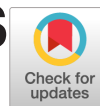# PREDICTIVE VALUE OF SPECIFIC CYTOKINES FOR LETHAL COVID-19 OUTCOME

N.A. Arsentieva[a], N.E. Liubimova[a], O.K. Batsunov[a,b], Z.R. Korobova[a,b], R.N. Kuznetsova[a,b],
A.A. Rubinstein[b], O.V. Stanevich[b], A.A. Lebedeva[b], E.A. Vorobyov[b], S.V. Vorobyova[b],
A.N. Kulikov[b], E.G. Gavrilova[b], D.E. Pevtcov[a], Yu.S. Polushin[b], I.V. Shlyk[b], A.A. Totolian[a,b]

[a] St. Petersburg Pasteur Institute, St. Petersburg, Russian Federation
[b] Pavlov First St. Petersburg State Medical University, St. Petersburg, Russian Federation

Abstract. In our study, we aimed to evaluate the significance of specific cytokines in blood plasma as predictive markers of COVID-associated mortality. *Materials and methods.* In plasma samples of 29 patients with PCR-confirmed COVID-19 we measured the concentrations of 47 molecules. These molecules included: interleukins and selected pro-inflammatory cytokines (IL-1α, IL-1β, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-9, IL-12 (p40), IL-12 (p70), IL-13, IL-15, IL-17A/CTLA8, IL-17-E/IL-25, IL-17F, IL-18, IL-22, IL-27, IFNα2, IFNγ, TNFα, TNFβ/Lymphotoxin-α(LTA)); chemokines (CCL2/MCP-1, CCL3/MIP-1α, CCL4/MIP-1β, CCL7/MCP-3, CCL11/Eotaxin, CCL22/MDC, CXCL1/GROα, CXCL8/IL-8, CXCL9/MIG, CXCL10/IP-10, CX3CL1/Fractalkine); anti-inflammatory cytokines (IL-1Ra, IL-10); growth factors (EGF, FGF-2/FGF-basic, Flt-3 Ligand, G-CSF, M-CSF, GM-CSF, PDGF-AA, PDGFAB/BB, TGFα, VEGF-A); and sCD40L. We used multiplex analysis based on xMAP technology (Luminex, USA) using Luminex MagPix. As controls, we used plasma samples of 20 healthy individuals. Based on the results, we applied Receiver Operating Characteristic (ROC) analysis and Area Under Curve (AUC) values to compare two different predictive tests and to choose the optimal division point for disease outcome (survivors/non-survivors). To find optimal biomarker combinations, we as used cytokines concentrations as dependent variables to grow a regression tree using JMP 16 Software. *Results.* Out of 47 studied cytokines/chemokines/growth factors, we picked four pro-inflammatory cytokines as having high significance in evaluation of COVID-19 outcome: IL-6, IL-8, IL-15, and IL-18. Based on the results received, we assume that the highest significance in terms of predicting the outcome of acute COVID-19 belongs to IL-6 and IL-18. *Conclusion.* Analyzing concentrations of IL-6 and IL-18 before administering treatment may prove valuable in terms of outcome prognosis

Key words: cytokines, decision trees, multiplex analysis, COVID-19, severity, interleukins.

ПРОГНОЗНАЯ ЗНАЧИМОСТЬ СПЕЦИФИЧЕСКИХ ЦИТОКИНОВ В ОТНОШЕНИИ ЛЕТАЛЬНОГО ИСХОДА COVID-19

Арсентьева Н.А.[1], Любимова Н.Е.[1], Бацунов О.К.[1,2], Коробова З.Р.[1,2], Кузнецова Р.Н.[1,2], Рубинштейн А.А.[2], Станевич О.В.[2], Лебедева А.А.[2], Воробьев Е.А.[2], Воробьева С.В.[2], Куликов А.Н.[2], Гаврилова Е.Г.[2], Певцов Д.Э.[2], Полушин Ю.С.[2], Шлык И.В.[2], Тотолян А.А.[1,2]

[1] ФБУН НИИ эпидемиологии и микробиологии имени Пастера, Санкт-Петербург, Россия
[2] Первый Санкт-Петербургский государственный медицинский университет им. И.П. Павлова, Санкт-Петербург, Россия

Резюме. Целью настоящего исследования была оценка значимости специфических цитокинов в плазме крови в качестве прогностических маркеров смертности, связанных с COVID-19. *Материалы и методы.* В образцах

Адрес для переписки:
Коробова Зоя Романовна
197101, Россия, Санкт-Петербург, ул. Мира, 14,
ФБУН НИИ эпидемиологии и микробиологии имени Пастера.
Тел.: 8 (921) 400-26-83. E-mail: zoia-korobova@yandex.ru

Contacts:
Zoya R. Korobova
197101, Russian Federation, St. Petersburg, Mira str., 14,
St. Petersburg Pasteur Institute.
Phone: +7 (921) 400-26-83. E-mail: zoia-korobova@yandex.ru

