

# C-C Chemokine Receptor Type 5 Links COVID-19, Rheumatoid Arthritis, and Hydroxychloroquine

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## Research

**Keywords:** COVID-19, SARS-COV-2, Hydroxychloroquine, rheumatoid arthritis

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**C-C chemokine receptor type 5 links COVID-19, Rheumatoid arthritis, and Hydroxychloroquine**

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## **Abstract**

### **Background**

Patients with rheumatoid arthritis (RA) represent one of the fragile patient groups that might be susceptible to coronavirus disease -19 (COVID-19) and its severe form. On the other side, RA patients have been found not to have an increased risk of COVID19 infection. Moreover, some of the Disease-Modifying Anti-Rheumatic Drugs (DMARDS) commonly used to treat rheumatic diseases like Hydroxychloroquine (HCQ) were proposed as a potential therapy for COVID19 with a lack of full understanding of their molecular mechanisms. This highlights the need for the discovery of common pathways that may link both diseases at the molecular side

### **Methods**

We used the in silico approach to investigate the transcriptomic profile of RA synovium compared to osteoarthritis and healthy controls to identify RA specific molecular pathways shared with that of severe acute respiratory syndrome-corona virus-2 (SARS-COV-2) infected lung tissue.

### **Results**

Our results showed upregulation of chemotactic factors, including CCL4, CCL8, and CCL11, that all shared CCR5 as their receptor, as a common derangement observed in both diseases; RA and COVID-19. Moreover, our results also highlighted that HCQ might interfere with the COVID-19 infection through its ability to upregulate specific immune cell populations like activated natural killer (NK) cells, besides blocking CCR5 rich immune cell recruitment to the SARS-COV-2 infected lungs

### **Conclusion**

Our results might explain some of the reports that showed beneficial effects and indicate the need for proper patients stratification on their immune profile before selecting the therapeutic protocol or clinical trial enrollment.

### **Keyword**

COVID-19, SARS-COV-2, Hydroxychloroquine, rheumatoid arthritis

## **Background**

Since the outbreak of Coronavirus disease-19 (COVID-19) disease, the clinical features of this disease showed significant variability between different subpopulations. Severe acute respiratory syndrome coronavirus 2, shortened to SARS-CoV-2, is the virus that causes COVID-19 disease. Initially, patients with chronic diseases, as well as immunodeficiencies, were considered as high-risk groups patients for the development of the more severe form of the COVID-19[1, 2]. Patients with rheumatoid arthritis (RA), a prevalent immune-mediated disease, are at higher risk of bacterial and viral infections due to its pathogenesis and the use of immunosuppressive agents as an RA treatment. As a result, RA patients represent one of those fragile patients groups that might be susceptible to the critical form of the COVID19 disease[3-5].

Unexpectedly, recent reports showed that patients with RA have no increased risk of COVID-19 infection. Moreover, some of the Disease-Modifying Anti-Rheumatic (DMARDs) that commonly used to treat rheumatic diseases like Hydroxychloroquine (HCQ) were proposed as potential therapies for COVID-19 [6-9]. HCQ used as monotherapy in mild RA cases, or it can be used as a combined treatment, particularly with methotrexate and sulphasalazine as Triple Disease Anti-Rheumatic Drugs (tDMARDs) regimen. Several mechanisms were proposed for HCQ to produce its action, and this includes the anti-inflammatory effect through lysosomal acidification interference and phospholipase A2 inhibition[10, 11]. Also, HCQ was proposed to modulate the inflammatory response through its inhibition of the toll-like receptors signal as well as the T and B cell receptors leading to inhibition of their cytokine production, including the interleukin (IL)-1 and IL-6[11, 12]. This cytokine inhibition was proposed as an essential mechanism that might explain the role of HCQ in reducing the cytokine storm essential in COVID-19 pathogenesis[13]. HCQ was also reported to inhibit viral replication[14].

The controversial results that recently linked to the efficacy of HCQ in COVID-19, in addition to the lack of full understanding of its molecular mechanisms, highlight the need for the discovery of common pathways that may link both diseases; COVID-19 and RA at the molecular side. This step is essential for the identification of possible targets that can block pathogenesis of RA and prevent severe forms of COVID-19. Also, it might help in

identifying the predictive biomarkers that can help in more efficient patient stratification to predict COVID-19 patient's responses to HCQ.

In this study, we used in silico approach to investigate the transcriptomic profile of RA synovium to identify shared molecular pathways with that of SARS-COV-2 infected lung tissue.

## **Materials and Methods**

### **RA synovium specific DEG**

The Gene Expression Omnibus (GEO) public repository was used to retrieve the gene expression profile of synovial tissue from 33 RA, 26 osteoarthritis (OA) patients, and 20 healthy controls from three datasets (GSE55235, GSE55457, GSE55584) as previously reported[15]. Raw cell files were reanalyzed using AltAnalyze tool (20) and in house pipeline for normalization and filtration as previously described[16] to identify novel synovium related biomarkers.

### **tDMARDs response in RA synovium**

We used the publicly available synovial tissue transcriptomic data to compare the infiltration of the immune cells at baseline and after six months of tDMARDs to identify subgroups that might not respond well to tDMARDs. RNAseq dataset (GSE97165) of synovial biopsies taken from 19 early RA (defined as within 12 months of the onset of symptoms) patients at baseline and after six months of tDMARDs treatment were retrieved and reanalyzed.

