Serum calprotectin: A circulating biomarker of the inflammatory state in Philadelphia-negative myeloproliferative neoplasms

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To the Editor:

Philadelphia-negative myeloproliferative neoplasms (MPN's), essential thrombocythemia (ET), polycythemia vera (PV) and primary myelofibrosis (PMF), are characterized by clonal myeloproliferation and a strong inflammatory milieu. This chronic inflammation causes debilitating symptoms associated with the disease, drives the selective expansion of the neoplastic clone, and might even be responsible for bone marrow (BM) fibrosis development [1].

Calprotectin is a major cytosolic protein of granulocytes and an acute phase reactant [2]. Through the amplification of inflammatory responses, calprotectin has been shown to induce tissue remodelling and fibrosis. In addition, calprotectin might also possess a tumor-promoting activity [3,4]. In everyday clinical practice, fecal calprotectin measurements are commonly used for monitoring inflammatory bowel diseases (IBDs) activity, assessment of treatment response and prediction of relapse [5]. During intestinal inflammation, fecal calprotectin is released by intestinal granulocytes. Thus, elevated fecal calprotectin levels help in differentiating gastrointestinal inflammation in IBDs from functional disorders. Similarly to MPN's, JAK-STAT inhibition has been shown to be safe and effective for the treatment of IBDs [6]. As MPN's are neoplasms characterized by JAK-STAT-induced chronic inflammation, which is proposed to be directly involved in disease progression and BM fibrosis, we found it relevant and timely to investigate serum calprotectin levels in MPN's.

This was a single-center, case-controlled study. We enrolled 43 newly diagnosed MPN patients (13 ET, 16 PV and 14 PMF). The diagnosis was made according to the WHO 2008 criteria. Patient's characteristics are shown in Table 1. Seventeen healthy blood donors served

as controls. The median age of controls was 67 years of age (range 60-70), and 10 were male (59%). There was no difference between patients and controls according to age or sex ($p>0.050$ for both analyses). BM fibrosis was graded according to the current European consensus [7]. Blood samples for measurement of blood counts, serum C-reactive protein (CRP) and serum lactate dehydrogenase (LDH) were taken at the time of study enrollment. Commercially available quantitative sandwich enzyme linked immunosorbent assay (ELISA) kit was used to measure serum calprotectin levels (Bühlmann Laboratories AG, Schönenbuch, Switzerland). All statistical analyses were performed with MedCalc Statistical Software®, version 18.11.6. Categorical variables were compared using chi-square test. The differences between independent samples were assessed with Kruskal-Wallis or Mann-Whitney U test. Receiver operating characteristic (ROC) curves were constructed for MPN's vs controls to assess the sensitivity and specificity of serum calprotectin and serum CRP levels for detection of MPN's. Spearman correlation coefficients were calculated to assess the correlations between serum calprotectin levels and different laboratory variables. P values <0.050 were considered statistically significant for all analyses.

All three MPN disorders [ET (median 2.32 µg/mL, range 0.42-9.00), PV (median 5.05 µg/mL, range 0.40-9.98) and PMF (1.92 µg/mL, range 0.50-23.19)] had higher serum calprotectin levels when compared to controls (median 0.65 µg/mL, range 0.40-2.19; overall $p<0.001$) (Figure 1.). There was no statistically significant difference in serum calprotectin levels between ET, PV and PMF patients ($p=0.220$).

The approximate areas under the ROC curves for serum calprotectin and serum CRP levels were 0.867 (STErr±0.047, 95% CI 0.775-0.958, $p<0.001$) and 0.723 (STErr±0.069, 95% 0.592-0.831, $p=0.001$), respectively. According to the ROC curve, when the cut–off value of serum calprotectin is >1.11 µg/mL, serum calprotectin has a sensitivity of 81.4% and a specificity of 88.2% for detection of MPN's. When two ROC curves were compared, serum

calprotectin had higher sensitivity and specificity than CRP for detection of MPN's (p=0.001) (Figure 2.).

