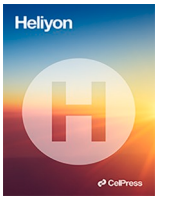
Heliyon 10 (2024) e25938

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon



Research article

Persisting IL-18 levels after COVID-19 correlate with markers of cardiovascular inflammation reflecting potential risk of CVDs development

Ivana Andrejčinová [a,b], Gabriela Blažková [a], Ioanna Papatheodorou [a,b], Kamila Bendíčková [a,c], Veronika Bosáková [a,b], Monika Skotáková [a], Roman Panovský [a,d], Lukáš Opatřil [a,d], Ondřej Vymazal [a,b], Petra Kovačovicová [a,e], Vladimír Šrámek [f], Martin Helán [a,f], Marcela Hortová-Kohoutková [a,c,**], Jan Frič [a,c,g,*]

[a] International Clinical Research Center, St. Anne's University Hospital, Brno, Czech Republic
[b] Department of Biology, Faculty of Medicine, Masaryk University, Brno, Czech Republic
[c] International Clinical Research Center, Faculty of Medicine, Masaryk University, Brno, Czech Republic
[d] 1st Department of Internal Medicine/Cardioangiology, Faculty of Medicine, Masaryk University, Brno, Czech Republic
[e] Department of Histology and Embryology, Faculty of Medicine, Masaryk University, Brno, Czech Republic
[f] Department of Anesthesiology and Intensive Care, Faculty of Medicine, Masaryk University, Brno, Czech Republic
[g] Institute of Hematology and Blood Transfusion, Prague, Czech Republic

ARTICLE INFO

Keywords:
COVID-19
Inflammation
CVDs
COVID-19 long-term consequences
IL-18

ABSTRACT

COVID-19 manifestation is associated with a strong immune system activation leading to inflammation and subsequently affecting the cardiovascular system. The objective of the study was to reveal possible interconnection between prolongated inflammation and the development or exacerbation of long-term cardiovascular complications after COVID-19. We investigated correlations between humoral and cellular immune system markers together with markers of cardiovascular inflammation/dysfunction during COVID-19 onset and subsequent recovery. We analyzed 22 hospitalized patients with severe COVID-19 within three timepoints (acute, 1 and 6 months after COVID-19) in order to track the impact of COVID-19 on the long-term decline of the cardiovascular system fitness and eventual development of CVDs. Among the cytokines dysregulated during COVID-19 changes, we showed significant correlations of IL-18 as a key driver of several pathophysiological changes with markers of cardiovascular inflammation/dysfunction. Our findings established novel immune-related markers, which can be used for the stratification of patients at high risk of CVDs for further therapy.

Abbreviations: BMI, body mass index; CVDs, cardiovascular diseases; FDR, False discovery rate; GFR, glomerular filtration rate; HF, heart failure; ICAM, intracellular adhesion molecule; IFN, interferon; IGFBP, insulin-like growth factor-binding protein; IQR, interquartile range; IL, interleukin; IRF, interferon regulatory factor; LIGHT, tumor necrosis factor superfamily member 14; LOS, length of stay; MCP, monocyte chemoattractant protein; MMP, matrix metalloproteinase; MPO, myeloperoxidase; NF-κB, nuclear factor κB; NGAL, lipocalin A; OPN, osteopontin; PlGF, placental growth factor; RT, room temperature; sRAGE, soluble receptor for advanced glycation end-products; sST2, soluble Suppression of Tumorigenicity 2 protein; TIE, tyrosine-protein kinase receptor; TNF, tumor necrosis factor; TP, timepoint; VCAM, vascular cell adhesion molecule.