плазмы 29 пациентов с ПЦР-подтвержденным COVID-19 проводилось определение концентрации 47 молекул: интерлейкинов и ряда провоспалительных цитокинов (IL-1α, IL-1β, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-9, IL- 12 (p40), IL-12 (p70), IL-13, IL-15, IL-17A/CTLA8, IL-17-E/IL-25, IL-17F, IL-18, IL-22, IL- 27, IFNα2, IFNγ, TNFα, TNFβ/лимфотоксин-α(LTA)); хемокинов (CCL2/MCP-1, CCL3/MIP-1α, CCL4/MIP-1β, CCL7/MCP-3, CCL11/эотаксин, CCL22/MDC, CXCL1/GROα, CXCL8/IL-8, CXCL9/MIG, CXCL10/IP-10, CX-3CL1/фракталкин); противовоспалительных цитокинов (IL-1Ra, IL-10); факторы роста (EGF, FGF-2/FGF-basic, Flt-3 Ligand, G-CSF, M-CSF, GM-CSF, PDGF-AA, PDGFAB/BB, TGFα, VEGF-A); и sCD40L. Для этого использовался мультиплексный анализ на основе технологии хMAP (Luminex, США) с на приборе Luminex MagPix. В качестве контроля использовались образцы плазмы 20 здоровых людей. Результаты исследования оценивались в ходе анализа операционных характеристик приемника (ROC) и значения площади под кривой (AUC) для сравнения двух разных прогностических тестов и выбора оптимальной точки разграничения для исхода заболевания (выжившие/невыжившие). Для поиска оптимальных комбинаций биомаркеров, в качестве зависимых переменных мы использовали концентрации цитокинов для построения дерева регрессии с применением программного обеспечения JMP 16. *Результаты*. Из 47 исследованных цитокинов/хемокинов/факторов роста мы выбрали четыре провоспалительных цитокина, имеющих большое значение для оценки исхода COVID-19: IL-6, IL-8, IL-15 и IL-18. На основании полученных результатов мы предполагаем, что наибольшую значимость с точки зрения прогнозирования исхода острого течения COVID-19 имеют IL-6 и IL-18. *Выводы*. Анализ концентраций IL-6 и IL-18 перед назначением лечения может быть важен с точки зрения оценки прогноза исхода COVID-19.

Ключевые слова: цитокины, деревья решений, мультиплексный анализ, COVID-19, тяжесть, интерлейкины.

Introduction

Novel coronavirus infection, later called COVID-19, started as an outbreak in December, 2019, and by March 2020 it had become a fully-grown pandemic [3]. The causative agent of COVID-19 is highly contagious; this infection's estimated mortality rate is 2%. It is caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), an enveloped, single-stranded RNA-virus of the genus *Betacoronavirus* [8]. Its virions attack target organs and induce local and systemic inflammation [2].

The clinical manifestation of the novel coronavirus can be described as an acute respiratory tract infection, although symptoms may vary drastically in patients: from asymptomatic to critically severe, i.e., acute respiratory distress syndrome (ARDS) pneumonia. ARDS and respiratory failure are the main causes of death in patients with COVID-19 [21].

So far, there is no certain explanation for the different clinical presentations of COVID-19. There are many theories concerning its pathogenesis, but it is evident that the main reason behind the clinical course of the disease is actually the host immune system [2, 23].

There is a lot of data concerning critically ill patients developing a so-called "cytokine storm" featuring pathological hyperinflammatory reactions with heavy cytokine release. Cytokines are regulatory peptides produced by cells of the body; the cytokine system consists of nearly 200 polypeptides. Their uncontrolled release in response to infection is damaging for multiple organs and body systems, including the respiratory tract, which can lead to progression of ARDS. It would seem there is a connection between severity of the disease, viral replication, and "cytokine storm" [28]. Based on that, the goal of our research was to evaluate the significance of specific cytokines as predictive markers of COVID-associated mortality.

Materials and methods

From May to December 2020, we studied 29 COVID-19 patients in the acute stage of the disease. Based on disease outcome, we divided them into two separate groups: survivors (n = 13), and non-survivors of COVID-19 (n = 16). As a control group, we chose healthy individuals (n = 20). The research was carried out at the Laboratory of Molecular Immunology, Saint Petersburg Pasteur Institute. The study protocol was approved by the Ethics Committee of the Saint Petersburg Pasteur Institute in accordance with the Declaration of Helsinki. All participants were informed of our study and willingly signed consent forms.

All patients were infected with the original Wuhan strain for the first time; no vaccines were available at the time of the study.

Overall, patients represented both sexes (58% male, 42% female), with ages from 31 to 83 (62.1±12.5). We divided them into: survivors (46% male, 54% female), aged from 31 to 77 (55.8±13.6); and non-survivors (69% male, 31% female), aged 45 to 83 years. Non-survivors' age median was 65±16.0, whereas for survivors it was 53±14.4. All patients were treated at the Pavlov First St. Petersburg State Medical University, a COVID-19 specialized hospital from May to July 2020. They were diagnosed with COVID-19 (U07.1), and the virus was identified via qualitative PCR (detection of SARS-CoV-2 RNA). Blood samples were taken in the acute phase of the disease at the time of admission (5−14 days from the first symptoms without any signs of recov-

ery). At hospital admission, 72.4% of individuals (21 patients) presented with a moderate course of the disease, while 27.6% (8 patients) presented with severe infection. Course of the disease was evaluated by administering doctors based on Russian Ministry of Healthcare guidelines on COVID-19 treatment.

All recovered patients presented moderate disease severity, while patients who did not survive developed either moderate or severe disease courses.

All patients developed typical COVID-induced changes (pneumonia) in lung tissues confirmed by CT-scan performed at the time of admission. Besides that, all of them presented fever, cough, joint and muscle paints, as well as blood oxygen saturation levels below 93 mm Hg and laboratory signs of inflammation such as higher WBC count and higher levels ESR, CRP compared to reference levels for their age and sex. They did not receive any treatment before administration to the hospital besides non-steroid anti-inflammatory drugs (Ibuprofen, Paracetamol, etc.) in small doses against fever and antiviral drugs (Umifenovirum) for 4 days or less. Some of the patients had underlying conditions, but we excluded patients with previous history of cancer, HIV, hepatitis, tuberculosis and lung pathology from the study, as well as chronic diseases in the acute stage.

Blood samples were taken at hospital admission, before initiation of therapy. We chose 20 healthy individuals living in Saint Petersburg as a control group (sex ratio: 35% male, 65% female) aged 25 to 65 years (44.1±10.3). These individuals did not have any inflammatory diseases, nor did they have any apparent chronic illnesses in the acute phase. Their SARS-CoV-2 PCR detection tests were negative, and they did not have specific SARS-CoV-2 IgG plasma antibodies.

Sample prepatation. We used peripheral blood as our study material. Blood samples were collected in vacuum tubes with EDTA anticoagulant reagent, followed by centrifugation (350g for 10 minutes). Plasma samples were transferred to cryotubes and frozen at −80°C before multiplex analysis.