Higher serum calprotectin levels were associated with older age (> 60 years of age) (p=0.007), presence of constitutional symptoms (p=0.006), poor (2-4) ECOG performance status (p<0.001), higher serum C-reactive protein levels (rho=0.672, p<0.001) (Figure 3.), and the need for hydroxycarbamide therapy (p=0.004). These associations remained significant when PMF, a MPN with a highest inflammatory burden, was excluded from analyses (p<0.050 for all aforementioned analyses). Although we found no difference in serum calprotectin levels between ET, PV and PMF patients, we further analysed if there is a statistically significant difference in serum calprotectin levels between MPN patients without BM fibrosis (MF-0), when compared to patients with BM fibrosis (MF-1-3). Fourteen MPN patients (9 ET and 5 PV) in our cohort had no signs of BM fibrosis (Table 1.). These patients had lower serum calprotectin levels, when compared to patients presenting with BM fibrosis (p=0.024). Similarly, ET and PV patients with reticulin fibres (MF-1) in the BM had higher serum calprotectin levels, when compared to ET and PV patients without reticulin fibrosis (MF-0) (p<0.001).

Serum calprotectin levels in MPN patients did not correlate with sex, erythrocyte, leukocyte, granulocyte and platelet counts, hemoglobin, hematocrit and LDH levels, history of thrombosis, or the presence of palpable spleen.

We additionally analysed the characteristics of MPN patients whose serum calprotectin levels were in the same range as controls (detected by serum calprotectin levels < 1.11 μg/mL, a cut-off value provided by the ROC curve analysis). Using this approach, we identified eight MPN patients (1 ET, 3 PV and 4 PMF). There were 3 male and 5 female patients; median age was 72 years of age (range 43-80). Five of these patients had Janus-kinase 2 (JAK2) mutation;

the remaining three harboured calreticulin (CALR) gene mutations. There was no difference between these patients when compared to MPN patients with higher serum calprotectin levels (>1.11 μg/mL), according to age, sex, driver mutations, ECOG performance status, or the need for hydroxycarbamide therapy ($p>0.050$ for all analyses). However, none of the MPN patients with low serum calprotectin levels had constitutional symptoms or history of thrombosis; these observations were statistically significant ($p<0.050$ for both analyses).

To the best of our knowledge, no previous studies have examined the role of serum calprotectin in hematological malignancies. We herein for the first time report elevated serum calprotectin levels in MPN's. As calprotectin is released mainly by granulocytes, constitutional JAK-STAT-driven granulocyte activation might be responsible for elevated serum calprotectin levels in MPN's. However, in our study serum calprotectin levels did not correlate with parameters indicative of stronger myeloproliferation, i.e. higher leukocyte, granulocyte, erythrocyte and platelet counts, higher hematocrit, hemoglobin and LDH levels, or larger spleen. On the other hand, higher serum calprotectin levels were associated with clinical and laboratory variables commonly regarded as indicators of the increased inflammatory state (presence of constitutional symptoms, poor performance status, higher CRP). In this perspective, elevated serum calprotectin levels in MPN's might primarily reflect the inflammatory state, not necessarily the extent of myeloproliferation. Our data also shows that higher serum calprotectin levels in MPN's are associated with the presence of reticulin fibres and/or BM fibrosis. Although our observations are limited by small number of patients, we speculate that calprotectin might, through inducing inflammation-induced tissue repair processes, have a role in promoting disease progression and BM fibrosis in MPN's.

We found no difference in serum calprotectin levels between ET, PV and PMF, which might limit the use of serum calprotectin in differentiating between these three disorders. On the other hand, our data shows that cut-off point of serum calprotectin of >1.11 μg/mL has

very good sensitivity (81.4%) and specificity (88.2%) for detection of MPN's (Figure 2). These observations might indicate a potential role of serum calprotectin as a diagnostic biomarker of MPN's. However, concomitant inflammatory disorders (i.e. IBDs, rheumatoid arthritis, systemic vasculitides, systemic lupus erythematosus) need to be excluded during the diagnostic process, as circulating calprotectin levels in various inflammatory conditions have been previously shown to strongly correlate with their disease activity indices [2,5,8]. Additional studies on larger series of MPN patients are needed to confirm our observations and to determine what would be the optimal cut-off of serum calprotectin for detection of MPN's. These studies could also unravel if serum calprotectin might help in discriminating between three disorders in the MPN spectrum. Limitations of our study are single-center study design, small number of patients included, assessment of spleen size by palpation, and the absence of additional controls with underlying inflammatory diseases that are not MPN’s. Nevertheless, our results indicate that serum calprotectin might be considered as a novel circulating biomarker of the inflammatory state in MPN's.

