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Clinical Correlates and Prognostic Significance of IL-8, sIL-2R, and Immunoglobulin-Free Light Chain Levels in Patients with Myelofibrosis

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Keywords

Cytokines · Immunoglobulin-free light chains · Primary myelofibrosis · Post-polycythemia vera myelofibrosis · Post-essential thrombocythemia myelofibrosis

Summary

Background: Chronic myeloproliferative neoplasms are characterized by clonal hematopoiesis and persistent inflammatory reaction. In this study, the clinical significance and prognostic impact of several inflammatory markers were evaluated in patients with BCR/ABL-negative myeloproliferative malignancies. **Methods:** Serum levels of interleukin-8 (IL-8) and lymphoid-associated activation markers – soluble interleukin-2 receptor (sIL-2R) and immunoglobulin-free light chains (FLC) – were evaluated in patients with primary myelofibrosis (MF), post-polycythemia vera MF, and post-essential thrombocythemia MF, and compared with the levels in healthy donors. **Results:** In 57 MF patients, sIL-2R excess correlated with transfusion-dependent anemia ($p = 0.03$) and splenomegaly ($p = 0.02$). There were no statistically significant correlations between sIL-2R and IL-8 levels, but the plasma concentration of κ-FLC positively correlated with the IL-8 level ($p = 0.027$). In univariate analysis, increased levels of IL-8 ($p = 0.016$) and sIL-2R ($p = 0.010$) significantly reduced 1-year overall survival. Only elevated sIL-2R rate retained significance ($p = 0.02$) in multivariate analysis when Dynamic International Prognostic Scoring System plus (DIPSSplus) risk stratification was added. **Conclusion:** We observed an association between FLC and proinflammatory cytokine hyperexpression. Serum cytokine levels and FLC might be a promising approach to predicting and monitoring treatment response in MF patients.

Introduction

Primary myelofibrosis (PMF) as well as a post-polycythemia vera (post-PV) and post-essential thrombocythemia (post-ET) myelofibrosis (MF) are BCR/ABL-negative chronic myeloproliferative neoplasms (MPNs) characterized by extramedullary hematopoiesis, splenomegaly, and bone marrow fibrosis [1]. The discovery of the new clonal molecular markers, *JAK2V617F*, *CALR*, and *MPL* mutations, has changed our understanding of the mechanisms involved in MPN pathogenesis, and has led to the development of new targeted drugs – Janus kinase inhibitors. Most of these agents exert their effect by inhibition of Janus kinases and suppression of the JAK-STAT signaling pathway in malignant and inflammatory cells [2–4]. Clinical response (alleviation of constitutional symptoms and splenomegaly) was associated with a reduction in proinflammatory cytokine burden [2]. According to these data, increased cytokine production and chronic inflammation play an important role in the pathogenesis of MPNs and support a crosstalk between clonal hematopoietic stem cells and the microenvironment. Therefore, an abnormal cytokine profile is believed to determine the clinical phenotype such as bone marrow

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stromal changes, extramedullary hematopoiesis, and constitutional symptoms.

Former studies showed that along with clinical factors, soluble interleukin-2 receptor (sIL-2R), interleukin-8 (IL-8), and immunoglobulin-free light chains (FLC) are among the most important predictors of outcome in patients with MF [5]. The production of IL-8 and IL-2 is regulated by the JAK-STAT signaling pathway [6–8], which is activated in patients with MPNs [9]. Increased levels of FLC might be a feature of immune imbalance and systemic lymphocyte activation in patients with MPNs. However, the association between increased FLC levels and proinflammatory cytokines was not investigated previously. In the current study, we evaluate the clinical significance of sIL-2R, IL-8, and FLC.

Patients and Methods

This prospective observational study included 57 patients with MF. The control group consisted of 16 healthy volunteer blood donors. In order to avoid age differences between investigated and control groups, only donors older than 50 years were included in the study. The survey was approved by the ethics committee of I.P. Pavlov's Medical University. All patients gave signed informed consent. In all patients, serum collection, trephine biopsy, and cytogenetic analysis were performed at the time of enrolment. To evaluate clinical risk factors, the Dynamic International Prognostic Scoring System plus (DIPSSplus) was used [10]. The diagnosis of PMF was made according to World Health Organization (WHO) 2016 criteria [11]. Patients with prefibrotic PMF were excluded from the analysis. The diagnosis of post-ET and post-PV MF was made according to International Working Group criteria [12]. Peripheral blood was collected from 57 patients with MF and 16 healthy donors; samples were centrifuged at 2,000 g for 15 min at +4 °C. Within 1 h of collection, serum aliquots were obtained and stored at –80 °C. The quantitative detection of human sIL-2R (CD25), IL-8, and FLC was performed with commercially available enzyme-linked immunosorbent assay kits (Cytokine, RF and Polygnost, RF, respectively; Affymetrix/eBioscience, Thermo Fisher Scientific, Waltham, MA, USA) according to the manufacturer's instructions.