* Corresponding author. International Clinical Research Center, Faculty of Medicine, Masaryk University, Brno, Czech Republic.
** Corresponding author. International Clinical Research Center, Faculty of Medicine, Masaryk University, Brno, Czech Republic.
E-mail addresses: 269300@mail.muni.cz (M. Hortová-Kohoutková), jan.fric@fnusa.cz (J. Frič).

https://doi.org/10.1016/j.heliyon.2024.e25938
Received 10 November 2023; Received in revised form 4 February 2024; Accepted 5 February 2024
Available online 13 February 2024

1. Introduction

Cardiovascular diseases (CVDs) together with obesity and diabetes have been associated with the more severe course of the COVID-19 progression and even increased mortality, almost since the very beginning of the pandemic. Furthermore, numerous reports showed that the development of CVDs during the course of disease was frequently associated with unfavorable prognosis [1–3]. Cardiovascular complications including acute myocardial injury during acute COVID-19 have been reported among recovered COVID-19 patients [4,5]. Currently a large number of prospective studies provided evidence for the rising number of new cardiac diagnoses related to post-acute COVID-19, employing tools such as echocardiography [6,7] or cardiac magnetic resonance [8,9].

Inflammation plays a crucial role in the pathogenesis of CVDs and the progression to acute and chronic heart failure (HF) [10–12]. Profound changes in the immune system are an inseparable part of COVID-19 pathophysiology, including dysregulated cytokine and cellular response. These changes can persist for an extensive amount of time, being an important component of COVID-19 long-term consequences. Although CVDs have been associated with chronic inflammation for a long time [13], the interconnection of CVDs and inflammation during COVID-19 and subsequent recovery is not sufficiently described.

Immune cells such as monocytes and macrophages are activated directly through infection [14,15] as well as indirectly in response to COVID-19 induced inflammation. The immunopathological manifestations of severe COVID-19 include lymphopenia [16], neutrophilia [17], cytokine dysregulation and disrupted type I IFN response [18]. The profound changes in cellular immunity have been reported in COVID-19, including the depletion of non-classical monocytes and higher frequencies of classical monocytes with reduced HLA-DR expression, as reported in severe COVID-19 cases [19–22]. Neutrophils in COVID-19 patients display upregulation of activation markers including CD11b and CD66b [23,24] and increased neutrophil counts is likely attributed to expansion of immature neutrophils reflecting disease severity [25].

Hyperinflammatory response results in vascular damage, metabolic dysregulation and endothelial dysfunction, hence damage in multiple organ systems [26]. Changes in cytokine networks can lead to development of CVDs [27,28]. Interleukin-1β (IL-1β) and interleukin-18 (IL-18) are IL-1 family members, both activated by caspase-1 following inflammasome formation. High serum levels of IL-18 [29] and IL-1β are associated with an increased risk of CVDs [30,31]. Interleukin-12 (IL-12) and interleukin-23 (IL-23), the two members of the IL-12 family, are important regulators of Th1 and Th17 cell responses [32]. Increased serum levels of IL-12 and IL-23 have been associated with adverse outcomes in patients with CVDs [33,34]. Induction of early innate immune response activates several transcription factors including interferon regulatory factor (IRF)-3, IRF-7 and nuclear factor κB (NF-κB), that control the production of type I interferons (IFNs) and pro-inflammatory cytokines [35]. In this study, we aimed to reveal possible interconnection between persistent inflammation and the development and progression of long-term cardiovascular complications after COVID-19.

2. Material and methods

2.1. Cohort design

Adult patients hospitalized in the St. Anne's University Hospital in Brno, Czech Republic with moderate to critical COVID-19 between October 2021 and January 2023 were recruited prospectively. All patients were treated according to The National Institute of Health Coronavirus Disease 2019 treatment guidelines [36]. Inclusion criteria were hospitalization due to COVID-19, laboratory-confirmed SARS-CoV-2 infection and 1 or more of the following: peripheral oxygen saturation (SpO_2) $\leq$ 94%, respiratory rate $\geq$30 per minute, lung infiltrates according to X-rays, signs of respiratory failure and septic shock. Patients with previous pathologies were excluded from the study. Specific SARS-CoV-2 variants were not identified. Written informed consents were obtained from all enrolled patients, and all procedures and protocols were approved by the institutional ethics committee of St Anne's University Hospital Brno (6G/2022). Blood samples from patients were collected in three timepoints (TPs): samples collected within 15 days from hospitalization were categorized as TP1 (acute phase of COVID-19), samples collected 1–2 months after hospital discharge as TP2 and 6 months after hospital discharge as TP3.

