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Tumor necrosis factor-α (TNF-α) and monocyte chemoattractant protein-1 (MCP-1) as biomarkers in chronic myeloid leukemia

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Abstract

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 work aimed to evaluate differences between TNF-α and MCP-1 plasma levels in 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 ELISA method. The levels of MCP-1 were higher in patients than 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.

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

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Faktor tumorske nekroze-alfa (TNF-a) i monocitni hemoatraktantni protein-1 (MCP-1) kao biomarkeri u hroničnoj mijeloidnoj leukemiji

Sažetak:

Hronične mijeloproliferativne neoplazme (HMP) su specifični klonski poremećaji hematopoetskih matičnih ćelija koji kontinuirano i bez prekida aktiviraju fiziološke puteve prenosa signala neophodne za normalnu i adekvatnu hematopoezu. Izražen proinflamatorni milje kod hronične mijeloidne leukemije (HML) predvođen je mnogim interleukinima. Cilj ovog rada je bio da se procene razlike između nivoa TNF-a i MCP-1 u plazmi kod pacijenata u hroničnoj fazi hronične mijeloidne leukemije i zdravih osoba kao kontrolne grupe. Studija je obuhvatila 50 uzastopnih pacijenata sa dijagnozom HML u hroničnoj fazi i na standardnoj terapiji inhibitorima tirozin kinaze (TKI), kao i 20 zdravih osoba u kontrolnoj grupi. Koncentracije faktora tumorske nekroze-alfa (TNF-a) i hemoatraktantnog proteina-1 monocita (MCP-1) u krvi merene su ELISA metodom. Nivoi MCP-1 bili su viši kod pacijenata nego kod kontrolne grupe (334,37 naspram 172,18 pg/ml, p=0,006), dok nije utvrđena razlika za TNF-a. Postoji veliki značaj MCP-1 i IL-6 kao novih, jakih i prediktivnih biomarkera u plazmi za remisiju HML bez lečenja. Dodatna i buduća istraživanja u ovoj oblasti biće od posebnog i velikog značaja za razumevanje patofiziologije i lečenja mijeloproliferativnih bolesti.

Ključne reči: mijeloproliferativne neoplazme, zapaljenje, faktor nekroze tumora-alfa, hemoatraktantni protein-1 monocita, inhibitori tirozin kinaze

Introduction

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. Chronic myeloid leukemia (CML), defined by the Philadelphia (Ph) chromosome, and three Ph-negative neoplasms, polycythemia vera, essential thrombocythemia, and primary myelofibrosis, belong to the group of four classic MPN (1). Seemingly benign diseases, but they 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 the 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. The most of patients are in chronic phase of disease, presented with a hight white cell count, splenomegaly, abdominal pain, with fatigue and symptoms of hyperviscosity. Smaller number of patients, 4%–5% are presented in accelerated phase CML, a transitional stage of disease associated with additional genetic mutations and instability, an increase in blasts and immature cells in peripheral blood resulting reatment resistance. And finally, the smallest number of patients is in blast phase CML 1%–2%, with poor-prognosis and with transformation into acute leukaemia, which may be myeloid, lymphoid or mixed phenotype. (2)

Inflammation has a very important part in the all levels of tumorogenesis from creation stipulations that influence on genetic mutations and making inflammatory microenvironment which approve 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, a number of 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 (TNF)-α, chemokines (IL-8, monocyte chemoattractant protein-1 (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 when a lot of mutations happen on genetic material led by 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, an all of them severly contributes 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 work aimed to evaluate differences between TNF-α and MCP-1 plasma levels in chronic-phase CML patients and healthy individuals as controls.

Material and methods

This prospective cross-sectional case-control investigation encompassed 50 consecutive patients diagnosed with CML in the chronic phase of diseases, and recruited at the Clinic of Hematology, Allergology and Clinical Immunology, University Clinical Center in Nis, Serbia, in January and February 2023. Clinical, molecular, and biochemical data was prospectively and retrospectively procured from the patients' medical history and through clinical examinations. All patients, which we have included, 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 are those who are 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 Nis, Serbia. Plasma samples, from patients and controls, were safely stored at -80 °C, until a final assessment and measurement of biomarkers by quantitative sandwich enzyme-linked immunoassay technique (ELISA kits), Levels of TNF-α and MCP-1 plasma concentrations were estimated using the adequate ELISA kits, the Quantikine® R&D Systems (Inc. Minneapolis, MN, USA) using the producer's protocol for each evaluated biomarker which is examined. 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 they participated in the study.

Ethical Statement

The work has been conducted out following 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 (Decision No. 12-1693-1/2-4 from 24.02.2025) of the Faculty of Medicine University of Nis, Serbia, and all the participants gave and signed informed consent.