Differences in the distribution of continuous variables between categories were analyzed by the Mann-Whitney test (for comparison of 2 groups) or the Kruskal-Wallis test (comparison of 3 or more groups). Patient groups with nominal variables were compared by χ^2 test. Correlations between 2 parameters were analyzed using Spearman's correlation coefficient. Post hoc correction for multiple comparisons was performed using the Bonferroni method for all the above calculations. Overall survival (OS) was considered as the period from the date of serum collection to the date of death (uncensored) or last contact (censored). OS curves were prepared using the Kaplan-Meier method, and the difference was compared using the log-rank test. A Cox proportional hazards regression model was used for multivariable analysis. p values of < 0.05 were considered significant. Pre-receiver operating characteristics (ROC) plots were used to determine cutoff levels for continuous variables of interest. SPSS (IBM Corp., Armonk, NY, USA) was used for all calculations.

Table 1. Patient and control group characteristics

	Myelofibrosis	Control group	p
Total, n	57	16	
Sex, n			0.8
Male	23	7	
Female	34	9	
Age, median (range), years	54 (22–76)	54 (50–67)	0.9
sIL-2R, median (range), ng/ml	5.2 (0.3–67)	3.3 (0.9–5.8)	0.0001
IL-8, median (range), ng/ml	0.50 (0.16–3.67)	0.27 (0.15–1.21)	0.049
κ-FLC median (range), μg/ml	9.7 (3.2–80.4)	6.1 (4.5–11.3)	0.0001
λ-FLC, median (range), μg/ml	10 (2.1–49.3)	7.3 (4.1–14.8)	0.0001