2.2. Blood sample processing and plasma preparation

Blood samples were processed within the 2 h of collection. For stabilized blood sample collection, 0.2 mL of whole blood was incubated with an equal amount of Whole Blood Cell Stabilizer (Cytodelics AB, Sweden) at room temperature (RT) for 10 min and then stored at −80 °C until further processing. Plasma samples were collected after 15-min centrifugation of the remaining whole blood at 2500×*g* and 4 °C, and immediately stored at −80 °C until usage.

2.3. Cytokine and vascular inflammation marker profiling (multiplex assay)

Measurement of the following human cytokines and chemokines was performed using LEGENDplex Human Inflammation panel 1 (BioLegend): interferon (IFN)-α2, IFN-γ, interleukin (IL)-1β, IL-6, IL-8, IL-10, IL-12p70, IL-17, IL-18, IL-23, IL-33, monocyte chemoattractant protein (MCP)-1 and tumor necrosis factor (TNF)-α. Measurement of vascular inflammation markers was performed using the LEGENDplex Human Vascular Inflammation Panel 1 specific for proteins: Cystatin C, intracellular adhesion molecule (ICAM)-1, insulin-like growth factor-binding protein (IGFBP)-4, matrix metalloproteinase, (MMP)-9, myeloperoxidase (MPO), Myoglobin, lipocalin A (NGAL), osteopontin (OPN) and vascular cell adhesion molecule (VCAM)-1; and LEGENDplex Human Vascular Inflammation

Panel 2 specific for proteins: soluble CD40 Ligand (sCD40L), tumor necrosis factor superfamily member 14 (LIGHT), placental growth factor (PlGF), soluble receptor for advanced glycation end-products (sRAGE), soluble Suppression of Tumorigenicity 2 protein (sST2), tyrosine kinase with immunoglobulin-like and EGF-like domains 1 (TIE-1) and tyrosine-protein kinase receptor (TIE-2). All experiments and plasma dilutions were performed according to the manufacturers' guidelines. Samples were acquired on a FACSCanto (BD Biosciences) and the data were analyzed using the LEGENDplex software.

2.4. Immunophenotyping of stabilized whole blood

All stabilized blood samples were processed according to the manufacturer's instructions. Briefly, stabilized blood samples were thawed at 37 °C followed by dilution 1:10 in Fixation buffer (Cytodelics AB, Sweden) and incubated at RT for 10 min. To lyse the red blood cells, samples were diluted 1:4 in Lysis buffer (Cytodelics AB, Sweden) and incubated at RT for up to 15 min. Cells were washed with Wash buffer (Cytodelics AB, Sweden) followed by washing in staining buffer (PBS with 2% FBS and 2 mM EDTA).

To obtain the phenotype of monocytes and neutrophils, cells were labeled using fluorophore-labeled antibodies against CD45 (PerCP/Cy5.5, clone HI30, BD Biosciences, Franklin Lakes, NJ, USA), CD11b (R718, clone M1/70, BD Biosciences, Franklin Lakes, NJ, USA), CD14 (FITC, clone M5E2, BD Biosciences, Franklin Lakes, NJ, USA), CD16 (PE/Cy5, clone 3G8, BioLegend, Inc, San Diego, CA, USA), CD66b (PE, clone G10F5, Thermo Fisher Scientific, Inc, Waltham, MA, USA), CD36 (PE/Cy7, clone 5–271, BioLegend, Inc, San Diego, CA, USA), CD39 (BV605, clone A1, BD Biosciences, Franklin Lakes, NJ, USA), HLA-DR (BV421, clone L243, Sony Biotechnology, Inc, San Jose, CA, USA), CD86 (BV785, clone IT2.2, BioLegend, Inc, San Diego, CA, USA) and CD33 (BV711, clone WM53, Sony Biotechnology, Inc, San Jose, CA, USA).

For phenotypic analysis of the T cells, NK cells and Treg cells, the following anti-human monoclonal antibodies were used: CD3 (BV650, clone UCHT1, Sony Biotechnology, Inc, San Jose, CA, USA), CD8 (PE/Cy5.5, clone 3B5, Thermo Fisher Scientific, Inc, Waltham, MA, USA), CD4 (BV510, clone SK3, BD Biosciences, Franklin Lakes, NJ, USA), CD25 (APC, clone BC96, BD Biosciences, Franklin Lakes, NJ, USA), CD56 (PE Dazzle 594, clone 5.1H11, BioLegend, Inc, San Diego, CA, USA) and CD127 (APC/eFluor 780, clone eBioRDR5, Thermo Fisher Scientific, Inc, Waltham, MA, USA).