Statistical Analysis

Statistical analysis was escored out with 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 $p < 0.05$.

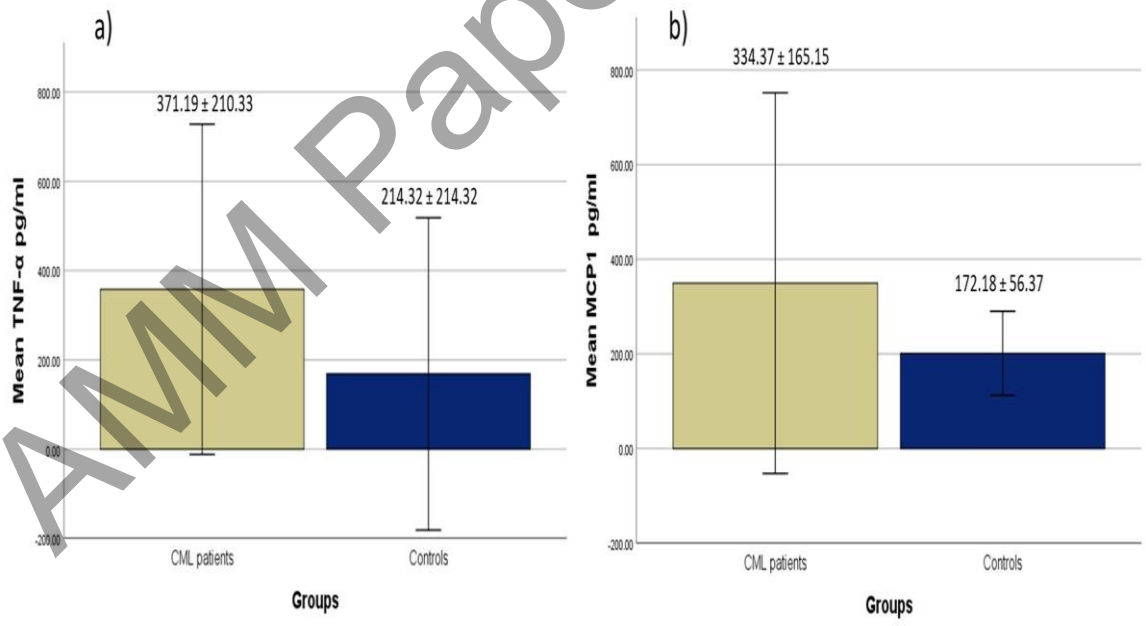
Results

The average age of study subjects was 58.46 ± 14.20 years for patients group and 56.02 ± 10.13 for a group of healthy subjects ($p > 0.05$). There were 46% males and 54% 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 the patients vs. 172.18 ± 56.37 pg/ml in the 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 groups, in patients 371.19 ± 210.33 pg/ml and controls 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.



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-a, IL-6, IL-8 and MCP-1 that improve cell proliferation, inhibit apoptosis, changes the pathophysiology of cells, stimulate neoangiogenesis and stimulate cell migration, which are actually the processes of tumorogenesis. On the other hand, the same cancer cells themselves can be source of inflammatory mediators that modify the microenviorment that can lead to cancer progression (11).

Tyrosine kinase inhibitors are a type of targeted therapy in CML, which attacks 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 (LSC) produce TNF-α at higher levels compared to their normal counterparts, which provides survival signals through the NF-κB/TNF-α feedback loop and, importantly, the 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 are a lot of information in the literature 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 very essential to examine the milieu of these cells as well as the production of cytokines and chemokines around them. LSC 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 for long been unknown.

Monocyte chemoattractant protein-1, as one of the first discovered human chemokine, is a one part of the CC-motif chemokine family, a very big group which includes cell signalling molecules and related receptors. MCP-1 is strong and powerful 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 has the ability to 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 had a negative impact on 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 is in accordance with our results of increased MCP-1 levels in CML patients that are detected in the stable chronic stage of the disease.

Conclusion

Myeloproliferative neoplasms are heavily supported by a chronic inflammation of the bone marrow. 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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Conflict of Interest: The Authors declare there is no conflict of interest for any author of this article.

