ORIGINAL ARTICLE

Chemokine (C-C motif) ligand-20 and Interleukin-15 as Novel Biomarkers in Rheumatoid Arthritis Women

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ABSTRACT

Background: Rheumatoid arthritis (RA) is an autoimmune disorder that causes chronic inflammation in the joints, leading to pain, swelling, and stiffness. The disease can also affect other organ systems, increasing the risk of cardiovascular complications. ***Objective:*** *This study aims to evaluate the immunological role of CCL20 and IL-15 in blood of women with rheumatoid arthritis*. ***Methodology:*** A case-control study was conducted at AL-Najaf Medical City, Iraq from October 2023 to February 2025. *This study enrolled 60 healthy participants as a control group, as well as 30 early RA women, 30 moderate RA women, and 30 severe RA women; all women who were diagnosed by the physician. Anti-CCP, RF, CCL20, and IL-15 have been measured using the ELISA technique.* ***Results:*** *Blood levels of anti-CCP (37.51 ± 1.397 U/ml) and RF (39.71 ± 2.007 IU/ml) were substantially higher ($P < 0.0001$) than those of the control group (4.108 ± 0.3711U/ml) and (8.333 ± 0.3359 IU/ml) respectively. The blood concentrations of IL-15 were significantly increased ($P < 0.05$) in moderate and severe RA women. In comparison, the blood levels of CCL20 were significantly increased ($P < 0.05$) in early, moderate, and severe RA subjects. A positive correlation was observed between blood concentrations of CCL20 and IL-15 in all women with RA.* ***Conclusion:*** *CCL20 and IL-15 are potential key indicators for diagnosing rheumatoid arthritis. Increased levels of these biomarkers may reflect disease severity. Furthermore, they could play a role in the treatment of rheumatoid arthritis female participants in our study.*

INTRODUCTION

Rheumatoid arthritis is a chronic autoimmune condition that primarily affects the joints, leading to inflammation, pain, and progressive joint damage[1]. As a systemic disease, it can also impact various organ systems, resulting in a range of extra-articular symptoms[2,3]. Although the exact cause is not fully understood, it most likely involves a complex interaction of genetic, environmental, and immune factors[4].

Common symptoms include joint pain, stiffness, and swelling, often involving the small joints of the hands and feet[5]. CCL20 (C-C motif chemokine ligand 20), a cytokine of the chemokine family, is an important factor of immune effectors activation and recruitment under infection or tissue injuries. Restricts particularly, the entrapment of leukocytes (T cells, dendritic cells, and immature B cells)[6].

CCL20 is noted to be potently regulated and induced by proinflammatory cytokines, environmental agents, and bacterial end products[1,2]. Its activities are mediated by the CCR6 receptor present on target cells. When this receptor is activated, intracellular signal transduction pathways are activated which results in the migration and activation of immune cells[7,8]. High levels of CCL20 have been detected in a series of inflammatory and autoimmune diseases, such as rheumatoid arthritis (RA). CCL20 further augments TNF-α-induced activation and recruitment of T cells and other inflammatory cells into the joints, promoting tissue destruction and chronic inflammation in RA[9,10].

Interleukin-15 (IL-15) recently emerged as another contributing factor in RA pathophysiology, which is elevated in the plasma and synovial fluid of patients with RA. IL-15 exists in soluble and membrane-bound forms, and is capable of enhancing the activation and proliferation of various immune cell subtypes to sustain chronic inflammatory processes that ultimately drive the pathogenesis of RA[11].

In this study, we aimed to measure CCL20 and IL-15 levels in females with early, moderate and severe rheumatoid arthritis. The main goals were to ascertain their immunological function, to assess their feasibility as biomarkers of disease severity[12], and to consider them as potential alternative therapeutic targets to conventional chemical agents[13].

METHODOLOGY

Female subjects (participants)

A case-control study was carried out at the Rheumatology and Arthritis Department, Al-Najaf Hospital, Al-Najaf City, Iraq (October 1, 2023 to February 1, 2025). A total of 150 subjects aged 18–60 years were enrolled in the study. The study cohort consisted of 30 women with early rheumatoid arthritis (RA), 30 with moderate RA, 30 with severe RA and a control group of 60 healthy individuals without clinical history or evidence of other diseases. Physicians confirmed the diagnoses for all the patients.

