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TUMOR NECROSIS FACTOR-α AND MONOCYTE CHEMOATTRACTANT PROTEIN-1 AS BIOMARKERS IN CHRONIC MYELOID LEUKEMIA

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Chronic myeloproliferative neoplasms (MPN) are specific clonal hematopoietic stem-cell disorders that continuously and without interruption activate the physiologic signal-transduction pathways necessary for normal and adequate hematopoiesis. A marked proinflammatory milieu in chronic myeloid leukemia (CML) is led by many interleukins. This study aimed to evaluate differences in plasma levels of tumor necrosis factor- α (TNF-α) and monocyte chemoattractant protein (MCP-1) between chronic-phase CML patients and healthy individuals as controls.

The study included 50 consecutive patients diagnosed with CML in the chronic phase and under the standard tyrosine kinase inhibitor (TKI) treatment, as well as 20 healthy controls. Blood concentrations of tumor necrosis factor-α (TNF-α) and monocyte Chemoattractant protein-1 (MCP-1) were measured by the enzyme-linked immunosorbent assay method. The levels of MCP-1 were higher in patients than in controls (334.37 vs. 172.18 pg/ml, p = 0.006), while no difference was determined for TNF-α.

There is great importance of MCP-1 and IL-6 as novel, strong, and predictive plasma biomarkers for treatment-free remission in CML. Additional and future research in this field will be of special and great importance in understanding the pathophysiology and treatment of myeloproliferative diseases.

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Key words: *myeloproliferative neoplasms, inflammation, tumor necrosis factor-α, monocyte chemoattractant protein-1, tyrosine kinase inhibitors*

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Introduction

Chronic myeloproliferative neoplasms (MPN) are specific clonal hematopoietic stem-cell disorders that continuously and without interruption activate the physiological signal-transduction pathways necessary for normal and adequate hematopoiesis. Chronic myeloid leukemia (CML), defined by the Philadelphia (Ph) chromosome, along with three Ph-negative neoplasms—polycythemia vera, essential thrombocythemia, and primary myelofibrosis—belongs to the group of four classic MPN (1). A seemingly benign disease, can turn into a more aggressive form, like acute myeloid leukemia, at any time. Chronic myeloid leukemia is a myeloid neoplasm with the reciprocal chromosome translocation between chromosomes 9 and 22, and resulting BCR-ABL1 fusion gene. It is a relatively rare type of myeloproliferative neoplasm, with an incidence of 0.7–1.0/100,000. CML is a hematologic disorder with three phases. Most patients are in the chronic phase of disease, presenting with a high white cell count, splenomegaly, abdominal pain, fatigue and symptoms of hyperviscosity. A smaller percentage of patients, 4–5% present in an accelerated phase CML. This transitional stage of disease is associated with additional genetic mutations and instability, along with an increase in blasts and immature cells in peripheral blood, resulting in treatment resistance. And finally, the smallest number of patients, approximately 1–2%, is in the blast phase of CML. This phase has a poor prognosis and involves transformation into acute leukemia, which may be myeloid, lymphoid or mixed phenotype (2).

Inflammation has played a very important part in all levels of tumorogenesis, from the creation of conditions that influence genetic mutations and making an inflammatory

microenvironment, which supports the development and proliferation of mutated cells. Activation of signaling pathways occurs due to the formation of oncoprotein BCR-ABL, including the RAS-RAF-MEK-ERK pathway, the phosphoinositide 3-kinase (PI3K)-AKT pathway, and the STAT5 pathway. Current research shows a connection between inflammation and the development of malignant processes, including myeloproliferative neoplasms. Cytokines, growth factors, and other modulatory mediators are pivotal for cooperation between the leukemia clone and the tumor microenvironment in the bone marrow (3, 4). Accordingly, several interleukins (IL) are determined in this communication, and make a proinflammatory milieu in CML, led by IL-1β, IL-6, IL-2R, tumor necrosis factor- α (TNF-α), chemokines IL-8, monocyte chemoattractant protein (MCP-1), interferon-induced protein 10, and growth factors (transforming growth factor (TGF)-β, platelet-derived GF, vascular endothelial GF), etc. (5, 6).

