

Expression profile analysis of COVID-19 patients

© Valentin S.Shimansky,^{1,2*} © Oleg S.Popov,^{1,2} © Tatiana G.Klochkova,¹ © Svetlana V.Apalko,^{1,2} © Natalya N.Sushentseva,^{1,2}
© Anna Yu.Anisenkova,^{1,2} © Sergey V.Mosenko,^{1,2} © Sergey G.Shcherbak,^{1,2}

¹ Saint Petersburg State Health Care Establishment the City Hospital No. 40 of Health Department of the Saint Petersburg Kurortny District Administration, 197706, Sestroretsk, Russian Federation

² Saint-Petersburg State University, Government of the Russian Federation, 199034, Saint-Petersburg, Russian Federation

*Correspondence: shimansky.valya@yandex.ru

ABSTRACT

1 **The course of COVID-19 is determined by various factors. Studies worldwide have shown a correlation between changes in**
2 **the expression profile and the severity of the disease. Thus, an in-depth study of differentially expressed genes will allow a**
3 **more detailed investigation of the metabolic changes occurring in the background of coronavirus infection. The technique**
4 **of RNA sequencing and subsequent bioinformatics analysis is suitable for such research tasks. In our study, we compared**
5 **groups of samples from patients with mild and severe disease course and identified a number of differentially expressed**
6 **genes. These genes are involved in metabolic pathways responsible for immune response, signaling, intercellular commu-**
7 **nication, metabolism of various compounds etc. We then identified master regulators whose function and role in pathways**
8 **enriched by differentially expressed genes makes them potential targets for biochemical and meta-studies.**

9 INTRODUCTION

10 Work on the peripheral transcriptomic signatures of COVID-19 is being conducted by many research groups. Many different as-
11 pects of the disease are being investigated. For example, in a study by Chinese scientists on the peculiarities of protein ubiquitina-
12 tion in COVID-19, comparing gene expression in peripheral blood mononuclear cells of 4 patients with severe disease and 4 healthy
13 controls, 268 differentially expressed genes were identified. As a result, we were able to identify 6 transcription factors and 2 mi-
14 croRNAs that are key factors in the regulation of ubiquitination in patients with severe COVID-19¹. A study of differentially expressed
15 matrix RNAs, microRNAs and long non-coding RNAs by researchers from China identified 25,482 mRNAs, 23 microRNAs and 410
16 long non-coding RNAs whose expression levels differed between COVID-19 patients and control donors. mRNAs that are overex-
17 pressed in samples of COVID-19 patients are mainly involved in antigen processing and endogenous antigen presentation, positive
18 regulation of T-cell-mediated cytotoxicity, and positive regulation of gamma delta T-cell activation. mRNAs with reduced expression
19 are mainly involved in glycogen biosynthesis².

20 A study of the blood transcriptome of people of different ages in the context of measuring the expression level of genes whose prod-
21 ucts interact with SARS-CoV-2 viral structures revealed five genes that change their expression with aging. They are involved in im-
22 mune response, inflammation, cellular component and cell adhesion, and platelet activation/aggregation. Moreover, the expres-
23 sion profile changed differently in men and women with aging³. In general, the transcriptomic signature of COVID-19 overlaps to a
24 large extent with that of influenza and other acute respiratory infections. Studies of dynamic changes in transcriptome of COVID-19
25 patients are beginning to appear. The data from these studies show that the blood transcriptome undergo dramatic and consistent
26 changes as in the early stage of recovery. And even months after clinical recovery, gene expression levels does not return to the val-
27 ues of a healthy person⁴. The development of interpretable transcriptome panels for profiling the immune response to SARS-CoV-2
28 infection is already underway, with target genes categorized into groups: immunologic relevance, role in disease progression, and
29 interaction with SARS structures⁵.

30 Transcriptomic analysis can be a powerful approach to assess the molecular response of the host and can provide valuable infor-
31 mation both on the pathophysiology of COVID-19 and find master regulators that could be potential targets for effective therapies.
32 The aim of this study was to analyse differential gene expression to identify a set of regulatory genes affecting key molecular and
33 cellular pathways involved in the pathogenesis of COVID-19.

34 All patients signed informed voluntary consent. The study was approved by the expert ethics board of the St. Petersburg State Health
35 Care Institution "City Hospital No. 40" (protocol No. 171 dated May 18, 2020). Biomaterial from the biobank collection in St. Peters-
36 burg State Health Care Institution "City Hospital No. 40" was used to fulfil the study objectives.