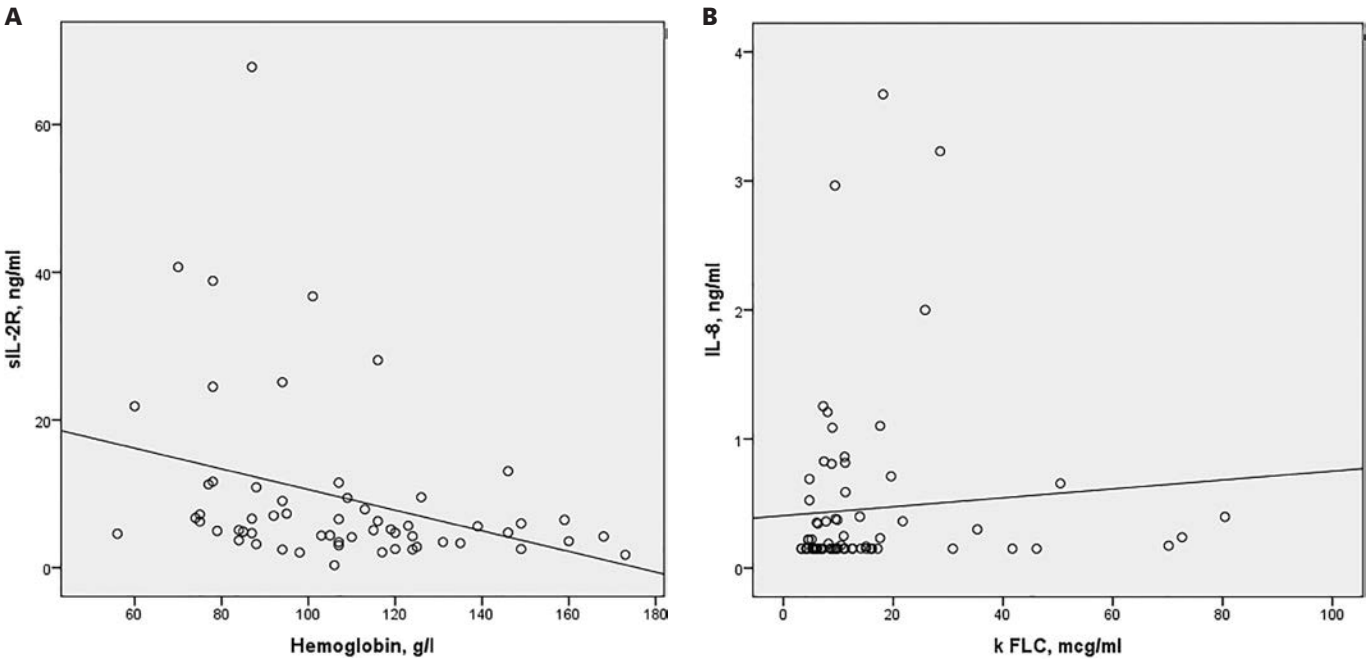

Fig. 1. A Correlation between sIL-2R and hemoglobin levels in patients with myelofibrosis (MF) (r = -0.380, p = 0.03); **B** correlation between IL-8 and κ-FLC levels in patients with MF (r = 0.288, p = 0.027).

Table 2. Relationship between levels of inflammatory markers and clinical features (post hoc correction was performed for every comparison)

Factor	sIL-2R, ng/ml		IL-8, ng/ml		κ-FLC, μg/ml		λ-FLC, μg/ml	
	r	p	r	p	r	p	r	p
Median age, years 54 (22–76)	0.143	0.55	0.020	0.86	0.155	0.35	0.209	0.12
Median hemoglobin, g/l 107 (56–173)	–0.380	0.03	–0.020	0.9	–0.130	0.35	0.249	0.61
Median leukocytes, $\times 10^9$/l 7.6 (1.5–73.0)	–0.156	0.43	0.172	0.20	–0.011	0.93	0.145	0.19
Median platelets, $\times 10^9$/l 188 (7–1,139)	–0.140	0.49	0.011	0.9	0.202	0.13	-0.256	0.55
	sIL-2R, ng/ml		IL-8, ng/ml		κ-FLC, μg/ml		λ-FLC, μg/ml	
	mean±SD	p	mean±SD	p	mean±SD	p	mean±SD	p
Sex, n		0.83		0.55		0.76		0.95
Male (23)	13.1±16.8		0.48±0.67		14.9±14.8		12.3±7.0	
Female (34)	7.1±6.5		0.53±0.81		16.7±17.8		12.6±8.4	
Constitutional symptoms, n		0.45		0.074		0.91		0.54
Yes (17)	6.6±5.1		0.61±0.87		15.9±16.6		12.9±8.3	
No (40)	16.4±19.3		0.27±0.24		16.2±17.0		11.6±6.6	
Blasts in peripheral blood, n								
< 1% (32)	6.4±4.0	0.14	0.46±0.71	0.64	16.2±18.2	0.83	2.3±0.5	0.47
≥ 1% (25)	13.6±16.9		0.56±0.81		15.3±14.6		2.4±0.6	
LDH, n		0.80		0.50		0.96		0.87
< 2 ULN (35)	11.7±19.5		0.39±0.37		9.6±3.2		10.3±5.1	
≥ 2 ULN (22)	10.1±11.4		0.75±1.07		13.0±10.1		11.0±5.9	
Transfusion dependency, n		0.04		0.23		0.56		0.64
No (36)	5.3±2.9		0.56±0.79		15.3±16.8		11.6±5.6	
Yes (21)	16.9±17.4		0.42±0.69		17.2±16.5		14.0±10.6	
Splenomegaly ≥ 10 cm below left costal margin, n		0.02		0.87		0.62		0.75
No (57)	7.4±3.1		0.52±0.78		15.2±16.7		12.1±5.7	
Yes (20)	13.6±16.6		0.48±0.70		16.6±16.7		13.1±10.9	
Unfavorable karyotype, n		0.43		0.37		0.32		0.53
No (6)	8.2±8.4		0.53±0.79		16.0±17.3		12.5±8.1	
Yes (51)	21.1±27.0		0.28±0.28		15.4±8.5		12.8±5.2	
JAK2V617 molecular status, n		0.21		0.10		0.13		0.05
Negative (30)	10.5±11.6		0.49±0.84		15.9±15.2		11.8±6.9	
Positive (27)	9.1±13.0		0.44±0.60		14.3±14.3		12.5±8.5	
Treatment, n		0.89		0.85		0.16		0.68
Treatment-naïve (15)	6.1±3.2		0.57±0.91		8.0±3.7		8.7±3.1	
On treatment (42)	11.2±14.3		0.50±0.73		14.4±10.2		13.1±8.5	

SD = Standard deviation; LDH = lactate dehydrogenase; ULN = upper limit of normal.