Tumor growth occurs due to numerous mutations in the genetic material, driven by the inflammatory process. Chronic inflammation, which constitutively affects the development of mutations in the genetic material of cells, is responsible for the process of tumorogenesis. Chronic inflammation can be caused by various infections, different types of irritants or autoimmune diseases, and all of them severely contribute to tumor formation by making an inflammatory microenvironment conducive to genetic mutations and inadequate cell proliferation and development. Inflammation, as an early stage of tumorogenesis, is strongly linked to neoangiogenesis, fast tumor growth, dissemination of tumor, environmental and general immunosuppression, and finally, unstable genetic material of cells (7, 8).

This study aimed to evaluate differences in plasma levels of TNF-α and MCP-1 between chronic-phase CML patients and healthy individuals as controls.

Materials and Methods

This prospective cross-sectional case-control investigation encompassed 50 consecutive patients diagnosed with CML in the chronic phase of the disease, and recruited at the Clinic of Hematology, Allergology and Clinical Immunology, University Clinical Center Niš, Serbia, in January and February 2023. Clinical, molecular, and biochemical data were prospectively and retrospectively procured from the patients' medical history and through clinical examinations. All included patients were diagnosed and classified according to the current WHO classification and were followed by hematological and cytological assessments per guidelines and measurement of BCR-ABL1 transcript levels using real-time quantitative polymerase chain reaction standardized to the international reporting scale (2, 9).

The patients selected for the study received at least 3 months of tyrosine kinase inhibitor (TKI) treatment. The control group consisted of 20 healthy, age-matched, and community-based adult volunteers who agreed to participate in the investigation. The collected EDTA blood samples were analyzed and examined at the Faculty of Medicine, University of Niš, Serbia. Plasma samples, from patients and controls, were safely stored at -80 °C until a final assessment and measurement of biomarkers by quantitative sandwich technique, enzyme-linked immunosorbent assay (ELISA) kits. The levels of TNF-α and MCP-1 plasma concentrations were estimated using adequate ELISA kits, the Quantikine® R&D Systems (Inc., Minneapolis, MN, USA) following the producer's protocol for each biomarker analyzed. Complete blood count (CBC) parameters—erythrocyte, leukocyte, platelet counts, and hemoglobin concentrations—were evaluated using the COULTER® AcT Diff Analyzer (Beckman Coulter Corporation, Hialeah, FL, USA) before their participation in the study.

Ethical Statement

The study was conducted according to the World Medical Association Code of Ethics (Declaration of Helsinki) for experiments on human subjects. The study was approved by the Faculty's Ethical Board of the Faculty of Medicine, University of Niš, Serbia (Decision No. 12-1693-1/2-4 of February 24, 2025), and all the participants gave and signed informed consent.

Statistical Analysis

Statistical analysis was performed using SPSS 25.0 software (SPSS Inc., Chicago, IL, USA). Data are reviewed as percentages and mean ± standard deviation (SD). The independent t-test was used, based on the normal distribution of the samples (Shapiro–Wilk test), all tests were two-tailed. The Pearson correlation coefficient test was also used. Significance was assumed at a value of $p < 0.05$.

Results

The average age of study subjects was 58.46 ± 14.20 years for the patient group and 56.02 ± 10.13 for the group of healthy subjects ($p > 0.05$). There were 46% of males and 54% of females in the patient group. All patients were under treatment with TKI, and in complete hematological and cytogenetic remission, with the major molecular response (BCR-ABL1 values of ≤ 0.1% IS) present in 78% (n = 39) at the last medical examination.

About half of them received TKI imatinib (52%) or nilotinib (48%). Mean plasma levels of MCP-1 were 334.37 ± 165.15 pg/ml in patients

vs. 172.18 ± 56.37 pg/ml in controls, p = 0.006 (95%CI [49.097–275.284]) (Figure 1a).

Conversely, no significant difference was determined in plasma concentrations of TNF-α between the patient group, 371.19 ± 210.33 pg/ml, and the control group, 214.32 ± 214.32 pg/ml, for p = 0.315 (Figure 1b).

There was no correlation between the measured cytokines.

The average values of all parameters of the CBC and leukocyte differential formula were in the normal range.