### **SARS-COV-2 and RA**

RNAseq dataset (GSE147507) were retrieved using the Gene Expression Omnibus (GEO) and used to identify DEGs between infected and uninfected lung samples using BioJupies tools [17].

### **Pathways and gene set enrichment**

Differentially expressed genes between the subgroups were defined, and gene set enrichment analysis was performed to identify the underlying pathways in each group using BioJupies tools. The DEGs were explored for common pathways using Metascape online tool (<http://metascape.org>) [10].

### **Estimating Immune and stromal cells in the synovium**

In order to achieve this goal, we used a recently available tool called ESTIMATE (Estimation of STromal and Immune cells in MAlignant Tumor tissues using Expression data) to estimate the difference in the infiltration of immune cells in healthy, OA and RA synovium. ESTIMATE R package was used to estimate the difference in immune cells' infiltration between the three groups using their transcriptomic profile,

### **Estimating infiltrating Immune cells and their activation status in the synovium**

The raw RNAseq data were used for in silico prediction of the immune cells' infiltration of the synovial tissue using CIBERSORT analytical tool to evaluate the pre versus post tDMARDs changes in the immune population and/or activation status. Then, patients were divided according to the level of alteration in immune cells percentage after the treatment. The immune cells that express a higher level of the identified receptor were explored using the Database of Immune Cell Expression (DICE) project tool (<https://dice-database.org/>). The expression of the chemokine receptor was searched in a microarray dataset (GSE77298) of synovial biopsies of RA and healthy controls.

## **Results**

### **RA synovium express genes related to immune cells activation, migration, signaling, and response to viruses**

For a better understanding of the RA disease pathogenesis, we reanalyze the gene expression profile of synovial tissue from 33 RA and compare to samples from 26 OA and 20 healthy controls. Our results showed that RA synovium expresses a specific signature that can differentiate it clearly from OA as well as healthy controls. This includes cytokine-mediated signaling pathway, positive regulation of cytokine production, Interleukin-2 family signaling, T cell receptor signaling pathway, leukocyte migration, negative regulation of chemotaxis, cellular response to interleukin-1, T cell activation, and regulation of morphogenesis of an epithelium. Moreover, pathways related to defense response to other organisms, antigen processing and presentation of peptide antigen via major histocompatibility complex (MHC) class I and response to the virus were also enriched specifically in RA synovium. (Figure 1, table1, table 2)

### **RA synovium express higher CCL5 and its receptor CCR5**

Next and in order to investigate the role of the main cytokines that control the immune response including cell number, activation, maturation, differentiation, and migration, we filtered the top DEGs between the three groups (healthy, OA, and RA) to look for chemokines and interleukins only. Interestingly, RA synovium showed significantly higher expression of important chemokines ligands (CCL18, CXCL9, CXCL10, CXCL13 CCL5, and its receptor CCR5. Moreover, RA synovium expresses higher interleukins related genes (IL21R, IL32, IL2RG) (Table 3).

### **RA synovium showed a higher infiltration of plasma cells, CD4 memory T cells, and gamma delta T cells but less dendritic and activated NK cells**

In order to decipher the effect of infiltrating immune cells to the synovium and their status of activation, which might mask the local gene expression and can explain the dynamics of immune cells in disease pathophysiology, we explored the immune infiltration using in silico tools. RA synovium showed a significantly higher level of infiltrating immune cells

compared to OA and healthy controls confirming the DEGs and pathways enrichment results. Specifically, RA synovium showed higher infiltration of plasma cells, CD4 memory T cells, and gamma delta T cells but less dendritic and activated NK cells (Figure 2).

### **SARS-COV-2 infected lungs express more CCL4, CCL8, and CCL11 that share CCR5 as a common receptor**

Next, we tried to understand some of the molecular mechanisms involved in SARS-COV-2 pathogenesis with potential interaction with the mechanisms and pathways involved in RA. Eighty-four DEGs were identified between uninfected and COVID-19 infected lung samples. These DEGs were enriched in pathways specific to (response to the virus, response to interferon, leukocyte activation, and chemotaxis) (Figure 3A). Interestingly, SARS-COV-2 infected lungs express more CCL4, CCL8, and CCL11; the three ligands shared the same receptor, which is CCR5 (Figure 3B). Top immune cells that express CCR5 were CD4 T memory T reg cells, Th17, Th1, and monocytes.

### **tDMARDs Treatment In Early RA Increase Synovial Activated Natural Killers And Resting Mast Cells But Decrease Plasma Cells And M1 Macrophages**

Next, we tried to investigate the effect of tDMARDs on immune modulation, which might improve our understanding of its role in the treatment of RA as well as other diseases like COVID19 infection. To achieve this, we investigated the effect of the treatment of tDMARDs on different immune cell populations of the synovium. Our results showed that four immune cell populations were significantly changed after six months of tDMARDs. This includes the resting mast cells and activated NK cells that were shown to be increased by 84% and 74% of patients, respectively. On the other hand, M1 macrophages and plasma cells were decreased after treatment in 68% and 58% of patients, respectively (Figure 4).