References

1. Fisher DAC, Fowles JS, Zhou A, Oh ST. Inflammatory pathophysiology as a contributor to myeloproliferative neoplasms. Frontiers in Immunology. 2021 Jun 1;12. doi: 10.3389/fimmu.2021.683401

2. Khoury JD, Solary E, Abla O, Akkari Y, Alaggio R, Apperley JF, et al. The 5th edition of the World Health Organization Classification of Haematolymphoid Tumours: Myeloid and Histiocytic/Dendritic Neoplasms. Leukemia. 2022 Jun 22;36(7):1703–19. doi: 10.1038/s41375-022-01613-1

3. Masselli E, Pozzi G, Gobbi G, Merighi S, Gessi S, Vitale M, et al. Cytokine profiling in myeloproliferative neoplasms: Overview on phenotype correlation, outcome prediction, and role of genetic variants. Cells. 2020 Sep 21;9(9):2136. doi: 10.3390/cells9092136

4. Kleppe M, Kwak M, Koppikar P, Riester M, Keller M, Bastian L, et al. JAK–STAT pathway activation in malignant and nonmalignant cells contributes to MPN pathogenesis and therapeutic response. Cancer Discovery. 2015 Jan 9;5(3):316–31. doi: 10.1158/2159-8290.CD-14-0736

5. Yu H, Pardoll D, Jove R. STATs in cancer inflammation and immunity: a leading role for STAT3. Nature Reviews Cancer. 2009 Oct 23;9(11):798–809. doi: 10.1038/nrc2734

6. Camacho V, Kuznetsova V, Welner RS. Inflammatory cytokines shape an altered immune response during myeloid malignancies. Frontiers in Immunology. 2021 Nov 3;12. doi: 10.3389/fimmu.2021.772408

7. Nishida A, Andoh A. The role of inflammation in cancer: Mechanisms of tumor initiation, progression, and metastasis. Cells [Internet]. 2025 Mar 25;14(7):488. doi: 10.3390/cells14070488

8. Hoermann G, Greiner G, Valent P. Cytokine regulation of microenvironmental cells in myeloproliferative neoplasms. Mediators of Inflammation. 2015 Jan 1;2015(1). doi: 10.1155/2015/869242

9. Branford S. Why is it critical to achieve a deep molecular response in chronic myeloid leukemia? Haematologica. 2020 Sep 17;105(12):2730–7. doi: 10.3324/haematol.2019.240739

10. Wang Y, Zuo X. Cytokines frequently implicated in myeloproliferative neoplasms. Cytokine X. 2019 Mar 1;1(1):100005. doi: 10.1016/j.cytox.2019.100005

11. Anand M, Chodda SK, Parikh PM, Nadkarni JS. Abnormal levels of proinflammatory cytokines in serum and monocyte cultures from patients with chronic myeloid leukemia in different stages, and their role in prognosis. Hematological Oncology . 1998 Dec 1;16(4):143–54. doi: 10.1002/(sici)1099-1069(199812)16:4<143::aid-hon628>3.0.co;2-u

12. Copland M. Treatment of blast phase chronic myeloid leukaemia: A rare and challenging entity. British Journal of Haematology. 2022 Jul 22;199(5):665–78. doi: 10.1111/bjh.18370

13. Wolf AM, Wolf D, Rumpold H, Ludwiczek S, Enrich B, Gastl G, et al. The kinase inhibitor imatinib mesylate inhibits TNF-α production in vitro and prevents TNF-dependent acute hepatic inflammation. Proceedings of the National Academy of Sciences. 2005 Sep 8;102(38):13622–7. doi: 10.1073/pnas.0501758102

14. Nievergall E, Reynolds J, Kok CH, Watkins DB, Biondo M, Busfield SJ, et al. TGF-α and IL-6 plasma levels selectively identify CML patients who fail to achieve an early molecular response or progress in the first year of therapy. Leukemia. 2016 Feb 22;30(6):1263–72. doi: 10.1038/leu.2016.34

15. Pavlovsky C, Cordoba BV, Sanchez MB, Moiraghi B, Varela A, Custidiano R, et al. Elevated plasma levels of IL-6 and MCP-1 selectively identify CML patients who better sustain molecular remission after TKI withdrawal. Journal of Hematology & Oncology. 2023 Apr 29;16(1). doi: 10.1186/s13045-023-01440-6

16. Cashman JD, Eaves CJ, Sarris AH, Eaves AC (1998) MCP-1, not MIP-1alpha, is the endogenous chemokine that cooperates with TGF-beta to inhibit the cycling of primitive normal but not leukemic (CML) progenitors in long-term human marrow cultures. Blood. 1998 Oct 1;92(7):2338-44.

17. Mulholland BS, Forwood MR, Morrison NA. Monocyte chemoattractant protein-1 (MCP-1/CCL2) drives activation of bone remodelling and skeletal metastasis. Current Osteoporosis Reports. 2019 Nov 11;17(6):538–47. doi: 10.1007/s11914-019-00545-7

18. Gallipoli P, Pellicano F, Morrison H, Laidlaw K, Allan EK, Bhatia R, et al. Autocrine TNF-α production supports CML stem and progenitor cell survival and enhances their proliferation. Blood. 2013 Sep 17;122(19):3335–9. doi: 10.1182/blood-2013-02-485607