-PV MF and PMF patients were not statistically significant (overall $p=0.989$), neither was the difference between PMF and SMF patients (median 1432.3 pg/mL, range 57.8-4000, $p=0.987$).

Characteristics of ET and PV patients are shown in Table 1. Higher serum YKL-40 levels in ET and PV patients were statistically significantly associated with older age (rho=0.264, $p=0.020$), higher CRP (rho=0.712, $p<0.001$) (Figure 2), presence of constitutional symptoms ($p=0.029$), poor performance status (ECOG ≥2-4; $p<0.001$), CV risk factors ($p=0.012$), BM reticulin fibrosis ($p=0.002$) and history of thrombosis ($p<0.001$). Treatment with low-dose acetylsalicylic acid was associated with lower YKL-40 levels ($p=0.038$), whereas warfarin use was associated with higher serum YKL-40 levels ($p=0.049$). We found no statistically significant associations in ET and PV patients between serum YKL-40 levels and sex, disease duration, driver mutational status, leukocyte, granulocyte, eosinophil, basophil and erythrocyte counts, hemoglobin, hematocrit and LDH levels, hydroxycarbamide therapy, or presence of palpable spleen.

Due to the aforementioned associations of serum YKL-40 with older age, history of thrombosis and CV risk factors, we further tested the hypothesis that higher serum YKL-40 levels might be associated with an increased risk of thrombosis. Median follow-up of ET and PV patients was 50 (range 11-65) months. Twenty-one (28%) patients developed thrombosis during the follow-up; seven (33.3%) were venous and 14 (66.7%) arterial ($p=0.126$) (Table 1). One patient developed splanchnic vein thrombosis. There was no difference in thrombosis incidence between ET (n=14) and PV (n=7) patients ($p=0.249$). For TFS analysis, ROC curve was

constructed with thrombosis as a classification variable to determine optimal cut-off of serum YKL-40 (>1714.4 pg/mL).

In univariate survival analyses, higher serum YKL-40 levels (HR 7.01, p<0.001) (Figure 3), history of thrombosis (HR 8.54, p<0.001), age >60 years (HR 3.22, p=0.008) and the presence of CV risk factors (HR 2.89, p=0.042) were associated with an increased risk of thrombosis. PV patients were also more likely to develop thrombosis, however, this association failed to reach statistical significance (HR 2.29, p=0.069). In the multivariate Cox proportional-hazards regression model, higher serum YKL-40 levels remained associated with a higher risk of thrombosis when adjusted for older age (>60 *vs*. ≤60 years), disease phenotype (ET *vs*. PV), history of thrombosis (yes *vs*. no), presence of JAK2 mutation (yes *vs*. no) and CV risk factors (yes *vs*. no) (Table 2).

Overview of MF patients is presented in Table 3. Higher serum YKL-40 levels (>1523.2 pg/mL) in MF patients were statistically significantly associated with older age (rho 0.362, p=0.029), higher CRP (rho 0.673, p<0.001), blast phase disease (p=0.003), higher peripheral blast cell count (rho=0.387, p=0.021), lower hemoglobin (<100 g/L, p=0.032), poor performance status (ECOG ≥2-4; p=0.028), presence of constitutional symptoms (p<0.001) and CV risk factors (p=0.045). We also noted a statistically significant trend for increase in serum YKL-40 levels across DIPSS categories (p<0.001) (Figure 4). Higher serum YKL-40 levels were observed in patients treated with hydroxycarbamide (p<0.001), whereas ruxolitinib use was associated with lower YKL-40 values (p=0.002). There were no statistically significant associations in MF patients between serum YKL-40 levels and sex, disease duration, driver mutational status, leukocyte, granulocyte, eosinophil, basophil and erythrocyte counts, hemoglobin, hematocrit and LDH levels, transfusion dependency, palpable spleen size or BM fibrosis grades.