Multiplex analysis. We measured the concentrations of 47 molecules in blood plasma: interleukins and selected pro-inflammatory cytokines (IL-1α, IL-1β, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-9, IL-12 (p40), IL-12 (p70), IL-13, IL-15, IL-17A/CTLA8, IL-17-E/IL-25, IL-17F, IL-18, IL-22, IL-27, IFNα2, IFNγ, TNFα, TNFβ/Lymphotoxin-α [LTA]); chemokines (CCL2/MCP-1, CCL3/MIP-1α, CCL4/MIP-1β, CCL7/MCP-3, CCL11/Eotaxin, CCL22/MDC, CXCL1/GROα, CXCL8/IL-8, CXCL9/MIG, CXCL10/IP-10, CX3CL1/Fractalkine); anti-inflammatory cytokines (IL-1Ra, IL-10); growth factors (EGF, FGF-2/FGF-basic, Flt-3 Ligand, G-CSF, M-CSF, GM-CSF, PDGF-AA, PDGFAB/BB, TGFα, VEGF-A); and sCD40L. The study was conducted with multiplex analysis based on xMAP technology (Luminex, Austin,

USA) with the Milliplex HCYTA-60k-PX48 reagent kit (Billerica, MA, USA) according to manufacturer's instructions. Data analysis was performed in Luminex MAGPIX (Luminex, Austin, USA).

Data analysis and statistics. Statistical analyses were conducted with GraphPad Prism 5.0 (GraphPad Software Inc.) and JMP 16.0 (SAS Institute Inc.). The data we received did not follow a normal distribution, so we used non-parametric statistical analysis methods. Between groups, we applied the Kruskal–Wallis test with Dunn's Multiple Comparison Test. We considered differences significant at $p < 0.05$. Median (Me) and interquartile range $(Q_{25}-Q_{75})$ were used as descriptive measures of metric data. We used receiver operating characteristic (ROC) analysis and calculated area under curve (AUC) values to compare two different predictive tests and to choose the optimal division point. To find optimal combinations of biomarkers, we used the decision tree building method with JMP 16.0 software.

Method limitations. As biological samples of our study we used plasma samples of patients with COVID-19 and without it. It must be noted that the number of participants in our study is relatively small (29 patients with COVID-19 and 20 controls). This may affect statistical analysis performed in this research. We also used only one detection method to measure cytokine levels in this study, since multiplex analysis technology provides a wider spectrum of possible markers to assess. There is a difference in sex ratio of healthy donors cohor.

Thus, interpretation of results of this study must be interpreted with the limitations listed above.

First, we analyzed the concentrations of 47 cytokines in the blood plasma of patients in the acute phase of COVID-19, relative to disease outcome, survivors and non-survivors (Table 1). In comparison with healthy donor controls, all patients with COVID-19 had elevated levels of: pro-inflammatory cytokines (IL-6, IL-7, IL-15, IL-18, IL-27, TNFα); CC-chemokines (CCL-2/MCP-1, CCL-3/MIP-1α, CCL7/MCP-3, CCL22/MDC); CXC-chemokines (CXCL8/IL-8, CXCL9/MIG, CXCL10/IP-10); anti-inflammatory cytokines (IL-1RA, IL-10); growth factors (FGF-2/FGF-basic, G-CSF); and sCD40L.

Moreover, in those infected who later died due to COVID-19, we noted elevated levels of IL-1α, CCL4/MIP-1β, CX3CL1/Fractalkine, M-CSF, and TGFα. Our study shows that IFNα2 levels are significantly higher in deceased group patients ($p < 0.05$), yet we did not find any differences in IFNγ concentrations between different groups. Analyzing differences in cytokine concentration based on disease outcome, we found 14-fold higher levels of CCL7/MCP-3 in deceased group patiaents compared to those who recovered ($p < 0.05$).

To identify potential markers of unfavorable COVID-19 outcome amongst 47 studied factors, we built ROC-curves for acute phase patients; these
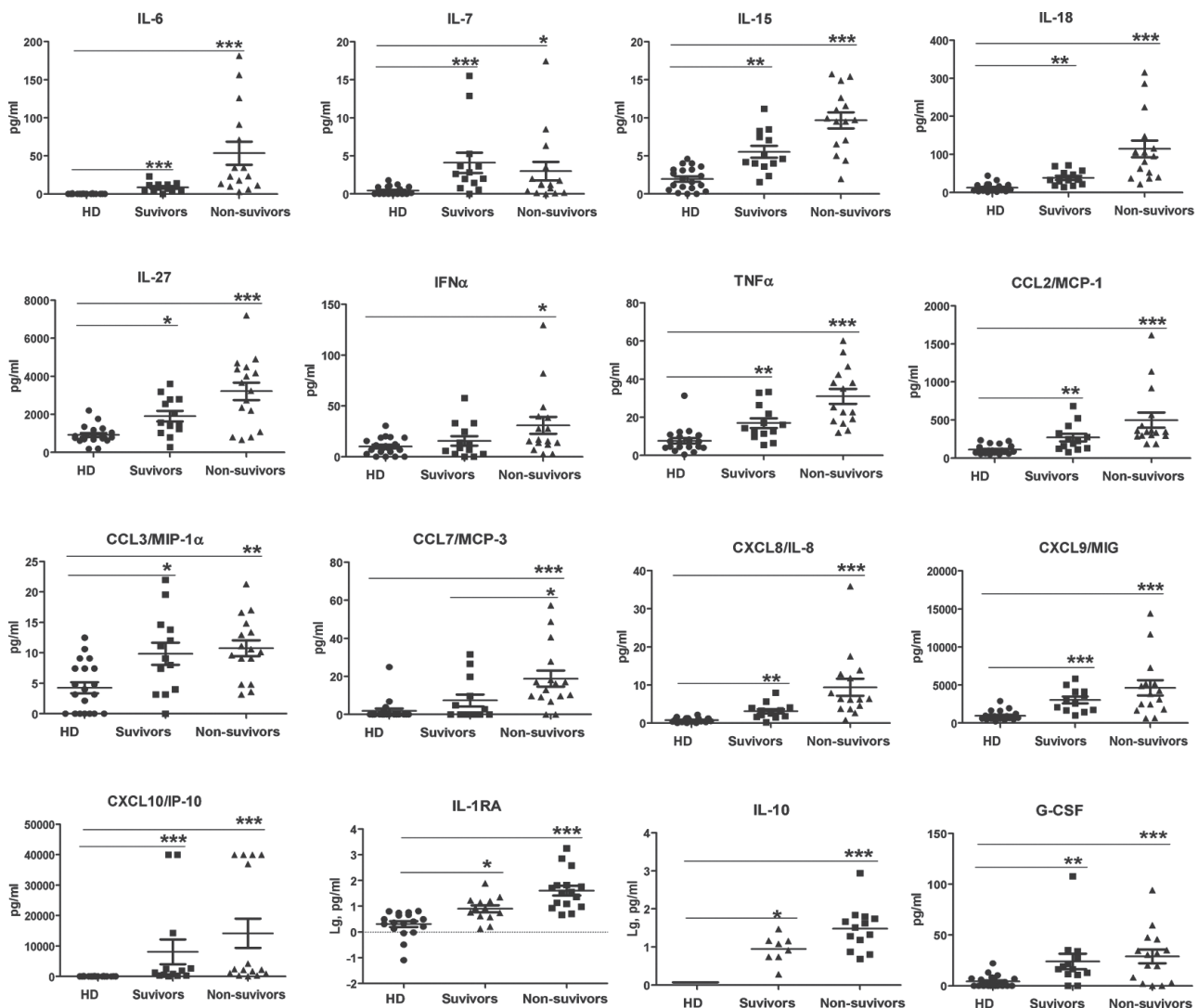
were divided into two groups based on disease outcome (survivors and non-survivors). We received statistically significant differences for 12 cytokines, as shown in Table 2.