Ethical Considerations

Approval for the study was obtained from the Institutional Ethics Committee of the College of Medicine at the University of Kufa and the Scientific Research Committee of the Najaf Health Department (7624/ 13/12/2023).

Measurement of blood levels of Anti-CCP, RF, CCL20, and IL-15

This test was conducted according to the manufacturing company instructions (Bioassay Technology Laboratory. Shanghai, China). Five ml of blood sample were collected from all the individuals and 2 ml of serum for each individual has been obtained by centrifugation at 8000 rpm/10 min. This serum concentration has been used for the measurement of Anti-CCP, RF, CCL20, and IL-15 by the enzyme-linked immunosorbent assay (ELISA)[14,15].

Statistical analysis

The mean and standard error (SE) were calculated for each value using GraphPad Prism. A p-value of less than 0.05 was considered statistically significant in the analysis[16]

RESULTS

Anti-CCP and RF

The study demonstrated a significant increase ($P < 0.0001$) in blood levels of anti-CCP (37.51 ± 1.397 U/ml) and RF (39.71 ± 2.007 IU/ml) among all female patients compared to the control group, which had levels of 4.108 ± 0.3711 U/ml and 8.333 ± 0.3359 IU/ml, respectively (Figure 1 and 2). There were highly significant differences ($p < 0.0001$) in anti-CCP levels among patients with early (27.12±2.442 U/ml), moderate (27.12 ± 2.442 U/ml), and severe (51.24 ± 3.116 U/ml) rheumatoid arthritis compared to the control group. The anti-CCP levels in patients with severe rheumatoid arthritis were significantly higher ($P < 0.0001$) compared to those with early and moderate rheumatoid arthritis (Figure 3). RF levels showed highly significant differences ($p<0.0001$) among female patients women with early (30.19±1.360 IU/ml), moderate (35.88 ± 1.465 IU/ml), and severe (67.11 ± 5.580 IU/ml) rheumatoid arthritis compared to the control group. Furthermore, patients with severe rheumatoid arthritis had significantly higher RF levels (p 0.0001) than those with early and moderate rheumatoid arthritis (Figure 4).