As serum YKL-40 levels in MF patients were associated with clinical and laboratory parameters indicative of more aggressive disease (higher DIPPS, blast phase disease, presence of constitutional symptoms), we further analyzed if higher serum YKL-40 levels might be associated with impaired survival. Median follow-up for the living MF patients was 39 months. Median OS was 18 months. For OS analysis, ROC curve was constructed with death as a classification variable to determine optimal cut-off of serum YKL-40 (>1523.2 pg/mL). In univariate survival analysis, MF patients with higher serum YKL-40 levels had inferior OS in comparison to MF patients presenting with lower YKL-40 levels (HR 5.35, $p<0.001$), as shown in Figure 5. This association remained significant (HR 4.31, 95% CI 1.10-4.10, $p=0.038$) in the multivariate Cox regression model after adjusting for DIPSS (HR 5.09, 95% CI 1.06-7.93, $p=0.024$) and sex (nonsignificant).

Discussion

Conflicting results have been reported on the circulating YKL-40 levels in ET and PV patients. One study has reported no significant difference in YKL-40 levels between ET or PV and healthy controls. The same study demonstrated elevated serum YKL-40 levels in PMF patients, however, without comparison to ET and PV patients [32]. In contrast, another study reports elevated serum YKL-40 levels in PV when compared with ET patients and healthy controls [33]. The present study performed on a larger number of MPN patients clearly demonstrated that all five MPN disorders, ET, post-ET MF, PV, post-PV MF and PMF, had higher serum YKL-40 levels when compared with healthy controls (Figure 1). However, we found no difference in serum YKL-40 levels among different MPN disorders, which might limit the use of circulating YKL-40 in differentiating diseases within the MPN spectrum. In addition, there was no difference between ET or PV and SMF patients; this observation limits the potential use of serum YKL-40 as a circulating biomarker of MF transformation.

Higher serum YKL-40 levels in MPN patients in our study were associated with parameters indicative of increased tumor burden and inflammatory state; higher CRP (Figure 2), constitutional symptoms and poor performance status. Interestingly, MF patients treated with ruxolitinib had lower serum YKL-40 values when compared to patients treated with hydroxycarbamide. Because ruxolitinib therapy has been shown to ameliorate various inflammation-linked symptoms in MF patients and to efficiently decrease the production of major proinflammatory cytokines [11,15], this observation might further substantiate its anti-inflammatory activity in MF patients. In contrast to the previously mentioned study in ET and PV patients treated with vorinostat therapy [33], we found no correlations between serum YKL-40 levels and parameters indicative of stronger myeloproliferation (i.e. higher leukocyte count, higher platelets, LDH levels or spleen size). This might be partly due to the fact that the majority of patients (60%) in our study were included in various time-points of their disease course after having been diagnosed with MPN. Hydroxycarbamide and ruxolitinib therapy almost certainly influenced blood cell counts and modulated different clinical variables, i.e. presence of constitutional symptoms and spleen size. Although it would have been preferable to include only newly diagnosed patients, it was not feasible. To minimize the effect of therapy or general patient condition, we included only patients that were clinically well, treated as outpatients, and on stable ruxolitinib or hydroxycarbamide dose. Nevertheless, our results indicated that elevated serum YKL-40 levels might indeed represent the increased tumor burden and advanced inflammatory state in MPNs.

BM fibrosis in MPNs is the result of complex and poorly understood interactions among megakaryocytes, fibroblasts, endothelial cells, inflammatory cytokines and marrow stroma. Although there is evidence suggesting that myelofibrosis osteoclasts and fibroblasts might be clonal and functionally impaired [38], the current dogma is that stromal changes are secondary to the cytokine release produced by the hematopoietic clone cells in MPN patients [13]. YKL-

40 has been shown to be a growth factor for fibroblasts and several studies have demonstrated an important role of YKL-40 in extracellular matrix remodeling [18,22,23]. In our study, higher serum YKL-40 levels were associated with the presence of reticulin fibers in the BM of ET and PV patients; reticulin fibrosis has been cited as a risk factor for MF transformation [13]. However, we did not detect positive correlation between serum YKL-40 levels and BM fibrosis grades in MF patients. It was most likely due to the limited number of MF patients included and because our study sample was predominated with MF patients presenting with grade 3 BM fibrosis (Table 3). In this perspective, positive association of serum YKL-40 levels with reticulin fibrosis in ET and PV might implicate the potential role of circulating YKL-40 in promoting disease progression and BM fibrosis in MPNs through inflammation-induced tissue repair processes. However, additional studies are needed to confirm our speculations and elucidate the cell(s) of serum YKL-40 origin in MPNs.