37 RESULTS

38 Participant Characteristics

39 The participants of the study were the patients of the infectious disease department of the St. Petersburg State Health Care Insti-
40 tution City Hospital No. 40, Kurortny District who were admitted for treatment with coronavirus infection (confirmed by polymerase
41 chain reaction). The studied patients were divided into two groups according to the severity of the disease (group 1 - mild and mod-
erate course, group 2 - severe and extremely severe course of the disease). Age-sex structure of the groups is presented in Table 1.

Severity	Number of patients	Number,M	Age,M	Number,F	Age,F
Severity 1	109	54	60, 9 ± 13, 3	55	60, 7 ± 13, 6
Severity 2	37	21	59, 4 ± 13, 8	16	60, 9 ± 13, 5

Table 1: Table 1.Age-sex structure of the groups

43 Results of differential expression analysis

44 We obtained the following results analysing blood samples from patients divided by severity. 4734 genes passed the 0.01 statistical significance level. 806 genes were upregulated and 3925 genes were downregulated in group 2 relative to group 1. Upregulated genes with the lowest p-value are listed in Table 2. Downregulated genes with the lowest p-value are listed in Table 3.

Gene name	p-value	logFC
<i>ALOX15</i>	2.57e-07	1.314
<i>SLC4A10</i>	4.96e-07	0.829
<i>PID1</i>	9.82e-07	0.516
<i>TRAV1-1</i>	1.24e-06	0.455
<i>SIGLEC8</i>	1.35e-06	1.33
<i>TRBV29-1</i>	1.49e-06	0.315
<i>GIMAP5</i>	1.67e-06	0.047
<i>FCER1A</i>	1.81e-06	0.538
<i>CCR3</i>	2.00e-06	0.690
<i>SMPD3</i>	2.55e-06	0.461

Table 2: Table 2. Top 10 upregulated genes by p-value.

Gene name	p-value	logFC
<i>TRBJ1-6</i>	2.12e-06	-1.17e-01
<i>NR3C2</i>	2.91e-06	-2.33e-02
<i>PLXDC1</i>	3.35e-06	-1.74e-01
<i>TRBJ1-3</i>	5.98e-06	-3.57e-03
<i>ADAMTS2</i>	7.67e-06	-5.69e-01
<i>LINC00649</i>	8.17e-06	-4.90e-01
<i>TRAJ10</i>	8.38e-06	-7.85e-02
<i>RORC</i>	8.48e-06	-5.51e-01
<i>LINC01550</i>	9.74e-06	-2.88e-01
<i>BACH2</i>	9.96e-06	-5.78e-01

Table 3: Table 3. Top 10 downregulated genes by p-value.

47 GO enrichment analysis showed that upregulated genes are grouped in pathways responsible for adaptive immune response, signalling, intercellular communication, and several others (Table 4).

Metabolic pathway	Number of hits	GO group size	p-value
Regulation of cell migration	39	922	3.14e-05
Chemotaxis	28	707	0.001
Cell part morphogenesis	29	724	0.001
cellular nitrogen compound metabolic process	11	150	0.002
Regulation of MAPK cascade	30	782	2.04e-05
Regulation of development process	76	2 860	1.68e-05
Response to growth factor	28	725	0.002
Ventral trunk neural crest cell migration	3	4	0.001
Regulation of signaling	96	3 927	0.002
Trigeminal nerve structural organization	3	5	4.47e-05
Adaptive immune response	50	978	3.14e-09
Trigeminal nerve morphogenesis	4	12	3.37e-05
Immune response	81	2 893	1.91e-04
Signaling	180	7 537	5.14e-07
Cell communication	179	7 554	8.51e-07
Signal transduction	167	6 942	2.58e-09
Response to stimulus	226	10 504	1.40e-06
Cell surface receptor signaling pathway	102	3 548	4.53e-09

Table 4: Table 4. Top GO terms, enriched with upregulated genes.

49 Downregulated genes are concentrated in molecular pathways responsible for the processes of cellular nitrogen and heterocycle
50 compound metabolism, gene expression, etc. (Table 5).

Metabolic pathway	Number of hits	GO group size	p-value
Regulation of cell migration	39	922	3.14e-05
Chemotaxis	28	707	0.001
Cell part morphogenesis	29	724	0.001
cellular nitrogen compound metabolic process	11	150	0.002
Regulation of MAPK cascade	30	782	2.04e-05
Regulation of development process	76	2 860	1.68e-05
Response to growth factor	28	725	0.002
Ventrul trunk neural crest cell migration	3	4	0.001
Regulation of signaling	96	3 927	0.002
Trigeminal nerve structural organization	3	5	4.47e-05
Adaptive immune response	50	978	3.14e-09
Trigeminal nerve morphogenesis	4	12	3.37e-05
Immune response	81	2 893	1.91e-04
Signaling	180	7 537	5.14e-07
Cell communication	179	7 554	8.51e-07
Signal transduction	167	6 942	2.58e-09
Response to stimulus	226	10 504	1.40e-06
Cell surface receptor signaling pathway	102	3 548	4.53e-09

Table 5: Table 5.Top GO terms, enriched with downregulated genes.