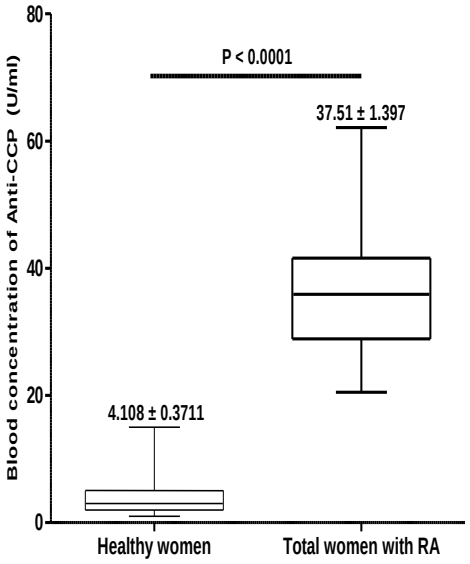

Fig.1: Anti-CCP blood levels in all women with RA compared to the control group

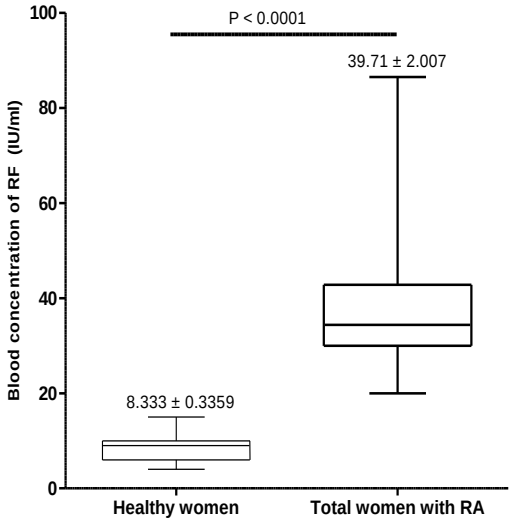

Fig. 2: RF blood levels in all women with RA compared to the control group

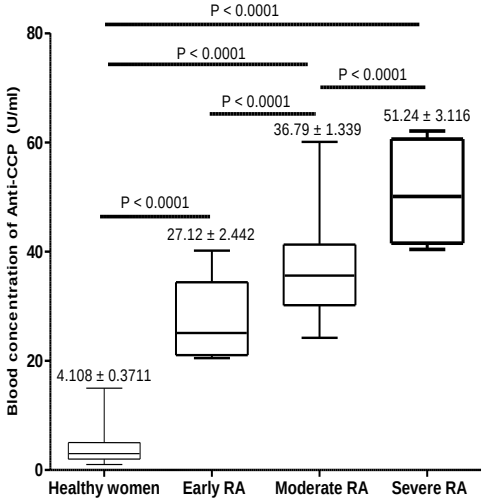

Fig.3: Anti-CCP blood levels in women with early, moderate, sever RA compared to the control group

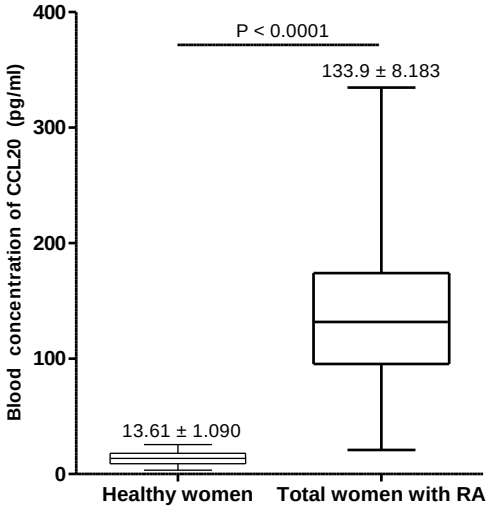

Fig.5: CCL-20 blood levels in all women with RA compared to the control group

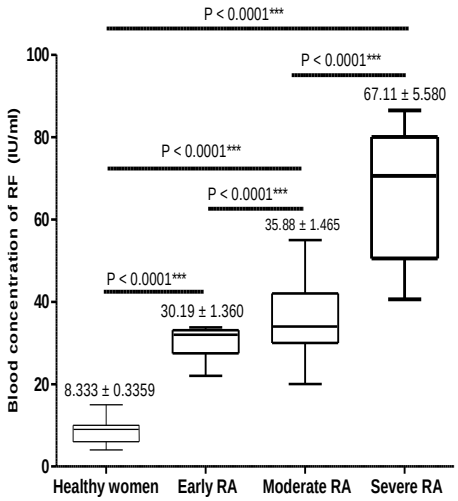

Fig.4: RF blood levels in women with early, moderate, sever RA compared to the control group

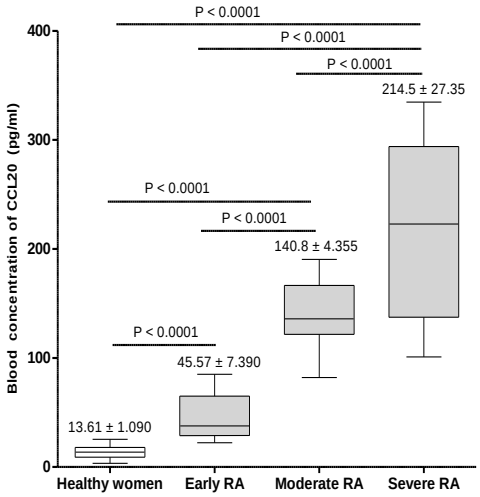

Fig.6: CCL-20 blood levels in women with early, moderate, sever RA compared to the control group

CCL-20

Results from this study showed a markedly higher serum levels of CCL20 in RA female patients (133.9 ± 8.183 pg/ml) when compared with healthy controls (13.61 ± 1.090 pg/ml, $p < 0.0001$) (Figure 5). CCL20 levels were significantly higher in patients with RA (early-stage) than in the control group (45.57 ± 7.390 pg/ml; $p < 0.0001$). Similarly, moderate RA patients also exhibited markedly higher levels of serum CCL20 (140.8±4.355pg/ml) than controls ($p<0.0001$). Moreover, female patients with severe RA had statistically significant higher levels of CCL20 than did patients with either early or moderate RA ($p < 0.0001$) (Figure 6).