The largest AUC values were found for the following cytokines: IL-6, IL-15, IL-18, and CXCL8/IL-8 (Fig. 2)

ROC analysis results showed little significance when testing separate plasma cytokine levels to predict disease outcome. Instead, we used a decision tree building method for that purpose. Based on the results received, we established that combined detection of IL-18 and IL-6 is more valuable in terms of COVID-19 outcome prediction. For these two cytokines, parameters were: AUC 0.97; sensitivity 94%; and specificity 100%. From this analysis, we received the following threshold values for prediction of COVID-19 outcome: IL-18 — 81.6 pg/ml; and IL-6 — 23.5 pg/ml (Fig. 3).

Discussion

Regulation of the antiviral immune response is conducted by different factors, mainly cytokines and chemokines. In our study of COVID-19 patient plasma, we noticed higher levels of: proinflammatory cytokines (IL-6, IL-7, IL-15, IL-18, IL-27, TNFα); chemokines (CCL2/MCP-1, CCL3/MIP-1α, CCL7/MCP-3, CCL22/MDC, CXCL8/IL-8, CXCL9/MIG, CXCL10/IP-10); anti-inflammatory cytokines (IL-1RA, IL-10); growth factors (FGF-2/FGF-basic, G-CSF); and sCD40L. Most of these factors play a role in virus-induced immune responses. Earlier this year, we proved that COVID-19 in the acute phase is followed by significantly higher levels of cytokines (both pro- and anti-inflammatory) in blood plasma [1]. This data further adds to previous findings of other researchers [9, 13, 14]. Age is a highly important factor for COVID-19 outcome,

Figure 1. Plasma cytokine levels in patients with acute phase COVID-19 and healthy donors (HD)
Note. COVID-19 patients are divided into two groups, depending on disease outcome: recovery (survivors), or death (non-survivors).

Table 1. Plasma cytokine concentrations in acute phase COVID-19 patients and healthy donors, Me (Q$_{25}$–Q$_{75}$)