### **DMARDs can block RA pathogenic CCR5 rich immune cell recruitment.**

Further analysis confirmed our previous finding that CCR5 was significantly upregulated in RA compared to healthy controls synovium ( $p=0.04$ ). Moreover, our results also showed that this receptor was dramatically downregulated after six months of tDMARDs

treatment ( $p=0.004$ ), as shown in figure (4). Those results highlighted a possible beneficiary effect of DMARDs in patients with COVID-19, through its ability to block CCR5 rich immune cell recruitment that we already found to be upregulated in the SARS-COV-2 infected lungs.

## **Discussion**

Since the outbreak of COVID19 infection, it was evident that this disease had a variable clinical impact on different subpopulations[1, 2]. Due to the immune dysregulation as well as the use of immune-modulating treatments, patients with rheumatic diseases were considered among the fragile subpopulations that might suffer from the more aggressive form of COVID19[3-5]. Interestingly, a group of disease-modifying anti-rheumatic drugs (DMARDS), including HCQ and IL6 inhibitors such as tocilizumab, was also proposed as a possible therapeutic option to treat COVID-19 patients[18]. However, the mechanisms through which those agents produce their effect is not fully understood.

Therefore, a better understanding of the relationship between RA and its associated therapies and COVID19 disease might help to improve the response to COVID 19 pandemic. Our results here highlight a possible link between RA and COVID 19, which might explain the molecular basis of the benefits of some of the DMARDS used for treating COVID19 infection.

Indeed, SARS-COV-2 infected lungs showed upregulation of chemotactic factors, including CCL4, CCL8, and CCL11, that all shared CCR5 as their receptor. This receptor is mainly expressed in the CD4 T memory T reg cells, Th17, Th1, and monocytes.

Recent reports showed the importance of this receptor in the pathogenesis of RA. Indeed, CCR5 were found to be highly expressed in RA synovium, in addition to massive infiltration of the synovium with T helper cell type 1 inflammatory cell. Moreover, an in vivo model using a non-functional form of the CCR5 receptor (CCR5- $\Delta$ 32) was shown to protect against RA[19, 20].

The similarity that we observe here in the pathogenesis of both diseases might provide evidence about the molecular pathways through which many of the commonly used drugs for RA treatment are proposed to have benefits in COVID-19 management[3].

Another observation we notice here is the finding that the tDMARDs used for RA treatment was able to significantly upregulate some immune cell populations, including resting mast cells and activated NK cells. The recent observation that during the COVID-19 infection, the main lymphocyte populations, including NK cells, were remarkably decreased, and this decrease were more prominent in the severe cases of COVID19 infection compared to mild cases as well as healthy controls[21, 22]. Moreover, another report also revealed that NK cells, in addition to the CD8+, were found to be important in modulating the anti-COVID-19 response[23].

This might explain the recent findings that patients with chronic arthritis treated with different forms of DMARD showed no evidence of increased risk of life-threatening or respiratory complications following the COVID-19 infection compared to the general population[3].

In contrast, our results clearly demonstrate a possible mechanism through which HCQ as a member of DMARDs might help in the management of COVID-19 infection through blocking CCR5 rich immune cell recruitment (Figure 6).

Further in vivo and in vitro is highly needed after the research laboratories back to their routine working schedule to explore the mechanistic effect of the drugs on lung secretion of chemokines, immune cell recruitments, and differential activation of antiviral immune cells like NK. The limitation of the work is that it is based on in silico reanalysis of the datasets that are limited in number; however, the rigorous and comprehensive approach can suggest the methodology to identify further unexplained mechanisms when the work on the virus-cell interaction is not available in most of the research labs.

## **Conclusion**

In summary, our results highlight common pathways that are involved in the pathogenesis of RA as well as COVID-19. Those pathways might represent ideal targets for the discovery of more efficient and targeted therapeutic options to treat RA and COVID-19. In addition, it might help to improve our understanding of the mechanisms through which some of the medications already used to treat COVID-19 infection, including the HCQ. Our results might explain some of the reports that showed beneficial effects and indicate

the need for proper patients stratification on their immune profile before selecting the therapeutic protocol or clinical trial enrollment.

### List of Abbreviations

CCL	C chemokine ligand
CCR	C chemokine receptor
COVID-19	coronavirus disease -19
CXCL	CX chemokine ligand
CXCR	CX chemokine receptor
DEGs	Differentially expressed genes
DICE	Database of Immune Cell Expression
DMARDs	Disease-Modifying Anti-Rheumatic Drugs
ESTIMATE	Estimation of STromal and Immune cells in MAalignant Tumor tissues using Expression data
GEO	The Gene Expression Omnibus
GSEA	Gene set enrichment analysis
HCQ	Hydroxychloroquine
IL-	Interleukin
ILC	Innate lymphoid cells
NK	Natural killer cells
OA	Osteoarthritis
PBMCs	Peripheral blood mononuclear cells
RA	Rheumatoid Arthritis
SARS-COV-2	Severe Acute Respiratory Syndrome-Corona Virus-2
WBC	White blood cells

**Ethics approval and consent to participate:** "Not applicable."

**Consent for publication:** "Not applicable."

**Availability of data and material:** All data generated or analyzed during this study are included in this published article

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**Authors' contributions:** all Authors contributed equally in designing and drafting the paper