Results

A total of 43 patients were diagnosed with PMF, 6 with post-PV MF, and 2 with post-ET MF. DIPSSplus risk distributions at the time of referral were as follows: 23% low, 18% intermediate-1, 40% intermediate-2, 19% high. Patient characteristics are detailed in tables 1 and 2. 15 patients did not receive any PMF-specific therapy at the time of blood collection and were treatment-naïve. 42 patients were treated with conventional disease-specific therapy: hydroxycarbamide, interferon, or prednisone. None of the patients received JAK inhibitor therapy at the time of sample collection. There was no significant statistical difference in sex (p = 0.8) and age (p = 0.9) between controls and the study group.

MF patients exhibited significantly higher levels of IL-8, sIL-2R, and FLC compared to the control group (table 1). In this study, we analyzed associations between different clinical characteristics and elevated cytokine levels. When correction for multiple testing was performed, only transfusion-dependent anemia (p = 0.03) (fig. 1 A) and splenomegaly (p = 0.02) (table 2) were significantly correlated with sIL-2R excess. We did not find any statistically significant correlations between sIL-2R and IL-8 levels; however, there was correlation between the IL-8 and the κ-FLC plasma concentration (r = 0.288; p = 0.027) (fig. 1 B).

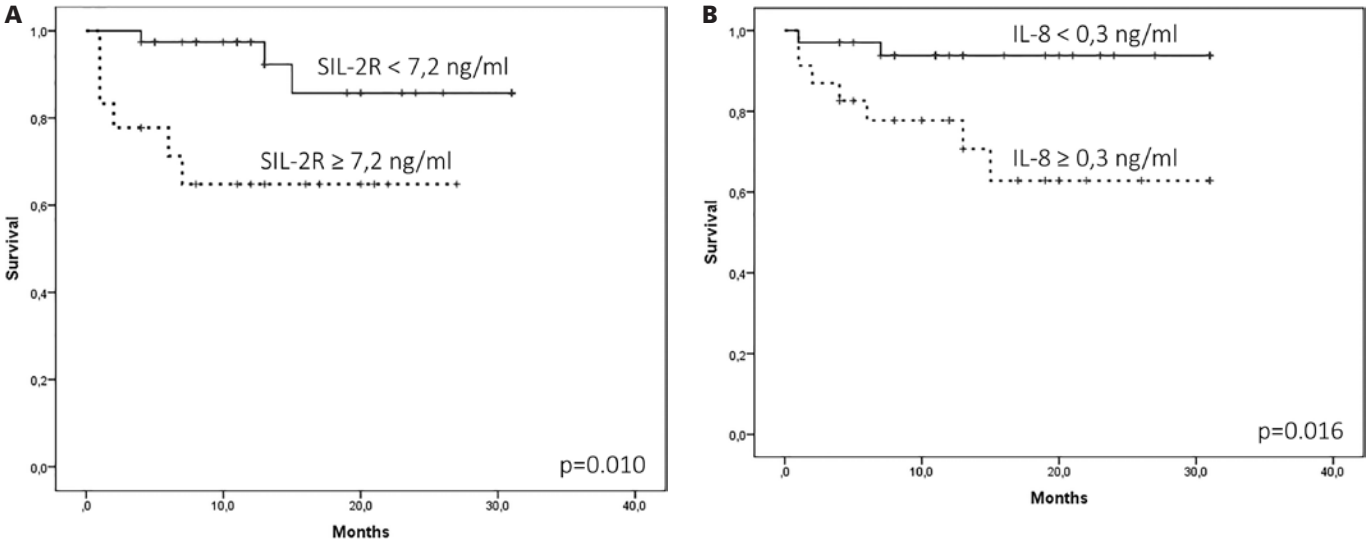

Fig. 2. 1-year overall survival in patients with myelofibrosis according to **A** sIL-2R and **B** IL-8 levels.

Table 3. Results of multivariate analysis of risk factors affecting overall survival

Factor	Hazard ratio (95% CI)	p
IL-8	3.2 (0.5–19.3)	0.2
sIL-2R	17.4 (1.5–196.6)	0.02
DIPSSplus	69.2 (4.6–137.3)	0.002

CI = Confidence interval.