Cytokine, pg/ml	Studied groups			p-value		
	Healthy donors (HD, n = 20)	COVID-19 survivors (n = 13)	COVID-19, non-survivors (n = 16)	HD vs non-survivors	HD vs survivors	non-survivors vs survivors
Interleukins and selected proinflammatory cytokines						
IL-1α	0.87 (0.22–3.923)	1.68 (0–4.705)	4.475 (2.198–5.773)	< 0.05	ns	ns
IL-1β	0.28 (0–2.798)	0.46 (0–4.925)	2.44 (0.44–5.193)	ns	ns	ns
IL-2	0 (0–0)	0 (0–0.005)	0 (0–0.3475)	ns	ns	ns
IL-3	0 (0–0)	0 (0–0)	0 (0–0)	ns	ns	ns
IL-4	0 (0–0)	0 (0–0.895)	0 (0–0.3625)	ns	ns	ns
IL-5	1.515 (0.905–2.283)	3.21 (1.515–6.31)	1.95 (1.373–4.203)	ns	ns	ns
IL-6	0 (0–0.17)	8.63 (3.55–12.21)	29.11 (11.92–117.3)	< 0.001	< 0.001	ns
IL-7	0.17 (0–0.92)	2.63 (1.1–4.47)	1.275 (0.22–5.653)	< 0.05	< 0.001	ns
IL-9	0 (0–5.32)	0 (0–10.35)	0 (0–3.06)	ns	ns	ns
IL-12 p40	10.56 (4.488–22.14)	15.02 (8.955–25.97)	19.85 (8.275–38.51)	ns	ns	ns
IL-12 p70	0 (0–0.5775)	0 (0–0.185)	0 (0–0.045)	ns	ns	ns
IL-13	6.12 (1.97–10.7)	12.45 (5.125–15.41)	10.26 (3.47–13.83)	ns	ns	ns
IL-15	1.765 (0.5925–3.19)	4.61 (3.795–7.88)	9.8 (6.678–14.34)	< 0.001	< 0.01	ns
IL-17A	0.125 (0–2.1)	0 (0–2.235)	1.4 (0.0625 — 3.55)	ns	ns	ns
IL-17E/IL-25	173.1 (0–469.4)	146.6 (37.81–335.4)	277.6 (143.2–415.5)	ns	ns	ns
IL-17F	0 (0–0)	0 (0–0)	0 (0–0)	not applicable		
IL-18	8.0 (4.898–19.7)	33.6 (20.15–54.31)	100.3 (43.47–140)	< 0.001	< 0.01	ns
IL-22	0 (0–0)	0 (0–0)	0 (0–0)	not applicable		
IL-27	821.9 (680.2–1141)	1568 (1130–2790)	3453 (1363–4463)	< 0.001	<0.05	ns
IFNα2	8.69 (3.258–17.85)	8.69 (2.72–28.93)	16.28 (12.6–39.63)	< 0.05	ns	ns
IFNγ	0 (0–0)	0 (0–21.99)	6.6 (0–35.75)	ns	ns	ns
TNFα	6.55 (4.078–10.29)	14.21 (10.57–24.12)	30.24 (18.3–45.56)	< 0.001	< 0.05	ns
TNFβ/Lymphotoxin-α	1.725 (0.5825–3.015)	2.7 (1.12–5.005)	3.25 (1.553–5.12)	ns	ns	ns
CC-chemokines						
CCL2/MCP-1	89.49 (64.15–153.3)	223.4 (129.2–376.3)	346.7 (296.6–534.7)	< 0.001	< 0.05	ns
CCL3/MIP-1α	3.585 (0–7.43)	9.1 (3.585–14.22)	10.38 (5.875–14.47)	< 0.01	< 0.05	ns
CCL4/MIP-1β	10.08 (6.433–14.04)	12.15 (8.48–16.78)	19.35 (11.5–32.4)	< 0.05	ns	ns
CCL7/MCP-3	0 (0–0)	0 (0–14.79)	14.56 (9.29–25.48)	< 0.001	ns	< 0.05
CCL11/Eotaxin	31.3 (24.67–52.75)	40.32 (28.4–52.13)	44.59 (23.3–80.22)	ns	ns	ns
CCL22/MDC	557.3 (335.6–625.3)	254.3 (184.4–370.9)	230.7 (132.1–346.1)	< 0.01	< 0.05	ns

Table 1. Plasma cytokine concentrations in acute phase COVID-19 patients and healthy donors, Me ($Q_{25}-Q_{75}$) (continued)

Cytokine, pg/ml	Studied groups			p-value		
	Healthy donors (HD, n = 20)	COVID-19 survivors (n = 13)	COVID-19, non-survivors (n = 16)	HD vs non-survivors	HD vs survivors	non-survivors vs survivors
CXC-chemokines						
CXCL1/GROα	3.62 (1.208–11.26)	18.11 (9.705–40.96)	21.14 (5.225–24.81)	ns	< 0.05	ns
CXCL8/IL-8	0.655 (0.2875–1.195)	2.64 (1.73–4.04)	6.4 (3.993–13.64)	< 0.001	< 0.01	ns
CXCL9/MIG	710 (593.2–1164)	3165 (1703–4572)	4061 (1987–6741)	< 0.001	< 0.001	ns
CXCL10/IP-10	65.56 (48.53–95.57)	1294 (373.9–8508)	2364 (1022–40 000)	< 0.001	< 0.001	ns
CX3C-chemokines						
CX3CL1/Fractalkine	43.79 (26.08–58.62)	57.04 (26.74–76.45)	96.91 (57.45–114.6)	< 0.001	ns	ns
Anti-inflammatory cytokines						
IL-1RA	2.225 (0.915–4.598)	8.59 (4.02–16.3)	34.67 (10.3–66.61)	< 0.001	< 0.05	ns
IL-10	0 (0–0)	5.52 (0–13.23)	20.21 (5.278 — 53.36)	< 0.001	< 0.05	ns
Growth factors						
EGF	32.57 (5.27–68.8)	94.8 (51.95–152.2)	48.71 (16.5–89.99)	ns	< 0.05	ns
FGF-2/FGF-basic	18.09 (11.01–26.48)	55.35 (23.64–100.8)	38.94 (21.98–77.04)	< 0.05	< 0.01	ns
FLT-3L	8.42 (0.97–15.06)	2.72 (0.425–8.86)	9.08 (6.403–11.14)	ns	ns	ns
G-CSF	0 (0–7.45)	16.4 (11.22–30.94)	31.42 (4.313–47.35)	< 0.001	< 0.01	ns
M-CSF	7.645 (0–38.02)	102.1 (5.28–243.8)	158.5 (113.8–315.5)	< 0.001	ns	ns
GM-CSF	0 (0–0)	0 (0–0)	0 (0–0)	not applicable		
PDGF-AA	1073 (872–2487)	1739 (1220–2501)	1034 (659.7–2488)	ns	ns	ns
PDGF-AB/BB	22 946 (18 269–31 171)	25 599 (21 487–32 255)	23 641 (15 760–29 899)	ns	ns	ns
TGFα	0 (0–0)	0.96 (0–2.275)	2.985 (1.753–6.778)	< 0.001	ns	ns
VEGF-A	37.12 (18.25–102.9)	166.7 (57.85–324.5)	119.3 (30.22–250.9)	ns	< 0.05	ns
Other soluble ligands						
sCD40L	1079 (423.9–1871)	2883 (1281–5589)	3475 (2044–5838)	< 0.01	< 0.05	ns

Table 2. Cytokines showing the most statistically significant concentrations within our study

Cytokine	AUC	p-value	Cut off, pg/ml	Sensitivity, %	Specificity, %
IL-6	0.8389	0.002	12.8	85	75
IL-15	0.8101	0.0047	9.0	92	69
IL-18	0.8606	0.001	51.8	77	75
TNFα	0.8173	0.038	22.3	77	69
CCL2/MCP-1	0.7596	0.018	301.3	69	69
CCL7/MCP-3	0.7452	0.025	5.8	69	87
CXCL8/IL-8	0.8413	0.0018	4.3	85	75
CX3CL1/Fractalkine	0.7525	0.021	73.7	69	69
IL-1Ra	0.7764	0.012	9.1	62	81
IL-10	0.7524	0.021	15.0	92	62
FLT-3L	0.7284	0.037	7.9	77	75
TGFα	0.7500	0.023	2.1	69	69

elderly people are at risk of developing a more severe course of disease [18]. We have also performed analysis of cytokine levels in different cohorts based on the age, and found very little statistically significant difference. We noted elevation of TNFα in all cohorts — HD, survivors and non-survivors in patients above 50 (p < 0.05, p < 0.05 and p <0.01, respectively). Due to that, we excluded TNFα out of decision tree analysis.