**Acknowledgments:** "Not applicable."

## References

1. Guan, W.J., et al., *Clinical Characteristics of Coronavirus Disease 2019 in China*. N Engl J Med, 2020. **382**(18): p. 1708-1720.
2. Wu, Z. and J.M. McGoogan, *Characteristics of and Important Lessons From the Coronavirus Disease 2019 (COVID-19) Outbreak in China: Summary of a Report of 72314 Cases From the Chinese Center for Disease Control and Prevention*. JAMA, 2020.
3. Favalli, E.G., et al., *COVID-19 infection and rheumatoid arthritis: Faraway, so close!* Autoimmun Rev, 2020. **19**(5): p. 102523.
4. Hsu, C.Y., et al., *Comparing the burdens of opportunistic infections among patients with systemic rheumatic diseases: a nationally representative cohort study*. Arthritis Res Ther, 2019. **21**(1): p. 211.
5. Mehta, B., et al., *Serious infection risk in rheumatoid arthritis compared with non-inflammatory rheumatic and musculoskeletal diseases: a US national cohort study*. RMD Open, 2019. **5**(1): p. e000935.
6. Kim, A.H.J., et al., *A Rush to Judgment? Rapid Reporting and Dissemination of Results and Its Consequences Regarding the Use of Hydroxychloroquine for COVID-19*. Ann Intern Med, 2020.
7. Meo, S.A., D.C. Klonoff, and J. Akram, *Efficacy of chloroquine and hydroxychloroquine in the treatment of COVID-19*. Eur Rev Med Pharmacol Sci, 2020. **24**(8): p. 4539-4547.
8. Million, M., et al., *Early treatment of COVID-19 patients with hydroxychloroquine and azithromycin: A retrospective analysis of 1061 cases in Marseille, France*. Travel Med Infect Dis, 2020: p. 101738.
9. Luo, P., et al., *Tocilizumab treatment in COVID-19: A single center experience*. J Med Virol, 2020. **92**(7): p. 814-818.
10. Loffler, B.M., et al., *Effects of antimalarial drugs on phospholipase A and lysophospholipase activities in plasma membrane, mitochondrial, microsomal and cytosolic subcellular fractions of rat liver*. Biochim Biophys Acta, 1985. **835**(3): p. 448-55.
11. Sperber, K., et al., *Selective regulation of cytokine secretion by hydroxychloroquine: inhibition of interleukin 1 alpha (IL-1-alpha) and IL-6 in human monocytes and T cells*. J Rheumatol, 1993. **20**(5): p. 803-8.
12. Ben-Zvi, I., et al., *Hydroxychloroquine: from malaria to autoimmunity*. Clin Rev Allergy Immunol, 2012. **42**(2): p. 145-53.
13. Zhao, M., *Cytokine storm and immunomodulatory therapy in COVID-19: Role of chloroquine and anti-IL-6 monoclonal antibodies*. Int J Antimicrob Agents, 2020. **55**(6): p. 105982.
14. Sinha, N. and G. Balayla, *Hydroxychloroquine and covid-19*. Postgrad Med J, 2020.
15. Hachim, M., et al., *Estimating the Infiltration of Immune Cells in Synovium of Rheumatoid Arthritis Compared to Osteoarthritis and Healthy Control Using Transcriptomic Profiling*. 2019.
16. Hachim, M.Y., et al. *Identifying Asthma genetic signature patterns by mining Gene Expression BIG Datasets using Image Filtering Algorithms*. in 2019 IEEE International Conference on Imaging Systems and Techniques (IST). 2019.
17. Torre, D., A. Lachmann, and A. Ma'ayan, *BioJupies: Automated Generation of Interactive Notebooks for RNA-Seq Data Analysis in the Cloud*. Cell Systems, 2018. **7**(5): p. 556-561.e3.
18. Jixin Zhong, et al., *The immunology of COVID-19: is immune modulation an option for treatment*. The Lancet Rheumatology, 2020.
19. Zhou, Y., et al., *Impaired macrophage function and enhanced T cell-dependent immune response in mice lacking CCR5, the mouse homologue of the major HIV-1 coreceptor*. J Immunol, 1998. **160**(8): p. 4018-25.

20. Takeuchi, T. and H. Kameda, *What is the future of CCR5 antagonists in rheumatoid arthritis?* Arthritis Res Ther, 2012. **14**(2): p. 114.
21. Qin, C., et al., *dysregulation of immune response in patients with COVID-19 in Wuhan, China.* Clin Infect Dis, 2020.
22. Zheng, Z., et al., *Risk factors of critical & mortal COVID-19 cases: A systematic literature review and meta-analysis.* J Infect, 2020.
23. Zheng, M., et al., *Functional exhaustion of antiviral lymphocytes in COVID-19 patients.* Cell Mol Immunol, 2020. **17**(5): p. 533-535.