51 Master regulator analysis showed the following results: *CDH2, CXCL9, KIT, IL13, EPHA4, FLT3, DUSP6, FGFR2, IL4* for upregulated
52 genes; *RHOH, NFATC3, FXN, ADRB2, NTRK1, NAA30, ZC3H12D, MAF, KLRD1* for downregulated genes.

53 MATERIALS AND METHODS

54 RNA extraction and sequencing

55 RNA was isolated manually from blood samples using the phenol-chloroform extraction method. Library preparation was performed
56 using KAPA RiboErase HMR reagents (Roche), RNA Hyper Prep (Roche), KAPA Universal Adapter (Roche), KAPA UDI Primer Mixes
57 (Roche). Conversion was performed using High-Throughput Sequencing Primer Kit (App-C) and MGIEasy Universal Library Conver-
58 sion Kit (App-A) (MGI) reagents. Sequencing of the resulting libraries was performed on an MGISEQ-2000 sequencer using a 100
59 base pair-end length read on a DNBSEQ-G400 High-throughput Sequencing Set (PE100, 360 GB) cell (MGI).

60 Bioinformatics analysis

61 Quality control was performed for raw reads using the FastQC program. Alignment to the human genome hg38 version was per-
62 formed using the STAR aligner with standard settings. Counting reads and gene annotation were performed with the featureCounts
63 program.

64 Statistical analysis

65 The nonparametric Mann-Whitney test was used to calculate statistically significant differences in gene expression, and the log fold
66 change (logFC) was used to measure the differential expression. Calculations were performed in the R software package. GO path-
67 way enrichment analysis and the master regulator analysis were performed on the [ge.genexplain.com](https://www.genexplain.com) platform.

DATA AND CODE AVAILABILITY

Personal genetic and clinical data are under restrictions and are available through collaboration with the St. Petersburg State Health
Care Institution City Hospital No. 40, Kurortny District hospital.

CONFLICT OF INTEREST

The authors declare that they have no competing interests.

ACKNOWLEDGMENTS

The authors are grateful to the study participants and the staff from the St. Petersburg State Health Care Institution City Hospital
No. 40, Kurortny District hospital. The authors would like to thank all authors of the included studies for their valuable contributions
to data collection. This work was supported by Saint Petersburg State University, project ID: 94029859.

REFERENCES

- [1] J. Huang, R. Li, J. Cheng, and P. Zhen, "Transcriptome analysis of peripheral blood mononuclear cells response in patients with severe covid-19 reveals crucial genes regulating protein ubiquitination," *Medical Science Monitor*, vol. 28, 8 2022.
- [2] C. X. Li, J. Chen, S. K. Lv, J. H. Li, L. L. Li, and X. Hu, "Whole-transcriptome rna sequencing reveals significant differentially expressed mrnas, mirnas, and lncrnas and related regulating biological pathways in the peripheral blood of covid-19 patients," *Mediators of inflammation*, vol. 2021, 2021. [Online]. Available: <https://pubmed.ncbi.nlm.nih.gov/33833618/>

- [3] L. G. de Almeida Chuffa, J. dos Santos Souza, M. C. de Mello, M. de Oliveira Neto, and R. F. Carvalho, "The aging whole blood transcriptome reveals a potential role of faslg in covid-19," *bioRxiv*, p. 2020.12.04.412494, 12 2020. [Online]. Available: <https://www.biorxiv.org/content/10.1101/2020.12.04.412494v1><https://www.biorxiv.org/content/10.1101/2020.12.04.412494v1.abstract>
- [4] Y. Zhang, J. Tian, W. Shen, P. Li, C. He, G. Peng, R. Ren, and Z. Zhao, "Long term longitudinal analysis of peripheral blood transcriptome in covid-19 patients," *SSRN Electronic Journal*, 8 2020. [Online]. Available: <https://europepmc.org/article/PPR/PPR304048><https://europepmc.org/article/ppr/ppr304048>
- [5] D. Rinchai, B. S. A. Kabeer, M. Toufiq, Z. Tatari-Calderone, S. Deola, T. Brummaier, M. Garand, R. Branco, N. Baldwin, M. Alfaki, M. C. Altman, A. Ballestrero, M. Bassetti, G. Zoppoli, A. D. Maria, B. Tang, D. Bedognetti, and D. Chaussabel, "A modular framework for the development of targeted covid-19 blood transcript profiling panels," *Journal of translational medicine*, vol. 18, 7 2020. [Online]. Available: <https://pubmed.ncbi.nlm.nih.gov/32736569/>