Sample acquisition was performed using BD FACSymphony™ A1 (BD Biosciences) and the data were analyzed using FlowJo® software (FlowJo, LLC Ltd, Ashland, OR, USA).

2.5. Flow cytometry analysis of NF-κB (p65) nuclear translocation in monocytes

Cells were labeled using lineage antibodies against CD66b (biotin, clone G10F5, BioLegend, Inc, San Diego, CA, USA), CD56 (biotin, clone CMSSB, Thermo Fisher Scientific, Inc, Waltham, MA, USA), CD3 (biotin, clone UCHT1, Thermo Fisher Scientific, Inc, Waltham, MA, USA), CD19 (biotin, clone HIB19, Thermo Fisher Scientific, Inc, Waltham, MA, USA), CD235α (biotin, clone HIR2, Thermo Fisher Scientific, Inc, Waltham, MA, USA), CD20 (biotin, clone 2HL, Thermo Fisher Scientific, Inc, Waltham, MA, USA), followed by streptavidin (eFluor450, Thermo Fisher Scientific, Inc, Waltham, MA, USA), CD14 (BV510, M5E2, Sony Biotechnology, Inc, San Jose, CA, USA), CD16 (APC, clone eBioCB16, Thermo Fisher Scientific, Inc, Waltham, MA, USA) and CD45 (PerCP/Cy5.5, clone HI30, BD Biosciences, Franklin Lakes, NJ, USA) staining.

For cytosolic staining, cells were washed twice in BD Cytofix/Cytoperm buffer (BD Biosciences, Franklin Lakes, NJ, USA) and incubated for 30 min at 4 °C with anti–NF–κB p65 antibody (PE, clone D14E12, Cell Signaling Technology, Danvers, MA, USA) or IgG Isotype Control antibody (PE, clone DA1E, Cell Signaling Technology, Danvers, MA, USA). For nuclear staining, cells were washed twice in BD Cytofix/Cytoperm buffer (BD Biosciences, Franklin Lakes, NJ, USA) and incubated for 1 h at RT with anti–NF–κB p65 antibody (AF488, clone D14E12, Cell Signaling Technology, Danvers, MA, USA) or IgG Isotype Control antibody (AF488, clone DA1E, Cell Signaling Technology, Danvers, MA, USA). Sample acquisition was performed using BD FACSymphony™ A1 (BD Biosciences, Franklin Lakes, NJ, USA) and the data were analyzed using FlowJo® software (FlowJo, LLC Ltd, Ashland, OR, USA)

2.6. Statistical analysis

Statistical analysis was performed using Prism® (GraphPad Software, LLC, Ltd, La Jolla, CA, USA) software (version 9.0.0) and the programming language R (version 4.2.1) in R studio. Continuous variables were presented as median (interquartile range). Categorical variables were presented as numbers with percentages. Data were tested for normality using Shapiro-Wilk test and graphically by Q-Q plots and histograms. Changes in parameters across the three timepoints were tested using the Friedman test and Dunn's post-hoc test. The Benjamini-Hochberg method was employed to correct for multiple testing errors. False discovery rate (FDR)-adjusted P values smaller than 0.05 were considered statistically significant. The level of statistical significance was determined: *($P < 0.05$), **($P < 0.01$) and ***($P < 0.001$). Spearman correlation coefficients were used to identify correlations between variables and the visualization was done in R studio by corrplot package.

3. Results

Thirty-four adult patients with moderate to critical COVID-19 were initially eligible for the study. Ten patients did not participate in the study due to loss to follow-up or withdrawal of consent. One patient was excluded from the analysis due to incomplete data. As a result, 23 patient samples were processed in the study. We were able to perform cytokine and vascular inflammation marker profiling (Multiplex assay) for 22 patients. The immunophenotyping analysis of the whole blood was performed on 19 patients of whom

stabilized blood was also collected during acute COVID-19 (Immunophenotype). Demographic and clinical characteristics of enrolled patients are summarized in Table 1. Demographic data of one patient were not available.