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**Figure (2): Estimating immune cells infiltration in the synovium using transcriptomics profile of rheumatoid arthritis (RA) patients versus healthy controls (N) and osteoarthritis (OA).**

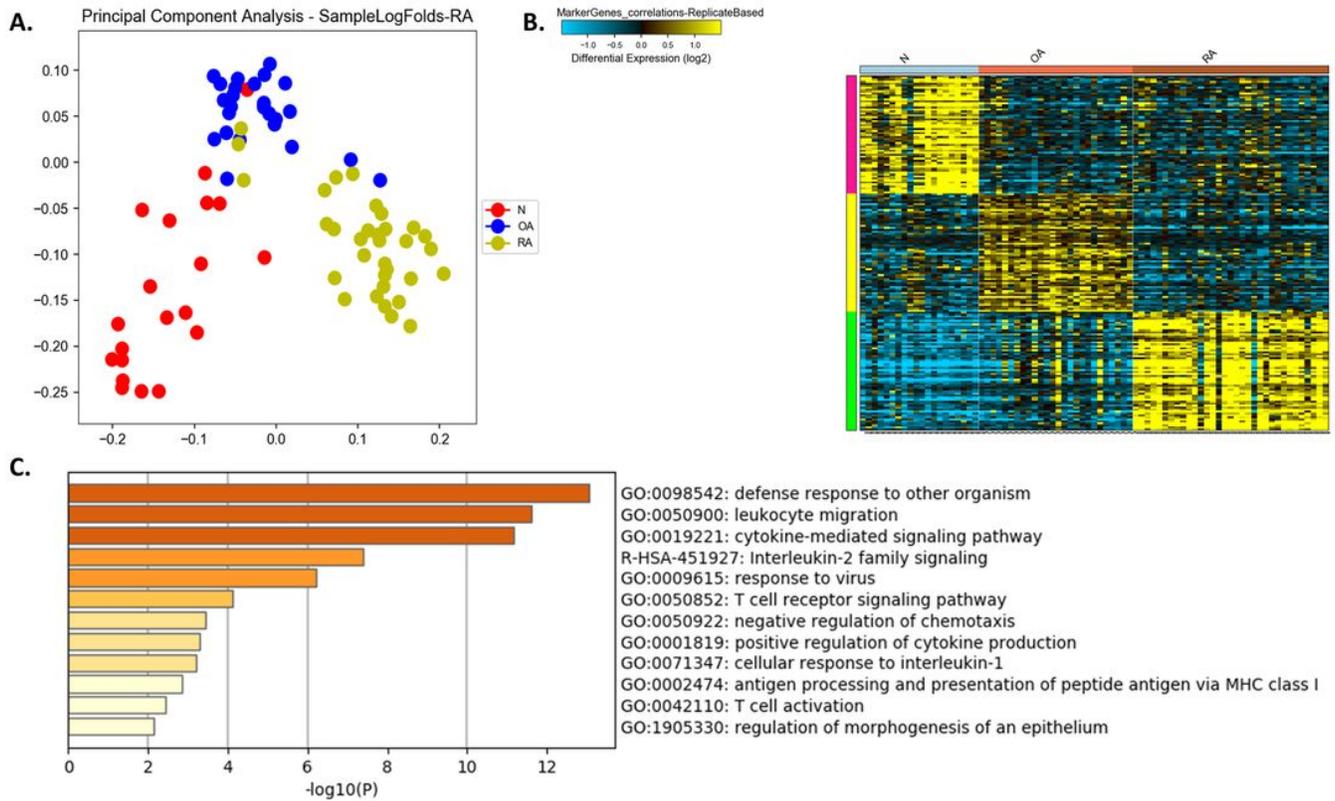
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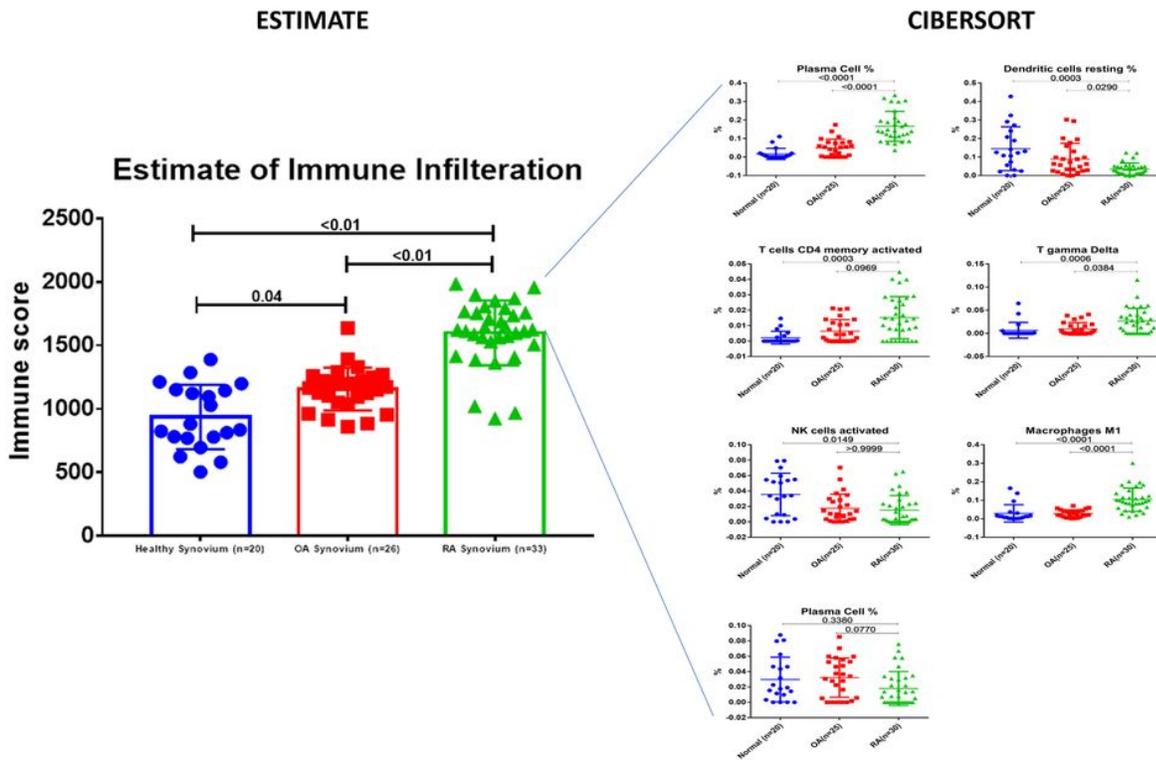
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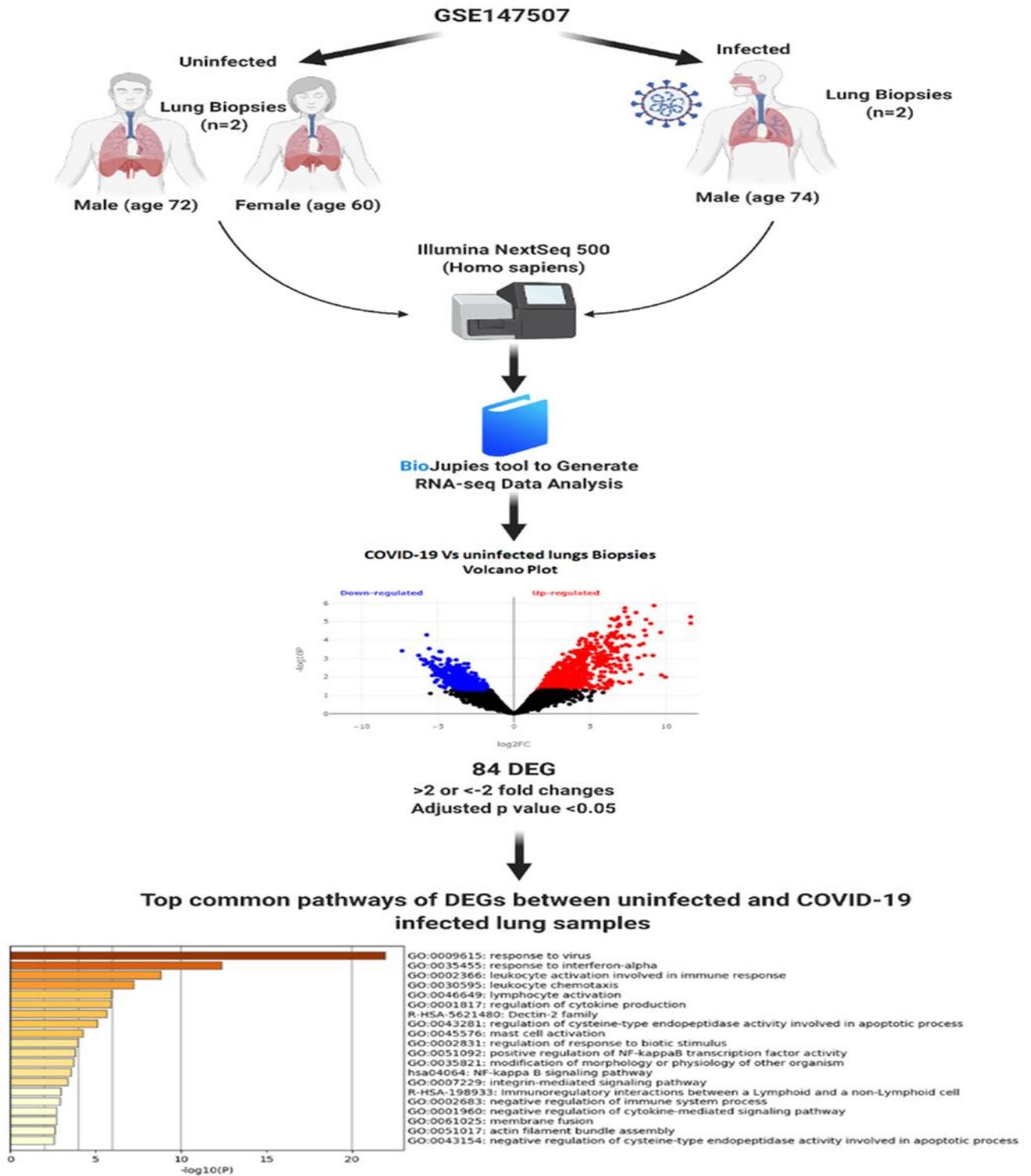
**Figure 1**