However, despite an absence of statistically significant differences, median values for concentrations of specific factors (IL-6, IL-15, IL-18, IL-27, TNFα, CCL2/MCP-1, CXCL8/IL-8, CXCL9/MIG, CXCL10/IP-10, IL-1RA, IL-10, G-CSF, sCD40L) in non-survivors were higher than those in survivors. Moreover, deceased patients showed higher levels of IL-1α, IFNα2, CCL4/MIP-1β, CX3CL1/Fractalkine, M-CSF and TGFα compared to controls, as noted in other reports [17].

Our data suggest activation of cytokine and chemokine systems in response to COVID-19, followed by systemic inflammation. On the other hand, elevation of cytokine concentrations in COVID-19 patient plasma points to development of a 'cytokine storm' causing organ damage; this may explain higher cytokine levels in patients who died of COVID. We noted higher levels of MCP-3, the only cytokine between cohorts of survivors and non-survivors showing significant differences between two groups. Based on the data discovered by previous researchers, this cytokine can, in fact, be a predictor for a cytokine

storm by itself [6]. However, we believe that single cytokine levels do not have enough prognostic value, and, therefore, there is a need for multiple factor-based evaluation. Such evaluation of the prognostic potential of cytokines based on disease outcome showed the significance of specific proinflammatory cytokines: IL-6, IL-15, IL-18, and CXCL8/IL-8.

IL-6. The IL-6 cytokine controls immune responses, cellular proliferation, and cellular differentiation. It is produced by different cell types: T-lymphocytes, macrophages, endothelial cells, fibroblasts, and monocytes. IL-6 targets B- and T-cells, basophiles, eosinophiles, and neutrophils. The effects of IL-6 on B-cells are mainly activation of differentiation and secretion of IgM, IgE, and IgA. However, it also controls activation, differentiation and survival of T-cells. This means that, after infection, cytokine storm induces activation and differentiation of T- and B-cells. IL-6 secretion causes antibody production by B-cells and increases auto-antibody formation. It also causes chronic inflammation and T-helper activation, potentially leading to autoimmune processes [22]. Diao et al. states that IL-6 levels correlate with severity of COVID-19. They also note elevation of this cytokine throughout the course of the disease with significantly lower levels after disease resolution, potentially marking depletion of IL-6 production and secretion [10]. In our study, patients with favorable COVID-19 outcomes presented moderate disease courses, while non-survivors, in 50% of cases, had severe infection. There
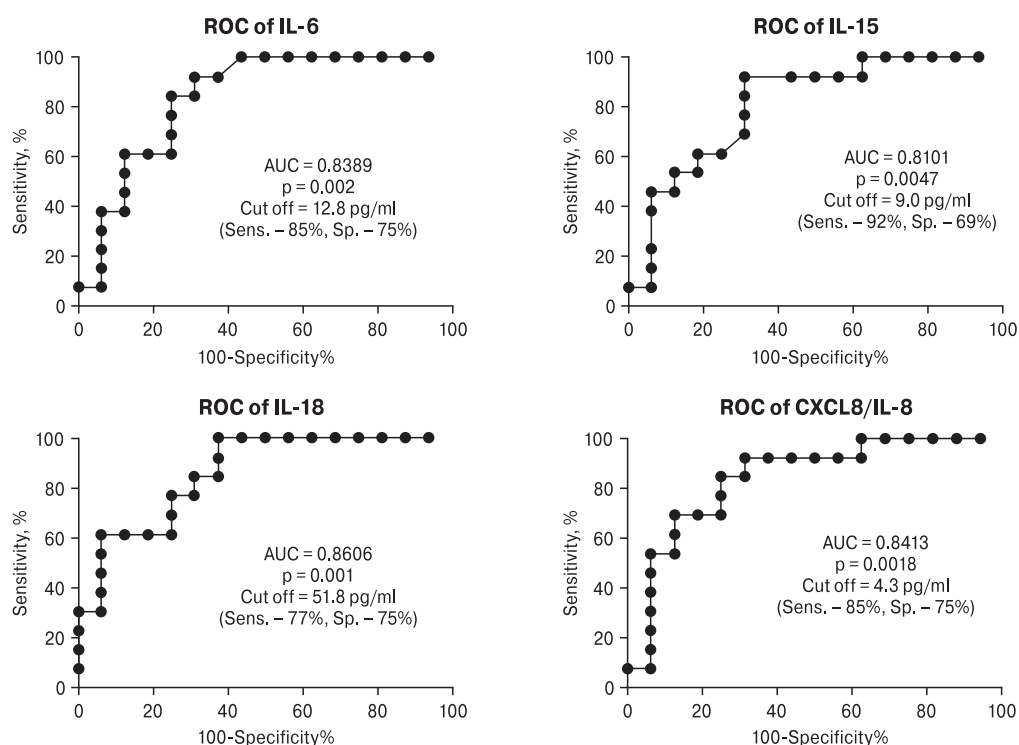
Figure 2. ROCs describing sensitivity and specificity for IL-6, IL-15, IL-18 and CXCL8/IL-8 comparison of patients with acute COVID-19 followed by different disease outcomes (recovery, death)
Note. AUC — area under curve; Sens. — sensitivity; Sp. — specificity.
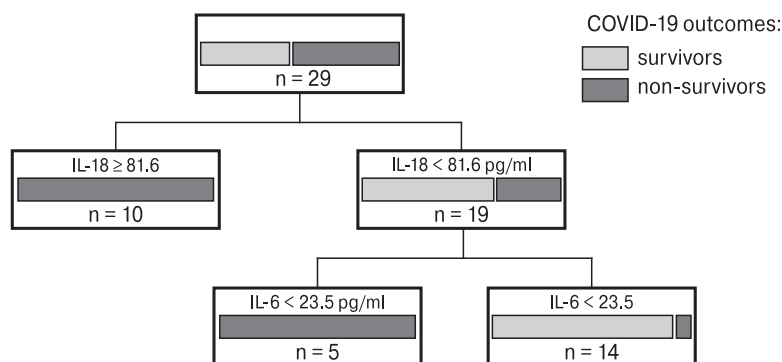
Figure 3. Decisions tree for division of patients with COVID-19 into two groups: survivors and non-survivors