Cytokine and chemokine levels measured across all three TPs are summarized in Table 2. From 13 measured cytokines, we found a significant decrease in IL-6 and IL-18 levels between TP1 and TP3. IL-18 also showed significant decrease between TP1 and TP2. Other cytokines did not show any changes in their levels during COVID-19 progression. Similarly, we measured the plasma levels of markers associated with cardiovascular inflammation/dysfunction (Table 3). We observed a significant decrease in the plasma levels of myoglobin and OPN in TP2 compared to TP1. OPN and MPO levels were continuously reduced also in TP3 compared to TP1. Plasma levels of ICAM-1 were decreased 6 months after COVID-19 onset (TP2 vs TP3). sST2 showed a significant decrease in TP3 compared to TP1. On the contrary, we observed increased levels of TIE-1 in TP2 and TP3 compared to TP1.

In order to investigate changes in the immune cells' frequency and functionality, we performed immunophenotyping of the whole blood samples from 19 patients of whom stabilized blood was also collected during acute COVID-19. The study design is depicted in Fig. 1A. We analyzed the most frequent leukocytes with possible role in COVID-19 based on their surface markers - neutrophils ($CD45^+$, $CD66b^+$, $CD16^+$), monocytes ($CD45^+$, $CD56^-$, $CD3^-$, $CD14^{+/low}$, $CD16^{+/-}$), T cells ($CD45^+$, $CD3^+$, $CD4^+$, $CD8^+$) and NK cells ($CD45^+$, $CD3^-$, $CD56^+$, $CD16^{+/-}$) (Supplementary Fig. S1). We observed changes in the frequency of neutrophils, T cells and NK cells within all TPs (Fig. 1B). Neutrophil frequency was reduced in the TP2. On the other hand, we observed an increase in the T cells and NK cell frequency in TP2 and TP3 compared to TP1. Contrary, the frequency of monocytes did not change during COVID-19 recovery (Fig. 1B). Next, we focused on the detailed profiling of leukocyte subsets. T cells (Fig. 1C) showed significant reduction of $CD4^+$ T cell frequency in TP2 compared to TP1. On the other hand, $CD8^+$ T cell frequency showed only increasing trends during COVID-19 resolution. We did not observe any changes in the frequency of $CD127^-$ $CD39^+$ Treg cells during COVID-19 resolution. We also did not see any changes in the NK cell subset frequency ($CD56^{bright}CD16^-$ nor $CD56^{dim}CD16^+$) (Fig. 1D). Although the total frequency of monocytes did not change (Fig. 1B), we observed significant decrease in the classical monocytes ($CD14^+CD16^-$) following acute COVID-19 (TP2) (Fig. 1E), whereas the frequency of non-classical monocytes ($CD14^{dim/-}$ $CD16^+$) increased in both TP2 and TP3 compared to TP1.

We also focused on the evaluation of functional abilities of selected leukocytes. Neutrophil CD11b expression increased in TP2 and TP3 compared to TP1, while CD66b expression levels did not change (Fig. 2A). Similarly, neither the expression of CD16 on NK cells (Fig. 2B) nor CD39 on Treg cells (Fig. 2C) were affected by COVID-19. Conversely, COVID-19 caused changes in monocyte functionality (Fig. 2D), represented by significant increase of HLA-DR in TP3. In addition, we observed trends in increasing CD36 surface expression on monocytes in TP2 and TP3 compared to TP1. Expression of CD86 on monocytes was not affected by COVID-19. In order to evaluate the monocyte's inflammatory activation ability, we then focused on NF-κB, since it is the most prominent pro-inflammatory transcription factor. We evaluated NF-κB activity in monocytes by measuring cytosolic and total levels of p65 during COVID-19 and its resolution (Fig. 2E). Calculation of relative cytosolic NF-κB levels (presented as the percentage of total) was feasible for 18 patients. NF-κB showed significant reduction in total p65 levels between TP2 and TP3, while cytosolic levels remained unchanged. However, some patients had increased levels of total NF-κB in TP3. We analyzed correlations between the total NF-κB p65 levels and markers of cardiovascular inflammation (Fig. 2F). We found negative correlations between NF-κB levels and markers OPN, IGFBP-4, VCAM-1 and Cystatin C in both TP2 and TP3. Strong positive correlations were found between total NF-κB levels and markers LIGHT and sCD40L in T2 and TP3, respectively.