Comparison between the synovium transcriptomics profile of rheumatoid arthritis (RA) patients versus healthy controls (N) and osteoarthritis (OA). (A) principle component analysis (PCA) showing that the top selected DEGs can cluster the groups precisely (B) Heatmap of the top markers that can differentiate the three groups and (C) shows top pathways enriched in RA specific markers identified



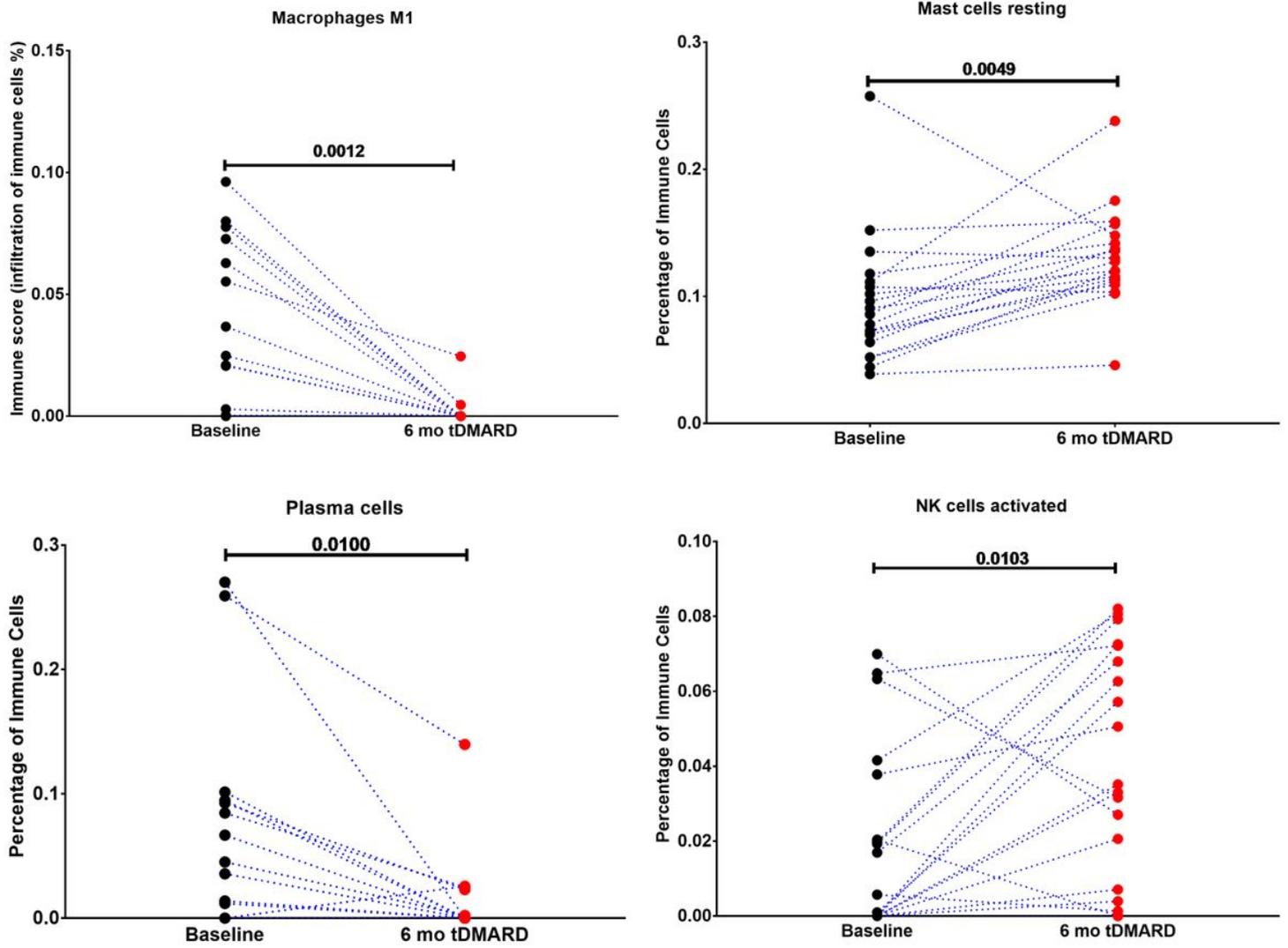
**Figure 2**

Estimating immune cells infiltration in the synovium using transcriptomics profile of rheumatoid arthritis (RA) patients versus healthy controls (N) and osteoarthritis (OA).



**Figure 3**

Percentage and activation state of lung natural killer cells in COVID-19 patient compared to healthy controls.



**Figure 4**

Effect of tDMARDs Treatment In Early RA synovial immune cells profile.