is another study that shows a correlation between higher levels of IL-6 and severity of lung injury [16]. Findings throughout the world have pinpointed high blood plasma concentrations of IL-6 in patients with COVID-19 and suggest administration of anti-IL-6 drugs [19].

IL-15. A study conducted by Angioni R et al. shows the presence of higher IL-15 levels in patients with severe COVID-19 and longer hospital stays. IL-15 may stimulate a rise in NK-cell subpopulations in elderly people compared to youth. On the other hand, long exposure of NK-cells to circulating IL-15 may reduce their cytolytic activity, potentially causing their depletion [4]. There is also a correlation between IL-15 and CXCL8/IL-8 concentrations.

CXCL8. CXCL8 (also called IL-8) is involved in inflammatory responses and attracts immune cells to the inflammatory site of viral infections. CXCL8/IL-8 plays an important role in the initial control of airway infection due to its activity against neutrophils and monocytes [15]. The level of CXCL8/IL-8 in nasal secretions correlates with the severity of symptoms in acute respiratory tract infections [12]. In addition, CXCL8/IL-8 is a chemokine considered to be a potential prognostic biomarker of acute respiratory distress syndrome (ARDS) [11]. Previously, it was found that CXCL8/IL-8 levels are increased in both plasma and bronchoalveolar lavage fluid of ARDS patients [7].

IL-18. IL-18 belongs to the IL-1 cytokine family and plays roles in: innate and adaptive immunity; fibrosis; and hematopoiesis [5]. IL-18 regulates Th1 and Th2 differentiation [20]; it also stimulates naïve T-cells, $CD8^+$ and NK cells to proliferate and to produce IFNγ [25]. Further, IL-18 promotes Th1 differentiation and increases NK-cell cytotoxicity by induction of Fas-ligand expression; this leads to destruction of infected cells through Fas-mediated apoptosis [26]. IL-18 can induce cytokine production in Th2, NK, and NKT-cells [27]. This makes IL-18 a unique type of cytokine: one that can stimulate various subpopulations of T and NK-cells. A study conducted by Satış et al. shows higher plasma levels of IL-18 in COVID-19 patients compared to healthy donors; the highest levels were noticed in patients with severe pneumonia. Those authors assert a correlation between disease outcome and IL-18 concentration [24].

Out of 47 studied factors, we found 4 pro-inflammatory cytokines having high significance in evaluation of COVID-19 outcomes: IL-6, CXCL8/IL-8, IL-15, and IL-18. The main significance, in terms of predicting the outcome of acute COVID-19, belongs to IL-6 and IL-18. The AUC value for these two cytokines is 0.97, with 94% sensitivity and 100% specificity. Analyzing concentrations of IL-6 and IL-18 before administering treatment may prove valuable in terms of outcome prognosis.

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Авторы:

Арсентьева Н.А., к.б.н., старший научный сотрудник лаборатории молекулярной иммунологии ФБУН НИИ эпидемиологии и микробиологии имени Пастера, Санкт-Петербург, Россия;

Любимова Н.Е., к.б.н., научный сотрудник лаборатории молекулярной иммунологии ФБУН НИИ эпидемиологии и микробиологии имени Пастера, Санкт-Петербург, Россия;

Бацунов О.К., младший научный сотрудник лаборатории молекулярной иммунологии ФБУН НИИ эпидемиологии и микробиологии имени Пастера, Санкт-Петербург, Россия; старший лаборант кафедры иммунологии ГБОУ ВПО Первый Санкт-Петербургский государственный медицинский университет им. акад. И.П. Павлова МЗ РФ, Санкт-Петербург, Россия;

Коробова З.Р., лаборант-исследователь лаборатории молекулярной иммунологии ФБУН НИИ эпидемиологии и микробиологии имени Пастера, Санкт-Петербург, Россия; старший лаборант кафедры иммунологии ГБОУ ВПО Первый Санкт-Петербургский государственный медицинский университет имени академика Павлова МЗ РФ, Санкт-Петербург, Россия;

Кузнецова Р.Н., к.м.н., врач-аллерголог-иммунолог медицинского центра ФБУН НИИ эпидемиологии и микробиологии имени Пастера, Санкт-Петербург, Россия; доцент кафедры иммунологии ГБОУ ВПО Первый Санкт-Петербургский государственный медицинский университет имени академика Павлова МЗ РФ, Санкт-Петербург, Россия;

Рубинштейн А.А., студент 6 курса ГБОУ ВПО Первый Санкт-Петербургский государственный медицинский университет имени академика Павлова МЗ РФ, Санкт-Петербург, Россия;

Станевич О.В., врач-инфекционист отдела эпидемиологии клиник ГБОУ ВПО Первый Санкт-Петербургский государственный медицинский университет имени академика Павлова МЗ РФ, Санкт-Петербург, Россия;