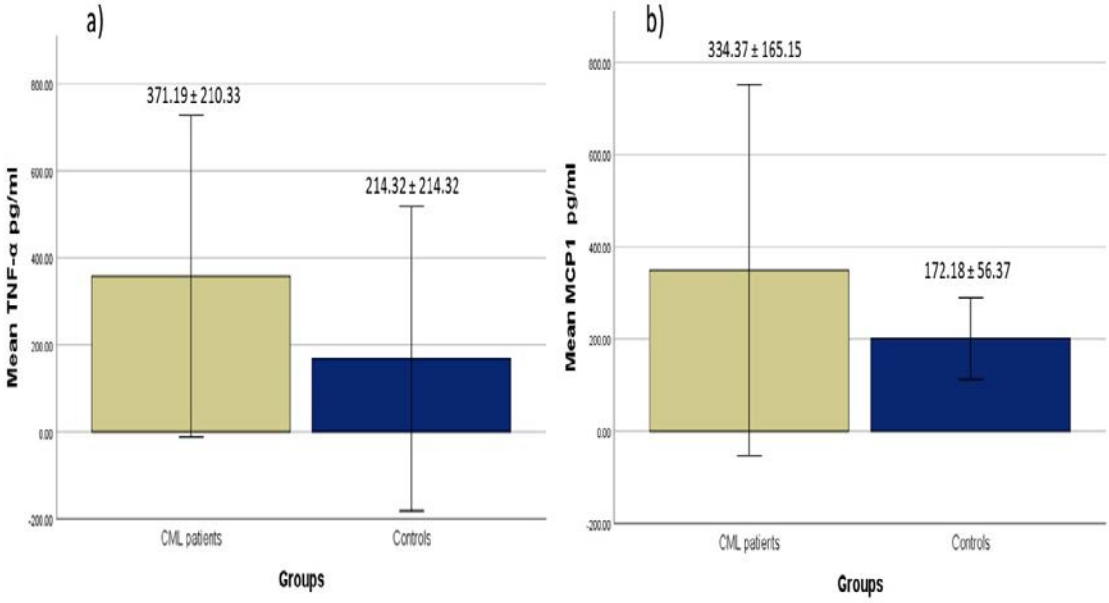

Figure 1. Plasma concentration of a) TNF-α, b) MCP-1 in patients with CML and control groups

Discussion

Myeloproliferative neoplasms are heavily supported by a chronic inflammation that transforms the bone marrow niche into a malignant clone-supporting environment. Patients suffering from myeloproliferative diseases often show elevated levels of proinflammatory cytokines (10). Chronic inflammation and autoimmune disorders affect tumor cells by contributing different levels of inflammatory cytokines such as TNF-α, IL-6, IL-8 and MCP-1 that improve cell proliferation, inhibit apoptosis, change the pathophysiology of cells, stimulate neoangiogenesis and stimulate cell migration, which are actually the processes of tumorogenesis. On the other hand, cancer cells themselves can be a source of inflammatory mediators that modify the microenvironment that may lead to cancer progression (11).

Tyrosine kinase inhibitors are a type of targeted therapy in CML, which attack specific types of tumor cells while causing less damage to normal cells. In CML, TKIs target the abnormal BCR-ABL1 protein that causes uncontrolled CML cell growth and block its function, causing the destruction of CML cells. It is possible that TKI-induced suppression of the feed-forward circles between STATs, IL-6, and NF-κB. For example, TKI was shown to downregulate IL-6 and IL-8 in primary CML cells in vitro, and imatinib mesylate inhibits TNF-α production by myeloid cells *in vitro* (12, 13). TNF-α and MCP-1 are well-known cytokines associated with CML. TNF-α levels were similar between CML patients on TKI treatment and healthy controls in our study, while MCP-1 showed significantly raised concentrations in CML patients. These results are in accordance with other studies with a similar patient population (CML on TKI with achieved MMR) (14, 15).

The pleiotropic function of TNF-α is reflected in the upregulation of multiple pro-inflammatory proteins via the canonical NF-κB and MAPK pathways. The CML stem/progenitor cells produce TNF-α at higher levels compared to their normal counterparts, which provides survival signals through NF-κB/TNF-α feedback loop and, importantly, TNF-α production is not BCR-ABL kinase-dependent (13, 16). Consistently, TNF-α is being activated in TKI-persisting quiescent LSC, and its plasma levels correlate with MPN progression (17). There is a lot of information in the literature on the nature of CML LSC, which are independent of the function of BCR-ABL1, but unfortunately, their eradication has remained largely elusive. It is considered that CML stem/progenitor cells are the source of resistance to therapy and relapse. This is precisely why it is

essential to examine the milieu of these cells as well as the production of cytokines and chemokines around them. Leukemia stem/progenitor cells show a distinct upregulation of inflammatory cytokines, which is associated with resistance to chemotherapy.