IL-15

IL-15 levels which showed significant elevation in female patients (RA) (105.3 ± 6.815 pg/ml) was compared with healthy controls (and (19.52 ± 2.180 pg/ml ($p < 0.0001$) (Figure 7) How ever there was no significant difference between healthy female and early-stage RA (25.76 ± 3.811 pg/ml, $p = 0.0793$). Levels of IL-15 were significantly higher in female with moderate RA (34.55 ± 4.354 pg/ml) when compared to healthy controls ($p = 0.0059$). IL-15 levels in ARA were notably elevated, with the most significant levels observed in those with severe RA (162.7 ± 5.684 pg/ml) compared to healthy individuals ($p<0.0001$). In addition, the severity of RA was associated with increased IL-15 levels ($p < 0.0001$) (Figure 8).

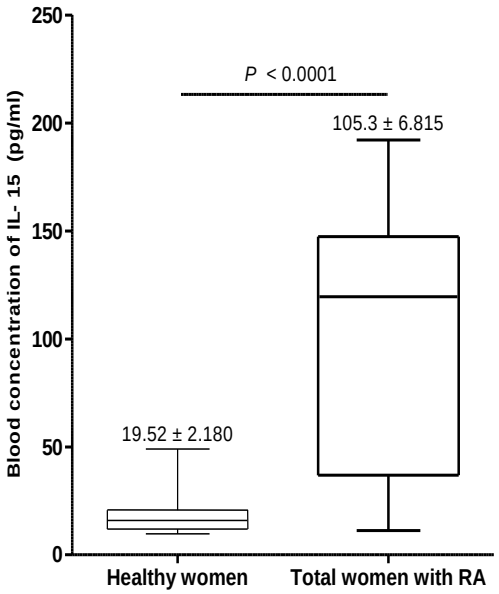

Fig.7: IL-15 blood levels in all women with RA compared to the control group

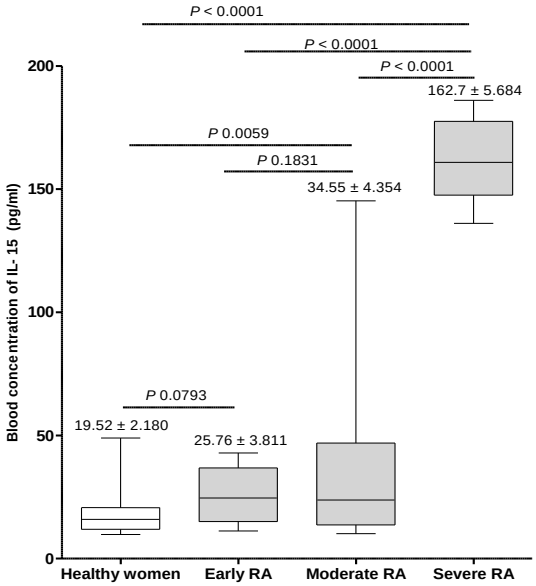

Fig.8: IL-15 blood levels in women with early, moderate, sever RA compared to the control group

DISCUSSION

Rheumatoid arthritis (RA) is a chronic autoimmune disease that primarily affects the joints, leading to inflammation, pain, and restricted mobility[17]. It is two to three times more prevalent in women than men, likely due to hormonal influences. The disease results from a complex interplay of genetic and environmental factors, many of which remain unidentified[18]. RA occurs when the immune system mistakenly triggers inflammation in the synovium, the joint lining, causing pain, swelling, and stiffness[19].