We also analyzed the prognostic relevance of inflammatory markers. Median follow-up from the time of serum collection was 12 months (range 1–31 months). During this time period, 9 deaths were documented. 5 patients died due to disease progression or acute myeloid leukemia transformation, 2 from infectious complications, 1 due to multiorgan failure, and 1 from hemorrhagic stroke.

Univariate analysis demonstrated that increased levels of IL-8 ($p = 0.016$) and sIL-2R ($p = 0.010$) significantly reduced the 1-year OS (fig. 2 A, B). The cutoff levels for sIL-2R and IL-8 were determined by ROC analysis. Only elevated sIL-2R rate retained significance in multivariate analysis when DIPSSplus risk stratification was added (table 3).

Discussion

PMF, as well as post-ET/PV MF, is characterized by abnormal hematopoietic stem cell proliferation and chronic inflammatory processes. The aim of our study was to evaluated the clinical and prognostic significance of proinflammatory markers such as IL-8, sIL-2R, and κ- and λ-FLC, many of which have been shown in recent studies to be involved in pathological processes [4]. Malignant cells induce complex cytokine interactions involving the microenvironment in the neoplastic process. Recent studies found that myeloid cells stimulate the growth of bone marrow cells derived from MF patients but not from healthy donors. They promote the proliferation and osteoblastic differentiation of bone marrow stromal cells and induce the expression of cytokines such as vascular endothelial growth factor, transforming growth factor beta 1, osteoprotegerin, and collagen type I. The latter facilitates fibrosis and bone formation, and contributes to neoangiogenesis [13]. Other cytokines such as IL-8, IL-6, and IL-1β are also overexpressed by malignant MPN cells and can cause activation of normal inflammatory cells [14].

We analyzed the serum concentrations of IL-8, sIL-2R, and FLC and found that the serum levels of all investigated markers were significantly higher in MF patients compared to healthy donors. There was no statistically significant difference in inflammatory markers between patients who received any MF-related treatment and treatment-naïve patients.

Increased levels of sIL-2R correlated with unfavorable clinical findings such as transfusion-dependent anemia and splenomegaly. The same data were documented in previous studies [15, 14]. However, in contrast to previous reports, we found only a trend towards a higher rate of constitutional symptoms in patients with increased IL-8 levels [15].

While we found no statistically significant correlation between sIL-2R and IL-8, the level of IL-8 positively correlated with the κ-FLC concentration, which was not investigated in previous studies.

Increased levels of FLC may reflect the activation of non-malignant lymphoid cells promoted by MF-associated cytokine release. This may explain why we observed an association between higher levels of FLC and proinflammatory cytokines like IL-8 and sIL-2R.

The level of sIL-2R showed the most significant impact on OS compared to the other investigated markers. The cutoff levels for IL-8 and sIL-2R were determined by ROC analysis. It should be mentioned that this study did not have any confirmatory cohort. Hence, the cutoffs were used just to determine the maximum survival difference and cannot be applied to the general patient population without further surveys.

In the multivariate analysis, sIL-2R retained significance when the clinical prognostic model DIPSSplus was added. IL-8 significantly affected OS only in univariate analysis. In 2 other studies, all markers (IL-8, sIL-2R, and FLC) influenced OS, irrespective of the DIPSSplus risk category [5]. A possible explanation for this finding may be that most of our patients were pretreated, especially those with post-PV/-ET MF. MF-related therapy could possibly reduce the rate of inflammatory cytokines. Some of the available treatment options have an immunomodulatory effect (lenalidomide, thalidomide) and downregulate the release of several cytokines [16, 17]. The JAK1/JAK2 inhibitor ruxolitinib is the only drug with a proven ability to significantly reduce cytokine expression (IL-6, TNF-α, MIP-1β) [2]. Its effect is believed to be associated not only with the direct suppression of the malignant clone but also with the interruption of the crosstalk between tumor cells and their microenvironment.

Conclusion

There is growing evidence that MPNs are accompanied by a chronic inflammatory state which is promoted by the malignant clone. We confirmed the prognostic significance of sIL-2R and IL-8 in MF patients, which was documented in previous studies. We also found an association between FLC and proinflammatory cytokine hyperexpression. Hence, the evaluation of inflammatory markers in patients with MF may be useful in clinical practice.

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Disclosure Statement

The authors declare no competing financial interests.

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