In recent years, a role of YKL-40 in promoting atherosclerosis has also been suggested. YKL-40 has been reported to be associated with coronary artery disease [29,30] and ischemic cerebrovascular disease [31,32]. In addition, positive association between elevated circulating YKL-40 levels and increasing levels of albuminuria has been described in patients with type 1 diabetes, indicating the role of YKL-40 in microvascular disease development [27]. Furthermore, several *in vitro* studies have confirmed the role of YKL-40 in atherosclerotic plaque formation and progression, as it induces vascular smooth muscle cell migration through the intima in response to exogenous signals and promotes branching tubule biogenesis by modulating the vascular endothelial cell morphology. These studies were supported by *in vivo* detection of YKL-40 protein and mRNA expression in human vascular smooth muscle cells and macrophages in atherosclerotic plaque [25,25]. In the current study, serum YKL-40 levels were significantly correlated in MPN patients with one of the inflammatory markers, CRP, and, in the MPN setting, higher CRP levels were previously associated with major CV events [9].

Moreover, higher serum YKL-40 levels in MPNs were associated with the presence of CV risk factors. Interestingly, patients treated with low-dose acetylsalicylic acid had lower serum YKL-40 values in comparison to patients treated with warfarin, probably due to the anti-inflammatory and atheroprotective properties of low-dose acetylsalicylic acid [39]. In addition, all patients on warfarin therapy included in our study also had atrial fibrillation as an additional indication for anticoagulation; higher serum YKL-40 levels were previously observed in patients with atrial fibrillation [28,29]. In this perspective, elevated serum YKL-40 levels in MPN patients might also reflect the subclinical inflammatory processes that underlie cardiovascular disease development. Furthermore, our data showed that higher serum YKL-40 levels in ET and PV patients were associated with a higher risk of thrombosis (Figure 3), independently of other well-known risk factors for thrombosis development (Table 2). These observations indicate that circulating YKL-40 might causally be involved in thrombosis development in MPNs. Therefore, information on circulating YKL-40 might aid in early identification of MPN patients at a high risk of future CV events and could help in improving thrombosis-risk prognostication of ET and PV patients. However, validation in a prospective cohort study is warranted to more precisely elucidate the role of circulating YKL-40 in promoting adverse CV events in MPNs.

In hematologic malignancies, increased YKL-levels have been previously shown to correlate with impaired OS in multiple myeloma [18] and acute myeloid leukemia [40]. To the best of our knowledge, our study is the first to report on clinical associations and strong prognostic properties of increased serum YKL-40 levels in MF patients. Higher serum YKL-40 levels in MF patients might integrate different pathophysiological processes, stronger myeloproliferation (blast phase disease, percentage of circulatory blasts, need of hydroxycarbamide therapy) and higher degree of inflammation (higher CRP, lower hemoglobin, poor performance status, presence of constitutional symptoms). We also observed

a trend to increase in serum YKL-40 across DIPSS categories (Figure 4), which might indicate the potential role of serum YKL-40 in promoting disease progression in MF patients. More importantly, serum YKL-40 levels were able to predict survival of MF patients independently of DIPSS. Therefore, assessing circulating YKL-40 in MF might have the potential to further improve prognostication of MF patients. However, prognostic properties of YKL-40 also need to be confirmed in larger prospective cohorts of uniformly treated MF patients.

Our study had several limitations, such as the limited number of patients included, short patient follow-up, assessment of spleen size by palpation, and the aforementioned heterogeneity of patients. Nevertheless, our results indicated that serum YKL-40 could be considered as a circulating inflammatory biomarker that might have a role in promoting disease progression, BM fibrosis and thrombosis in MPNs. Therefore, assessing circulating YKL-40 might have a good potential for improving prognostication of MF patients and could help in the early identification of ET and PV patients at a high risk of future CV events. Additional studies on larger series of MPN patients are needed to unravel if YKL-40 might be a new therapeutic target in MPNs.