Our study reported significantly higher blood levels of anti-CCP antibodies and rheumatoid factor (RF) in females with RA compared to healthy controls.

They are well-established biomarkers for the diagnosis of rheumatoid arthritis (RA) and disease activity[20]. Anti-CCP antibodies have a high specificity for RA and are significantly associated with disease severity and progression. That higher frequency indicates a persistent autoimmune reaction to citrullinated proteins which plays a role in the inflammation and injury of joints[21]. Autoantibodies such as rheumatoid factor (RF), a type of autoantibody that binds to the Fc portion of IgG, is frequently observed in RA patients and is associated with chronicity and extra-articular complications[22]. According to previous studies, the serum levels of TNF-α, IL-1β, IL-6, and sTNFRs have been shown to be significantly higher in women with rheumatoid arthritis and may contribute to rheumatoid arthritis diagnosis and follow-up. Due to its higher prevalence in women, hormones and genetic predisposition (and consequent immune dysregulation) are thought to cause increased levels of biomarker levels in RA patients[23]. In short, future studies into the relationships between these autoantibodies and disease severity, response to treatment and outcome could provide important insights into the pathogenesis of RA, its management and outcomes.

This study further showed that women with rheumatoid arthritis had considerably elevated plasma levels of CCL20 and IL-15 compared to healthy controls, thus suggesting their potential as biomarkers of RA activity. CCL20, an inflammatory chemokine, recruits immune cells such as Th17 cells and dendritic cells into inflamed joints[24]. Such increased levels in the RA patient represents the ongoing inflammation and is one of the important contributors to disease progression. CCL20 is a potentially attractive target for therapeutics, given its clear correlation with synovial inflammation[25].

IL-15 is a T-cell activation, survival, and proliferation cytokine that promotes inflammation. It also acts to stimulate macrophages and natural killer (NK) cells, thereby promoting the inflammatory response in rheumatoid arthritis[26]. Increased levels of IL-15 are associated with chronic T-cell activation that contributes to a progressive disease course and joint destruction. Furthermore, IL-15 is believed to drive autoreactive T cells and increase other proinflammatory mediators[27], which contributes to the persistent microenvironment of inflammation driven by the chronic local inflammation seen in RA.

In women with rheumatoid arthritis, there are grossly elevated levels of CCL20 and IL-15 secretion,

indicating its potential role in the severity and progression of the disease[28]. More research is required to investigate their role as therapeutic targets to suppress inflammation and delay joint destruction[29].

Recent evidence highlights the IL-2/15Rβ chain, the shared receptor subunit of IL-2 and IL-15, as a novel therapeutic target in RA [17]. Targeting the IL-15 signaling pathway has also been shown to reduce inflammatory responses and joint pathology, suggesting a potential therapeutic avenue in AIA[30]. These data indicate that blockade of IL-15 mediated immune activation may represent an important therapeutic approach for limiting inflammation and erosive damage in RA women[31].

CONCLUSION

CCL20 and IL-15 could be core determinants in diagnosing women with rheumatoid arthritis. Increased levels of these biomarkers are associated with disease severity. Moreover, these markers can be useful in the treatment of female patients with rheumatoid arthritis.