Лебедева А.А., врач-терапевт стационарного отделения скорой медицинской помощи ГБОУ ВПО Первый Санкт-Петербургский государственный медицинский университет имени академика Павлова МЗ РФ, Санкт-Петербург, Россия;

Воробьев Е.А., врач-нефролог, ассистент кафедры нефрологии ГБОУ ВПО Первый Санкт-Петербургский государственный медицинский университет имени академика Павлова МЗ РФ, Санкт-Петербург, Россия;

Воробьева С.В., врач-терапевт, ассистент кафедры пропедевтики внутренних болезней ГБОУ ВПО Первый Санкт-Петербургский государственный медицинский университет имени академика Павлова МЗ РФ, Санкт-Петербург, Россия

Куликов А.Н., д.м.н., профессор, зав. кафедрой пропедевтики внутренних болезней ГБОУ ВПО Первый Санкт-Петербургский государственный медицинский университет имени академика Павлова МЗ РФ, Санкт-Петербург, Россия;

Гаврилова Е.Г., к.м.н., врач анестезиолог-реаниматолог высшей категории, доцент кафедры анестезиологии и реаниматологии ГБОУ ВПО Первый Санкт-Петербургский государственный медицинский университет имени академика Павлова МЗ РФ, Санкт-Петербург, Россия;

Певцов Д.Э., руководитель отделения переливания крови, врач-трансфузиолог НИИ детской онкологии, гематологии и трансплантологии им. Р.М. Горбачевой ГБОУ ВПО Первый Санкт-Петербургский государственный медицинский университет им. акад. И.П. Павлова МЗ РФ, Санкт-Петербург, Россия;

Полушин Ю.С., академик РАН, д.м.н., профессор, зав. кафедрой анестезиологии и реанимации ГБОУ ВПО Первый Санкт-Петербургский государственный медицинский университет имени академика Павлова МЗ РФ, Санкт-Петербург, Россия;

Шлык И.В., д.м.н., профессор, зам. главного врача отделения неотложной помощи ГБОУ ВПО Первый Санкт-Петербургский государственный медицинский университет им. акад. И.П. Павлова МЗ РФ, Санкт-Петербург, Россия;

Тотолян А.А., академик РАН, д.м.н., профессор, директор ФБУН НИИ эпидемиологии и микробиологии имени Пастера, Санкт-Петербург, Россия; зав. кафедрой иммунологии ГБОУ ВПО Первый Санкт-Петербургский государственный медицинский университет им. акад. И.П. Павлова МЗ РФ, Санкт-Петербург, Россия.

Authors:

Arsentieva N.A., PhD (Biology), Senior Researcher, Laboratory of Molecular Immunology, St. Petersburg Pasteur Institute, St. Petersburg, Russian Federation;

Liubimova N.E., PhD (Biology), Researcher, Laboratory of Molecular Immunology, St. Petersburg Pasteur Institute, St. Petersburg, Russian Federation;

Batsunov O.K., Junior Researcher, Laboratory of Molecular Immunology, St. Petersburg Pasteur Institute, St. Petersburg, Russian Federation; Senior Laboratory Assistant, Department of Immunology, Pavlov First St. Petersburg State Medical University, St. Petersburg, Russian Federation;

Korobova Z.R., Investigator (Biologist), Laboratory of Molecular Immunology, St. Petersburg Pasteur Institute, St. Petersburg, Russian Federation; Senior Laboratory Assistant, Department of Immunology, Pavlov First Saint Petersburg State Medical University, St. Petersburg, Russian Federation;

Kuznetsova R.N., PhD (Medicine), Allergologist-Immunologist, Medical Center of St. Petersburg Pasteur Institute, St. Petersburg, Russian Federation; Associate Professor, Department of Immunology, Pavlov First St. Petersburg State Medical University, St. Petersburg, Russian Federation;

Rubinstein A.A., 6th year Student, Pavlov First St. Petersburg State Medical University, St. Petersburg, Russian Federation;

Stanevich O.V., Infectious Disease Physician, Department of Epidemiology of Clinics, Pavlov First St. Petersburg State Medical University, St. Petersburg, Russian Federation;

Lebedeva A.A., Physician, Emergency Department, Pavlov First St. Petersburg State Medical University, St. Petersburg, Russian Federation;

Vorobyov E.A., Nephrologist, Assistant Professor, Department of Nephrology, Pavlov First St. Petersburg State Medical University, St. Petersburg, Russian Federation;

Vorobyova S.V., Physician, Assistant Professor, Department of Propaedeutics of Internal Diseases, Pavlov First St. Petersburg State Medical University, St. Petersburg, Russian Federation;

Kulikov A.N., PhD, MD (Medicine), Professor, Head of the Department of Propaedeutics of Internal Diseases, Pavlov First St. Petersburg State Medical University, St. Petersburg, Russian Federation;

Gavrilova E.G., PhD (Medicine), Anesthesiologist-Resuscitator of the Highest Category, Assistant Professor, Resuscitation and Anaesthesiology Department, Pavlov First St. Petersburg State Medical University, St. Petersburg, Russian Federation;

Pevtcov D.E., Transfusiologist, Head of the Blood Transfusion Department, Raisa Gorbacheva Memorial Research Institute of Children Oncology, Hematology and Transplantation, Pavlov First St. Petersburg State Medical University, St. Petersburg, Russian Federation;

Polushin Yu.S., RAS Full Member, PhD, MD (Medicine), Professor, Head of the Resuscitation and Anaesthesiology Department, Pavlov First St. Petersburg State Medical University, St. Petersburg, Russian Federation;

Shlyk I.V., PhD, MD (Medicine), Professor, Deputy Head Physician, Emergency Department, Pavlov First St. Petersburg State Medical University, St. Petersburg, Russian Federation;

Totolian A.A., RAS Full Member, PhD, MD (Medicine), Professor, Director, St. Petersburg Pasteur Institute, St. Petersburg, Russian Federation; Head of the Department of Immunology, Pavlov First St. Petersburg State Medical University, St. Petersburg, Russian Federation.