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Tables and figures

Table 1. Overview of essential thrombocythemia and polycythemia vera patients

ET=essential thrombocythemia, PV=polycythemia vera, JAK2=Januse Kinase 2, CALR=calreticulin, ECOG=Eastern Cooperative Oncology Group, CV=cardiovascular, LDH=lactate dehydrogenase, CRP=C-reactive protein, IQR=interquartile range

Number of patients	75
Diagnosis	
ET (%)	42 (56%)
PV (%)	33 (44%)
Newly diagnosed patients (%)	29 (39%)
Age, years	67 IQR (39-81)
Disease duration, years	2 IQR (0-18)
Sex	
Male (%)	31 (41%)
Female (%)	44 (59%)
Driver mutation status	
JAK2 (%)	52 (69%)
CALR (%)	12 (16%)
Negative (%)	11 (15%)
Constitutional symptoms (%)	31 (41%)
ECOG	
0-1 (%)	60 (80%)
2-4 (%)	15 (20%)
Palpable splenomegaly (%)	28 (37%)
CV risk factors (%)	56 (25%)
Reticulin fibrosis (%)	25 (67%)
Hydroxycarbamide (%)	43 (57%)
Acetylsalicylic acid (%)	61 (82%)
Warfarin (%)	6 (8%)
Previous thrombosis (%)	16 (21%)
Leukocytes (x10e9/L)	8.1 IQR (3.2-17.6)
Granulocytes (x10e9/L)	5.3 IQR (1.1-17.0)
Basophils (x10e9/L)	0.1 IQR (0-2)
Eosinophils (x10e9/L)	0.2 IQR (0-8.1)

Erythrocytes (x10e12/L)	4.7 IQR (3.0-7.9)
Hemoglobin, g/L	138 IQR (87-202)
Hematocrit (%)	0.4 IQR (0.3-0.6)
Platelets (x10e9/L)	502 IQR (142-1413)
LDH (IU/L)	233 IQR (130-696)
CRP (mg/L)	2.6 IQR (0.2-8.9)

Table 2. Higher serum YKL-40 levels (>1714.4 pg/mL) in essential thrombocythemia and polycythemia vera were associated with an increased risk of thrombosis in the multivariate Cox regression model

Variable	HR	95% CI	p
Serum YKL >1714.4 pg/mL	4.64	[1.10-8.64]	*0.031*
CV risk factors	0.19	[0.19-13.45]	0.662
PV phenotype	4.04	[0.11-0.97]	*0.043*
History of thrombosis	4.79	[1.11-7.41]	*0.028*
JAK2 mutation	0.20	[0.29-2.14]	0.650
Age > 60 years	4.33	[1.08-13.82]	*0.037*
HR=hazard ratio, CI= confidence interval, CV=cardiovascular, PV=polycythemia vera			

Table 3. Overview of myelofibrosis patients

PMF=primary myelofibrosis, SMF=secondary myelofibrosis, JAK2=Januse Kinase 2, CALR=calreticulin, ECOG=Eastern Cooperative Oncology Group, DIPSS=Dynamic International Prognostic Scoring System, CV=cardiovascular, LDH=lactate dehydrogenase, CRP=C-reactive protein, IQR=interquartile range

Number of patients	36
Diagnosis	
SMF (%) PV (%)	17 (47%) 19 (53%)
Newly diagnosed patients (%)	16 (44%)
Age, years	65 IQR (33-80)
Disease duration, years	5 IQR (0-15)
Sex	
Male (%) Female (%)	19 (53%) 17 (47%)
Driver mutation status	
JAK2 (%) CALR (%) Negative (%)	25 (70%) 8 (22%) 3 (8%)
Constitutional symptoms (%)	21 (61%)
ECOG	
0-1 (%) 2-4 (%)	16 (44%) 20 (56%)
DIPSS	
Low risk (%) Intermediate-1 risk (%) Intermediate-2 risk (%) High risk (%)	6 (17%) 9 (25%) 13 (36%) 8 (22%)
Blast phase disease, %	18 (50%)
Transfusion dependency, %	12 (33%)
Spleen size, cm	7 (0-26)
Bone marrow fibrosis	
MF-2 (%) MF-3 (%)	6 (17%) 30 (83%)
CV risk factors (%)	21 (58%)
Hydroxycarbamide (%)	17 (47%)