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REFERENCES

1. Radu AF, Bungau SG. Management of rheumatoid arthritis: an overview. Cells 2021;10(11):2857; doi: https://doi.org/10.3390/cells10112857
2. Huang J, Fu X, Chen X, Li Z, Huang Y, Liang C. Promising therapeutic targets for treatment of rheumatoid arthritis. Front Immunol 2021;12:686155; doi: https://doi.org/10.3389/fimmu.2021.686155
3. McInnes IB, Schett G. The pathogenesis of rheumatoid arthritis. N Engl J Med 2011;365(23):2205–19. doi: https://doi.org/10.1056/NEJMra1004965
4. Smith MH, Berman JR. What is rheumatoid arthritis? Jama 2022;327(12):1194–. doi: https://doi.org/10.1001/jama.2022.1767
5. Tamara KM Alkhalidi and Aljanaby AAJ. Vascular Endothelial Growth Factor and Neutrophil Gelatinase-Associated Lipocalin as Critical Biomarkers in Patients with Burn Injury. Egypt J Med Microbiol 2025; 34.
6. Meitei HT, Jadhav N, Lal G. CCR6-CCL20 axis as a therapeutic target for autoimmune diseases. Autoimmun Rev 2021;20(7):102846. doi: https://doi.org/10.1016/j.autrev.2021.102846
7. Wang D, Yang L, Yu W, Wu Q, Lian J, Li F, Liu S, Li A, He Z, Liu J, Sun Z. Colorectal cancer cell-derived CCL20 recruits regulatory T cells to promote chemoresistance via FOXO1/CEBPB/NF-κB signaling. J Immunother Cancer 2019;7:1–5. doi: https://doi.org/10.1186/s40425-019-0699-1
8. Meitei HT, Jadhav N, Lal G. CCR6-CCL20 axis as a therapeutic target for autoimmune diseases. Autoimmun Rev 2021;20(7):102846. doi: https://doi.org/10.1016/j.autrev.2021.102846
9. Ola AA Alkhirsan and Aljanaby AAJ. Azurocidin and CXCL9 as Novel Biomarkers in Women with Asymptomatic Bacteriuria. Egypt J Med Microbiol 2025; 34.
10. Kaneko S, Kondo Y, Yokosawa M, Furuyama K, Segawa S, Tsuboi H, Kanamori A, Matsumoto I, Yamazaki M, Sumida T. The RORγt-CCR6-CCL20 axis augments Th17 cells invasion into the synovia of rheumatoid arthritis patients. Mod Rheumatol 2018;28(5):814–25. doi: https://doi.org/10.1080/14397595.2017.1340753
11. Reyes-Pérez IV, Sánchez-Hernández PE, Muñoz-Valle JF, Martínez-Bonilla GE, García-Iglesias T, González-Díaz V, García-Arellano S, Cerpa-Cruz S, Polanco-Cruz J, Ramírez-Dueñas MG. Cytokines (IL-15, IL-21, and IFN-γ) in rheumatoid arthritis: association with positivity to autoantibodies (RF, anti-CCP, anti-MCV, and anti-PADI4) and clinical activity. Clin Rheumatol 2019;38:3061–71. doi: https://doi.org/10.1007/s10067-019-04672-4
12. Park MK, Her YM, La Cho M, Oh HJ, Park EM, Kwok SK, Ju JH, Park KS, Min DS, Kim HY, Park SH. IL-15 promotes osteoclastogenesis via the PLD pathway in rheumatoid arthritis. Immunol Lett 2011;139(1-2):42–51. doi: https://doi.org/10.1016/j.imlet.2011.04.004
13. Kurowska W, Przygodzka M, Jakubaszek M, Kwiatkowska B, Maslinski W. Interleukin-15 as a biomarker candidate of rheumatoid arthritis development. J Clin Med 2020;9(5):1555. doi: https://doi.org/10.3390/jcm9051555
14. Mohammad AK, Aljanaby AAJ. A key role of interleukin 34 in patients with chronic kidney disease and lupus nephritis. Egypt J Med Microbiol 2025;34(1):289–93.
15. Al-janabi DR, Aljanaby AAJ. Bacteriological investigation of pyelonephritis in AL-Najaf Governorate, Iraq: a cross-sectional study. In: BIO Web Conf 2024;84:03014. EDP Sciences.
16. Toma RS, Aljanaby AAJ, Al-Hadraawy SK. Evaluation level of interleukin IL-33 and IL-19 in patients infected with Entamoeba histolytica and H. pylori. In: AIP Conf Proc 2023;2977(1):1–5. AIP Publishing.