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Tables and figures

Table 1. Patients's characteristics.

Number of patients	43
Sex	
Female	23 (53%)
Male	20 (47%)
Age (years)	72 IQR (37-94)
Disease	
ET	13 (30%)
PV	16 (37%)
PMF	14 (33%)
Mutational status	
JAK2	37 (86%)
CALR	6 (14%)
Bone marrow fibrosis grade	
MF-0	14 (33%)
MF-1	15 (35%)
MF-2	3 (7%)
MF-3	11 (25%)
Leukocytes ($x10^9$/L)	9 IQR (3.20-55.3)
Granulocytes ($x10^9$/L)	5.9 IQR (1.11-35.7)
Erythrocytes ($x10^{12}$/L)	4.39 IQR (2.17-6.80)
Hemoglobin (g/L)	129 IQR (69-196)
Hematocrit (%)	0.42 IQR (0.21-0.63)
Platelets ($x10^9$/L)	387 IQR (24-1509)
CRP (mg/L)	2.6 IQR (0.21-13)
LDH (IU/L)	277 IQR (132-1925)
Palpable splenomegaly	
No	21 (49%)
Yes	22 (51%)
ECOG	
0	12 (28%)
1	21 (49%)
2	5 (11%)
3	4 (9%)
4	1 (3%)
Constitutional symptoms	
No	28 (65%)
Yes	15 (35%)
Hydroxycarbamide therapy	

No	11 (26%)
Yes	32 (74%)

IQR=interquartile range, ET=essential thrombocythemia, PV=polycythemia vera, PMF=primary myelofibrosis, JAK2=Janus Kinase 2, CALR=Calreticulin, CRP=Serum C-reactive protein, LDH=lactat dehydrogenase, IU/L=international units per liter, ECOG=Eastern Cooperative Oncology Group performance status

Figure 1. Box-and-whisker plot. Serum calprotectin levels were significantly higher in all three MPN disorders when compared to healthy controls. Boxes represent the length of the interquartile range, from the first quartile to the third quartile. Within the boxes, horizontal lines represent median values of serum calprotectin. Dots are outliers, data values far away from the quartiles. ET=essential thrombocythemia, PV=polycythemia vera, PMF=primary myelofibrosis.