To further elaborate on possible stratification potential of some markers as well as their biological relevance for development of CVDs, we focused in detail on several candidates. CRP levels, creatinine levels and leukocyte counts decreased significantly in TP2 compared to TP1, indicating resolution of acute inflammation. IL-6 levels were significantly reduced in TP3 compared to TP1. On the other hand, creatinine levels increased in TP3 compared to TP2 (Fig. 3A). Glomerular filtration rate (GFR) represents one of the markers with promising potential to distinguish patients developing CVDs with cut-off values for patient risk stratification established

Table 1
Demographic and clinical characterization of patients.

Characteristic		Total (Multiplex assay)	Total (Immunophenotype)
Recruited patients, n		22	19
Sex, n (%)	Female	14 (63.6%)	11 (57.9%)
	Male	8 (36.4%)	8 (42.1%)
Age	Median (IQR)	55.50 (39.00–64.00)	56.00 (39.00–67.00)
BMI	Median (IQR)	29.93 (26.67–33.21)	29.00 (26.00–33.00)
Hospital LOS, days	Median (IQR)	7.5 (6.00–15.00)	7.0 (6.00–10.00)
COVID-19 vaccination, n (%)			
number of doses			
0		15 (68.18%)	11 (57.89%)
2		5 (22.73%)	5 (26.32%)
3		1 (4.55%)	1 (5.26%)
4		1 (4.55%)	2 (10.53%)
Pneumonia, n (%)		17 (80.95%)*	13 (72.22%)#
Oxygen therapy, n (%)		15 (71.43%)*	12 (66.67%)#
Mechanical ventilation, n (%)		4 (19.05%)*	0 (0%)#

Abbreviations: BMI, body mass index; IQR, interquartile range; LOS, length of stay. Asterisks and hashes indicate out of 21 and 18 patients, respectively (demographic data were not available for one patient).

Table 2
Plasma cytokine levels during COVID-19 resolution.

Marker		TP1	TP2	TP3
IL-1β pg/mL	Median (IQR)	2.20 (0.13–16.20)	0.87 (0–7.77)	1.70 (0.15–8.22)
IFN-α pg/mL	Median (IQR)	2.56 (1.12–8.23)	2.26 (0.43–5.28)	3.66 (1.46–4.99)
IFN-γ pg/mL	Median (IQR)	3.21 (1.07–5.64)	2.26 (0.00–4.69)	2.35 (0.00–7.37)
TNF-α pg/mL	Median (IQR)	24.59 (6.44–75.21)	39.8 (0.00–95.29)	28.39 (0.00–85.00)
MCP-1 pg/mL	Median (IQR)	425.56 (256.69–594.74)	567.80 (236.10–907.99)	430.41 (315.27–647.47)
IL-6 pg/mL	Median (IQR)	22.03 (4.17–53.16)	10.18 (4.14–23.22)	9.04 $^{\$\$}$ (2.68–25.48)
IL-8 pg/mL	Median (IQR)	52.51 (19.66–134.10)	29.32 (6.59–102.52)	33.00 (2.86–88.15)
IL-10 pg/mL	Median (IQR)	17.09 (6.38–29.61)	8.57 (1.45–36.00)	15.98 (0.71–24.22)
IL-12p70 pg/mL	Median (IQR)	6.74 (1.23–21.08)	4.45 (1.56–18.61)	5.95 (1.50–19.47)
IL-17 pg/mL	Median (IQR)	0.43 (0.21–1.07)	0.42 (0.19–0.98)	0.22 (0.07–0.84)
IL-18 pg/mL	Median (IQR)	611.29 * (431.40-1192.22)	479.89 (295.18–782.03)	369.88 $^{\$\$\$}$ (177.42–614.73)
IL-23 pg/mL	Median (IQR)	20.48 (4.60–40.09)	16.21 (1.14–57.23)	12.10 (2.78–54.25)
IL-33 pg/mL	Median (IQR)	89.45 (27.30–327.30)	64.88 (13.10–171.00)	90.03 (25.25–136.20)