Ruxolitinib (%)	10 (28%)
Acetylsalicylic acid (%)	8 (22%)
Warfarin (%)	0
Leukocytes (x10e9/L)	10.8 IQR (2.1-91.0)
Granulocytes (x10e9/L)	9.5 IQR (0.8-49.1)
Basophils (x10e9/L)	0.6 IQR (0-4.7)
Eosinophils (x10e9/L)	0.3 IQR (0-8.0)
Erythrocytes (x10e12/L)	3.4 IQR (2.2-7.2)
Hemoglobin, g/L	97 IQR (69-198)
Hematocrit (%)	0.3 IQR (0.21-0.53)
Platelets (x10e9/L)	145 IQR (5-1120)
Peripheral blast cell count (%)	0.5 IQR (0-12)
LDH, (IU/L)	452 IQR (164-1987)
CRP (mg/L)	3.2 IQR (0.8-17.9)

Figure 1. Serum YKL-40 levels were higher in all disease categories when compared with healthy controls. The Kruskal-Wallis test was used.

ET=essential thrombocythemia, PV=polycythemia vera, MF= myelofibrosis, PMF=primary myelofibrosis

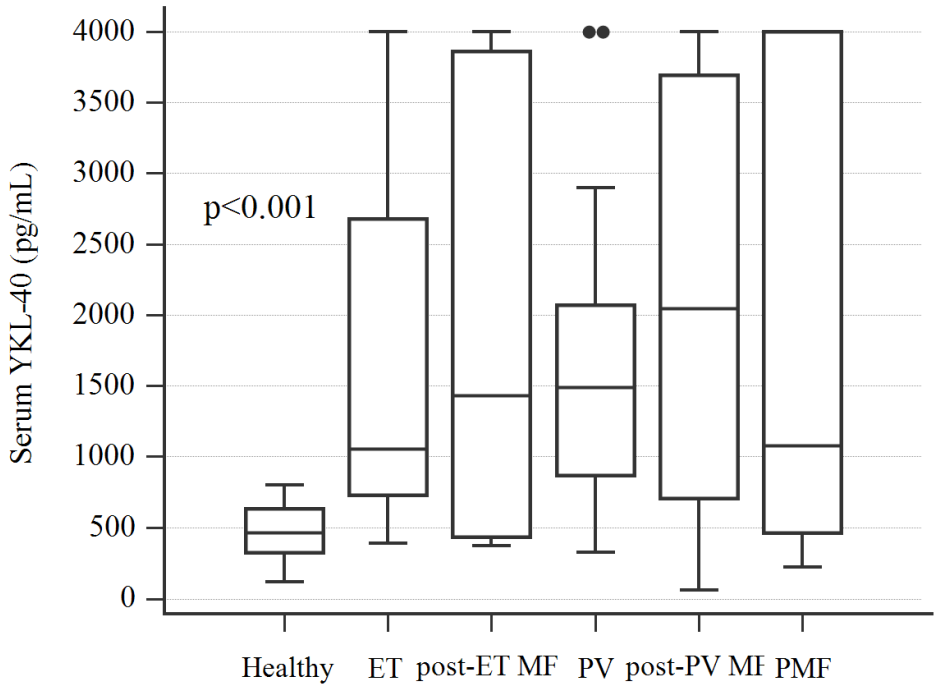

Figure 2. Statistically significant positive correlation between serum YKL-40 and serum C-reactive protein levels in essential thrombocythemia and polycythemia vera.