17. Yang XK, Xu WD, Leng RX, Liang Y, Liu YY, Fang XY, Feng CC, Li R, Cen H, Pan HF, Ye DQ. Therapeutic potential of IL-15 in rheumatoid arthritis. Hum Immunol 2015;76(11):812–8. doi: https://doi.org/10.1016/j.humimm.2015.08.003
18. Aletaha D, Smolen JS. Diagnosis and management of rheumatoid arthritis: a review. Jama 2018;320(13):1360–72. doi: https://doi.org/10.1001/jama.2018.13103
19. Jang S, Kwon EJ, Lee JJ. Rheumatoid arthritis: pathogenic roles of diverse immune cells. Int J Mol Sci 2022;23(2):905. doi: https://doi.org/10.3390/ijms23020905
20. Vossenaar ER, van Venrooij WJ. Anti-CCP antibodies, a highly specific marker for (early) rheumatoid arthritis. Clin Appl Immunol Rev 2004;4(4):239–62.
21. Van Venrooij WJ, Van Beers JJ, Pruijn GJ. Anti-CCP antibodies: the past, the present and the future. Nat Rev Rheumatol 2011;7(7):391–8. doi: https://doi.org/10.1038/nrrheum.2011.56
22. Karimifar M, Moussavi H, Babaei M, Akbari M. The association of immunoglobulin A, immunoglobulin G and anti-cyclic citrullinated peptide antibodies with disease activity in seronegative rheumatoid arthritis patients. J Res Med Sci 2014;19(9):823.
23. Nair N, Wilson AG, Barton A. DNA methylation as a marker of response in rheumatoid arthritis. Pharmacogenomics 2017;18(14):1323–32. doi: https://doi.org/10.2217/pgs-2017-0073
24. Mrabet D, Laadhar L, Sahli H, Zouari B, Haouet S, Makni S, Sellami S. Synovial fluid and serum levels of IL-17, IL-23, and CCL-20 in rheumatoid arthritis and psoriatic arthritis: a Tunisian cross-sectional study. Rheumatol Int 2013;33:265–6. doi: https://doi.org/10.1007/s00296-012-2445-6
25. Guziejko K, Czupryna P, Pancewicz S, Świerzbińska R, Dunaj J, Kruszewska E, Król ME, Zajkowska J, Grygorczuk S, Żebrowska A, Kondrusik M. Analysis of CCL-4, CCL-17, CCL-20 and IL-8 concentrations in the serum of patients with tick-borne encephalitis and anaplasmosis. Cytokine 2020;125:154852. doi: https://doi.org/10.1016/j.cyto.2019.154852
26. Zekri L, Hagelstein I, Märklin M, Klimovich B, Christie M, Lindner C, Kämereit S, Prakash N, Müller S, Stotz S, Maurer A. Immunocytokines with target cell–restricted IL-15 activity for treatment of B cell malignancies. Sci Transl Med 2024;16(737):eadh1988. doi: https://doi.org/10.1126/scitranslmed.adh1988
27. Lee H, Park SH, Shin EC. IL-15 in T-cell responses and immunopathogenesis. Immune Netw 2024;24(1):e11. doi: https://doi.org/10.4110/in.2024.24.e11
28. Khalafi M, Maleki AH, Symonds ME, Sakhaei MH, Rosenkranz SK, Ehsanifar M, Korivi M, Liu Y. Interleukin-15 responses to acute and chronic exercise in adults: a systematic review and meta-analysis. Front Immunol 2024;14:1288537. doi: https://doi.org/10.3389/fimmu.2024.1288537
29. Liu GY, Jia QY, Li ZJ, Li YG, Zhang MM, Liu MY, Wang XL, Li Y. Adenovirus-mediated CCL20/IL-15 gene transfer enhances antitumor immunity in mice. Immunobiology 2014;219(6):475–81. doi: https://doi.org/10.1016/j.imbio.2014.02.003
30. Harris KM, Fasano A, Mann DL. Monocytes differentiated with IL-15 support Th17 and Th1 responses to wheat gliadin: implications for celiac disease. Clin Immunol 2010;135(3):430–9. doi: https://doi.org/10.1016/j.clim.2010.05.001
31. Castillo EF, Schluns KS. Regulating the immune system via IL-15 transpresentation. Cytokine 2012;59(3):479–90. doi: https://doi.org/10.1016/j.cyto.2012.02.002