The process of onco-inflammation is now well known. In various tumors, the participation of cytokines, chemokines and cancer matrix clearly indicates the process of tumorigenesis, but in hematological malignancies, it has long been unknown.

Monocyte chemoattractant protein-1, as one of the first discovered human chemokines, is part of the CC-motif chemokine family, a very big group which includes cell signaling molecules and related receptors. MCP-1 is strong chemotactic factor for monocytes. It is secreted by a lot of dissimilar cell species, such as endothelial, epithelial, immune system cells, smooth muscle, tumor cells, mesangial, monocytic, CNS and fibroblastic cells. MCP-1 can be produced continuously or induced behind to oxidative stress, some activity of cytokines or after responding to a certain factor (17).

The primary role of the MCP-1 chemokine is the regulation of migration and infiltration of monocytes/macrophages. MCP-1 also signals through the NF-κB pathway and is one of the key elements at the intersection of the pathway signaling in MPNs (15, 18). It can arrest the activation of primitive normal progenitor cells, while not affecting the cycling of primitive CML progenitors (16). A recent study identified MCP-1 and IL-6 as novel, strong, and predictive plasma biomarkers for treatment-free remission in CML, among 20 cytokines tested. MCP-1 and IL-6 levels were markedly increased in CML patients in treatment-free remission compared to others. The low MCP-1/IL-6 levels harmed relapse-free survival and showed a significant prediction (8-fold higher risk) of relapse compared to high MCP-1 levels. The results are supposed to arise from a distinct TKI-associated mechanism that is not directed at killing LSC (18). The evidence aligns with our results of increased MCP-1 levels in CML patients who are diagnosed in the stable chronic stage of the disease.

Conclusion

A chronic inflammation of the bone marrow heavily supports myeloproliferative neoplasms. Many proinflammatory cytokines have been investigated. There is great importance of MCP-1 and IL-6 as novel, strong, and predictive plasma biomarkers for treatment-free remission in CML. Additional and future research in this field will be of special and great importance in understanding the pathophysiology, proinflammatory cytokines, and treatment of CML.

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FAKTOR NEKROZE TUMORA-α I MONOCITNI HEMOATRAKTANTNI PROTEIN-1 KAO BIOMARKERI U HRONIČNOJ MIJELOIDNOJ LEUKEMIJI

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Hronične mijeloproliferativne neoplazme (engl. *chronic myeloproliferative neoplasms* – CMPNs) predstavljaju specifične klonske poremećaje hematopoetskih matičnih ćelija koji kontinuirano i bez prekida aktiviraju fiziološke puteve prenosa signala neophodne za normalnu i adekvatnu hematopoezu. Izražen proinflamatorni milje u hroničnoj mijeloidnoj leukemiji (engl. *chronic myeloid leukemia* – CML) predvode mnogi interleukini. Cilj ovog rada bio je da se procene razlike između nivoa faktora nekroze tumora-α (engl. *tumor necrosis factor* – TNF-α) i monocitnog hemoatraktantnog proteina-1 (engl. *monocyte chemoattractant protein* – MCP-1) u plazmi pacijenata u hroničnoj fazi hronične mijeloidne leukemije i zdravih osoba kao kontrolne grupe. Studija je obuhvatila pedeset pacijenata sa dijagnozom CML-a u hroničnoj fazi i na standardnoj terapiji inhibitorima tirozin kinaze (engl. *tyrosine kinase inhibitors* – TKIs) i dvadeset zdravih osoba u kontrolnoj grupi. Koncentracije TNF-α i MCP-1 u krvi merene su metodom ELISA (engl. *enzyme-linked immunosorbent assay*). Nivoi MCP-1 bili su viši kod pacijenata nego kod zdravih osoba (334,37 naspram 172,18 pg/ml; p = 0,006). S druge strane, nije utvrđena razlika kada je ispitivan TNF-α. Velik je značaj MCP-1 i interleukina-6 (IL-6) kao novih, jakih i prediktivnih biomarkera u plazmi za remisiju CML-a bez lečenja. Dodatna i buduća istraživanja u ovoj oblasti biće posebno važna za razumevanje patofiziologije i lečenja mijeloproliferativnih bolesti.

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***Ključne reči**: mijeloproliferativne neoplazme, zapaljenje, faktor nekroze tumora-α, monocitni hemoatraktantni protein-1, inhibitori tirozin kinaze*

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