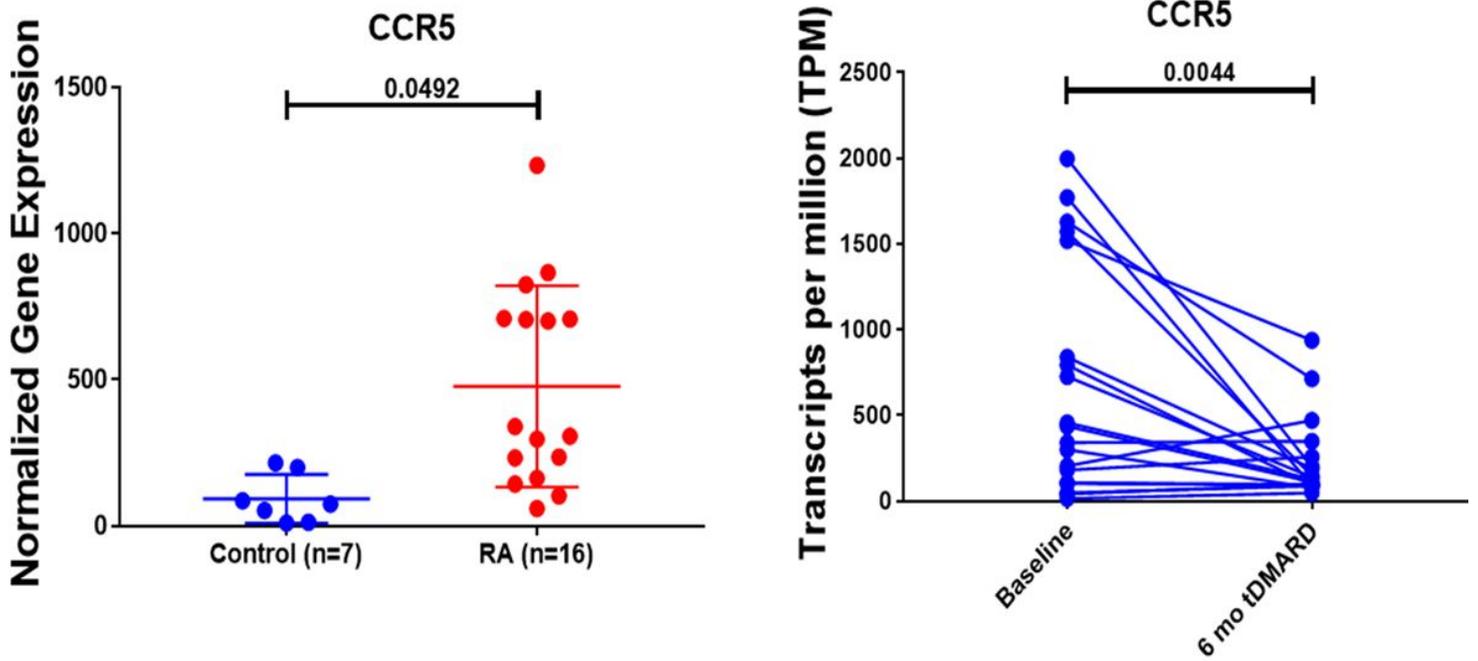


Figure 5

CCR5 expression in synovial biopsies of RA and control and CCR5 expression at baseline and after six months of tDMARDs treatment.

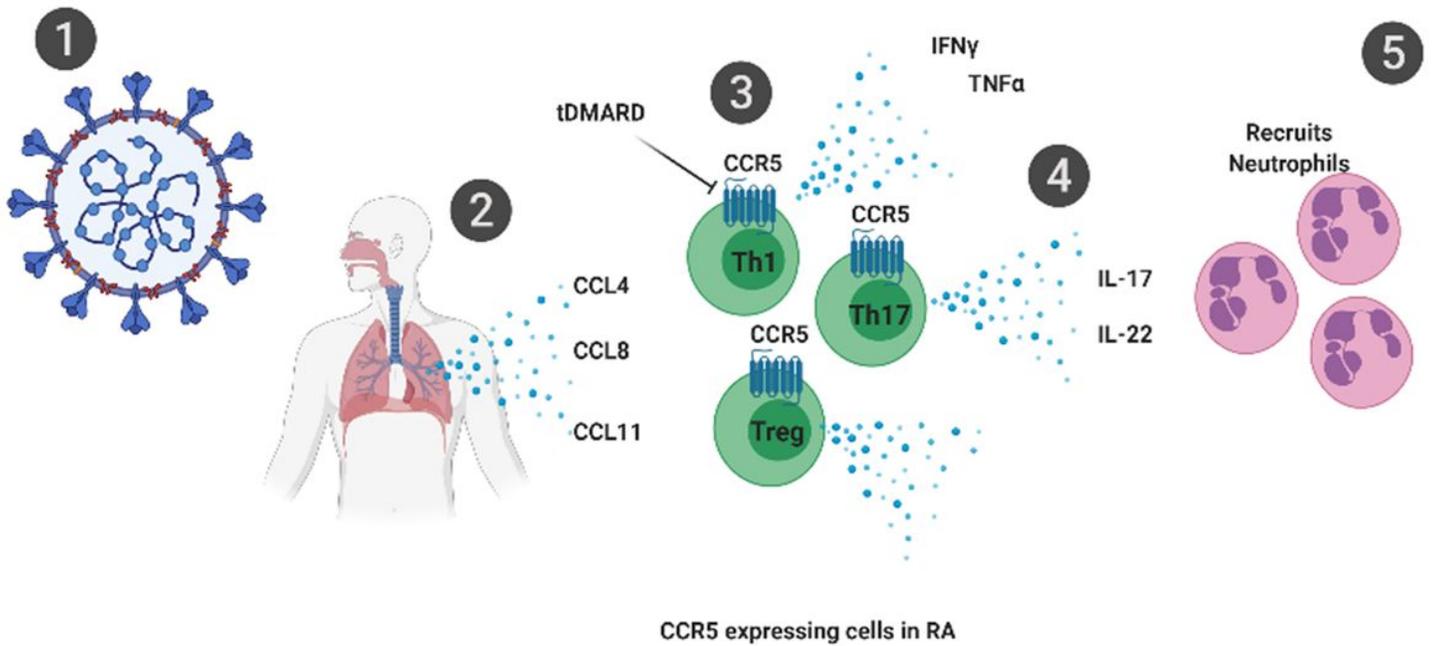


Figure 6

A working hypothesis for RA and COVID-19 interactions

## Supplementary Files

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