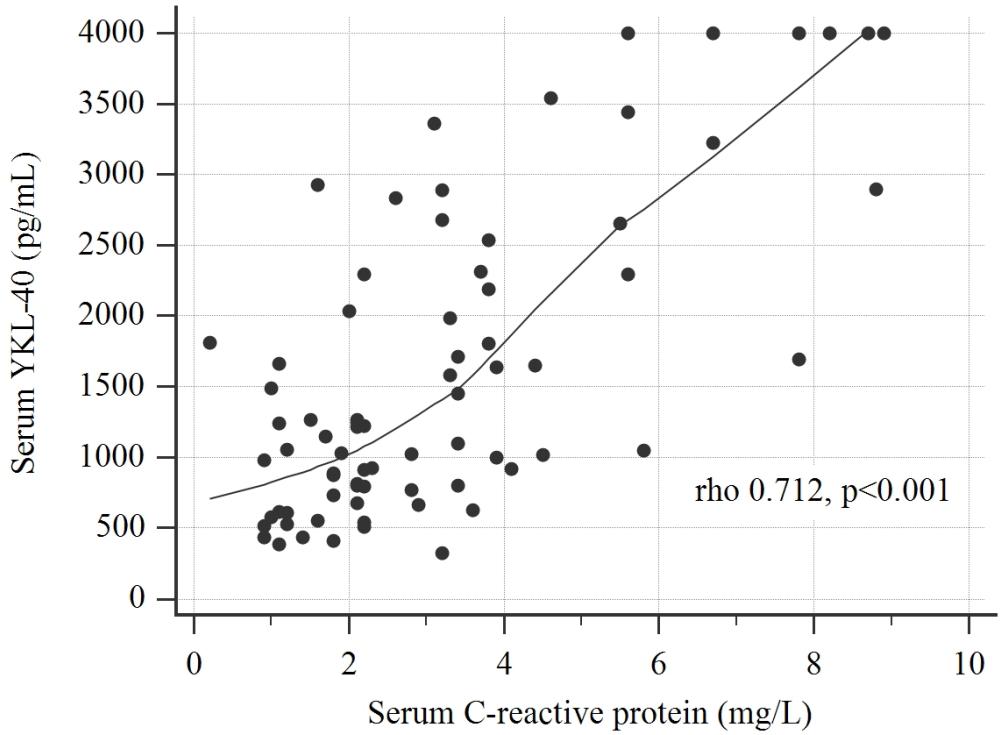

Figure 3. Higher serum YKL-40 levels (>1714.4 pg/mL) in essential thrombocythemia and polycythemia vera were associated with a higher risk of thrombosis. Log rank test was used.