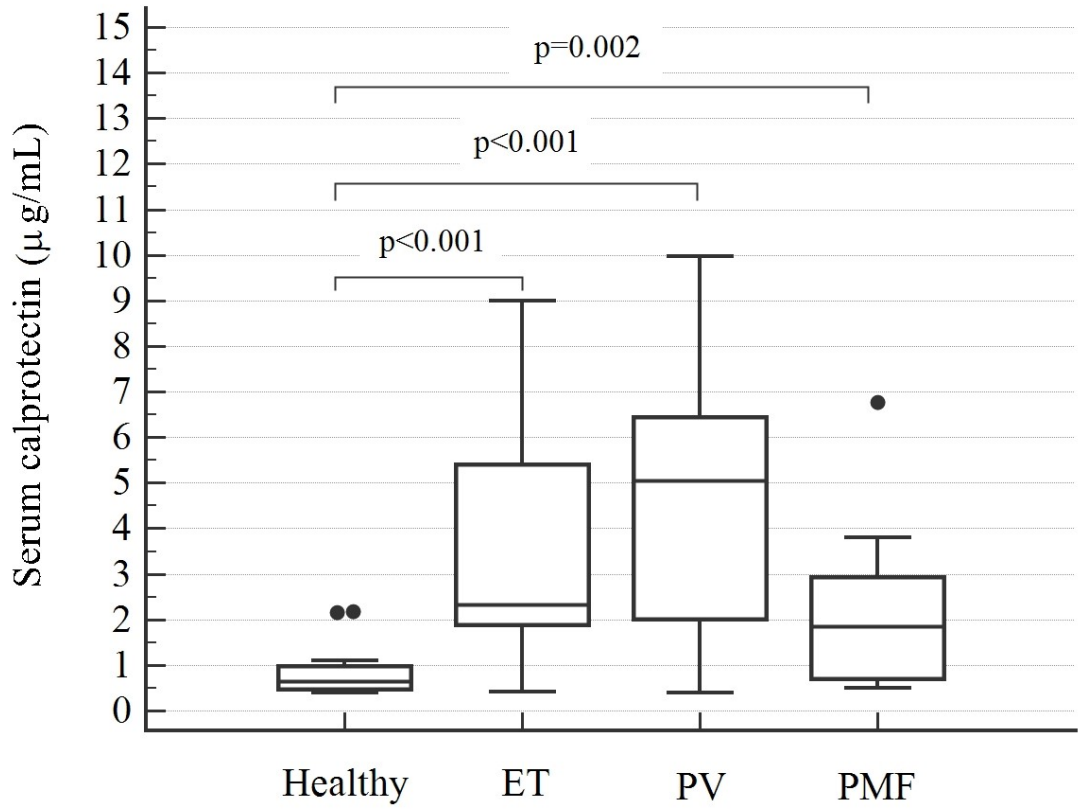

Figure 2. Receiver operating characteristic (ROC) curves were constructed for MPN's versus controls to assess the sensitivity and specificity of serum calprotectin and serum serum C-reactive protein (CRP) levels for detection of MPN's. When two ROC curves were compared, serum calprotectin had higher sensitivity and specificity than CRP for detection of MPN's. According to the ROC curve, when the cut-off value of serum calprotectin is >1.11 μg/mL, serum calprotectin has a sensitivity of 81.4% and a specificity of 88.2% for detection of MPN's.

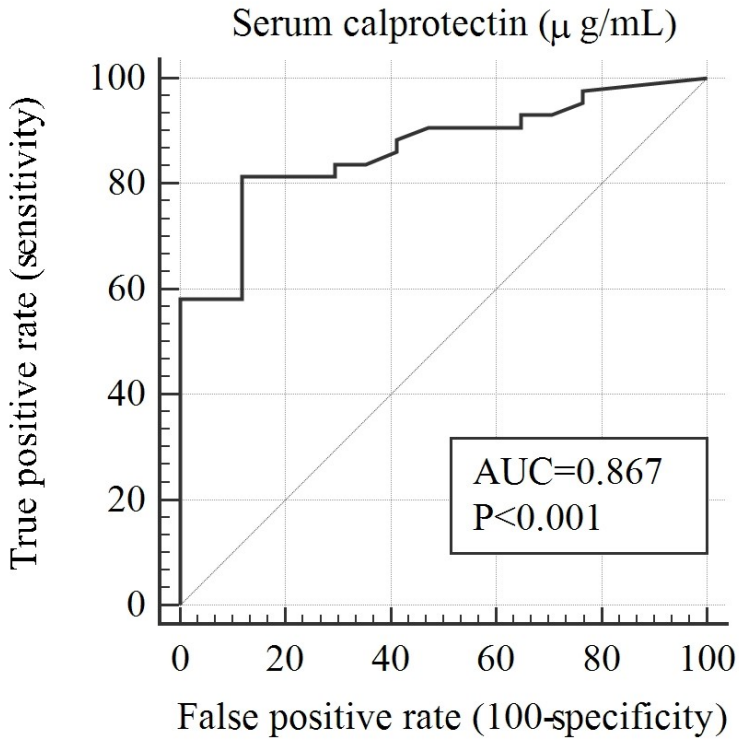

Figure 3. Statistically significant positive correlation was found in MPN patients between serum calprotectin levels and serum C-reactive protein levels.