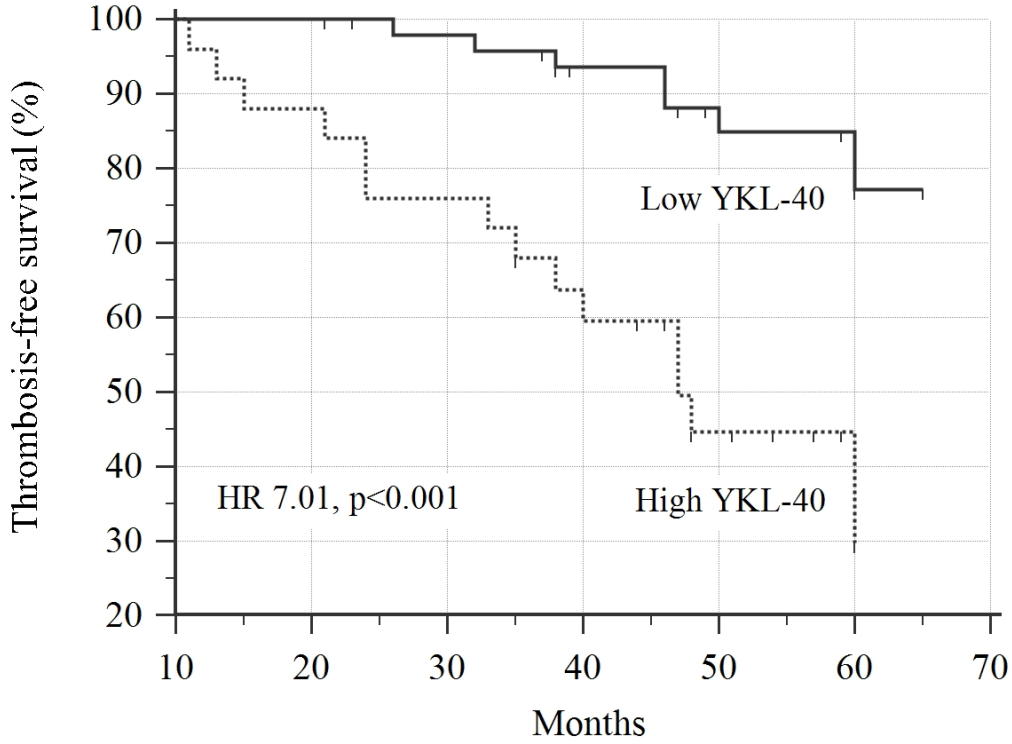

Figure 4. A trend of increase in serum YKL-40 levels was observed in myelofibrosis patients across Dynamic International Prognostic Scoring System (DIPSS) risk categories. The Jonckheere-Terpstra trend test was used. Int=intermediate

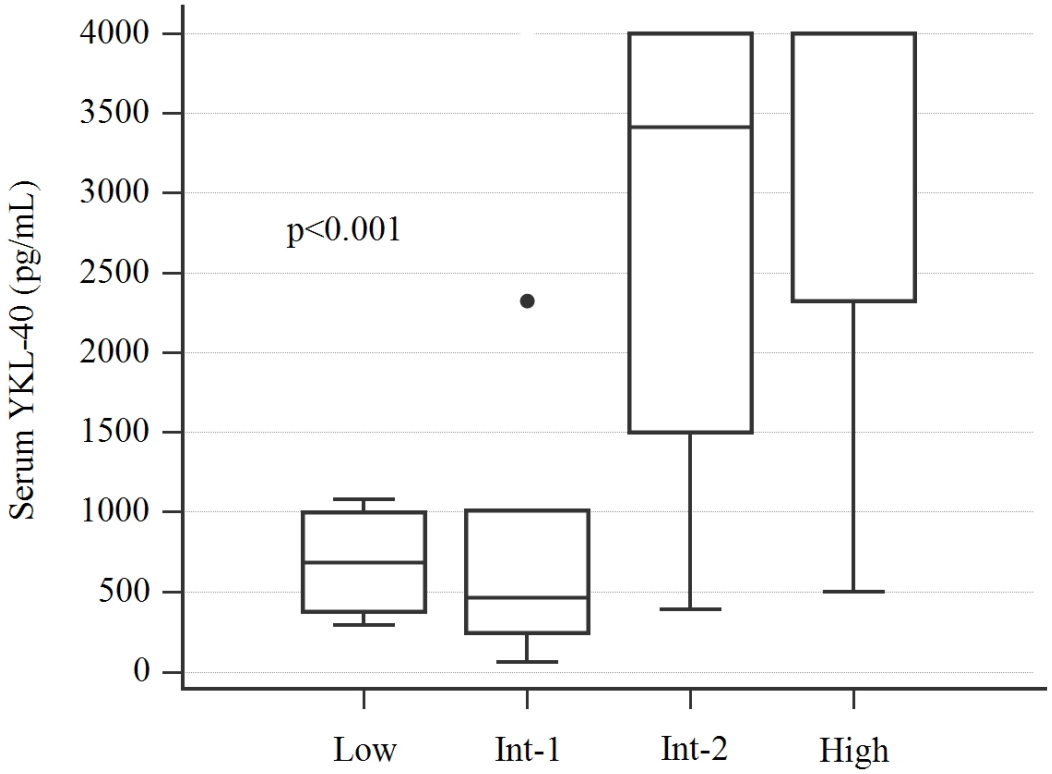

Figure 5. Myelofibrosis patients presenting with higher serum YKL-40 levels (>1523.2 pg/mL) had inferior overall survival. Log rank test was used.