TP1 – within the first 15 days post admission (acute phase), TP2 – 1 to 2 months after hospital discharge, TP3 – 6 months after hospital discharge. Data were tested using the Friedman test and Dunn's post-hoc test. The Benjamini-Hochberg method was employed to correct for multiple testing errors. Bold text indicates significant difference among the three TPs ($P < 0.05$). Symbols denote FDR-adjusted *P* values: asterisks and dollars indicate level of significance for comparisons between TP1 and TP2 ($^{*}P < 0.05$), and between TP1 and TP3 ($^{\$\$}P < 0.01$, $^{\$\$\$}P < 0.001$), respectively. Abbreviations: FDR, False Discovery Rate; IFN, interferon; IL, interleukin; IQR, interquartile range; MCP, monocyte chemoattractant protein; TNF, tumor necrosis factor; TP, timepoint.

Table 3
Cardiovascular inflammation/dysfunction marker levels during COVID-19 resolution.

Marker		TP1	TP2	TP3
Myoglobin ng/mL	Median (IQR)	115.93 ** (81.26–237.69)	63.63 (42.68–98.93)	96.89 (65.91–130.48)
OPN ng/mL	Median (IQR)	164.57 *** (101.68–250.99)	59.80 (32.89–119.46)	57.21 $^{\$\$\$}$ (28.35–76.96)
MPO ng/mL	Median (IQR)	332.42 (147.26–492.72)	188.49 (101.71–322.04)	129.63 $^{\$\$\$}$ (87.24–172.02)
NGAL ng/mL	Median (IQR)	231.72 (136.77–359.23)	222.17 (157.96–328.24)	186.61 (127.58–263.03)
IGFBP-4 ng/mL	Median (IQR)	240.27 (121.12–340.25)	169.88 (141.00–303.40)	202.14 (110.22–305.49)
ICAM-1 ng/mL	Median (IQR)	71.42 (58.56–105.31)	73.27$^{\#}$ (58.03–88.49)	49.59 (39.57–85.23)
VCAM-1 ng/mL	Median (IQR)	3501.27 (2800.25-4759.64)	3154.62 (2493.47-4577.22)	3178.28 (2206.09-3501.11)
MMP-9 ng/mL	Median (IQR)	255.92 (109.80–482.22)	173.26 (93.20–224.67)	120.92 (91.42–189.89)
Cystatin C ng/mL	Median (IQR)	374.24 (213.21–695.17)	285.11 (129.74–529.06)	312.57 (141.69–530.15)
sST2 pg/mL	Median (IQR)	1828.25 (1320.60-2943.05)	1505.18 (1248.20-2202.10)	1565.65 $^{\$}$ (1071.65-2225.95)
sRAGE pg/mL	Median (IQR)	2309.09 (1055.92-9087.70)	2594.03 (1671.60-4625.54)	1996.69 (1535.54-2945.45)
TIE-2 ng/mL	Median (IQR)	10.40 (8.20–13.42)	12.17 (10.16–15.37)	12.92 (10.43–15.72)
sCD40 L pg/mL	Median (IQR)	602.19 (166.62-1054.45)	287.84 (196.39–392.10)	333.26 (172.54–410.06)
TIE-1 ng/mL	Median (IQR)	13.30 * (9.89–18.99)	16.03 (10.56–20.47)	17.95 $^{\$}$ (11.59–21.75)
LIGHT pg/mL	Median (IQR)	249.36 (88.62–546.26)	298.76 (104.42–609.91)	301.27 (85.19–639.55)
PlGF pg/mL	Median (IQR)	28.89 (17.58–48.93)	31.12 (23.50–47.46)	28.41 (19.65–45.37)

TP1 – within the first 15 days post admission (acute phase), TP2 – 1 to 2 months after hospital discharge, TP3 – 6 months after hospital discharge. Data were tested using the Friedman test and Dunn's post-hoc test. The Benjamini-Hochberg method was employed to correct for multiple testing errors. Bold text indicates significant difference among the three TPs ($P < 0.05$). Symbols denote FDR-adjusted P values: asterisks, hashes and dollars indicate level of significance for comparisons between TP1 and TP2 ($^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$), TP2 and TP3 ($^{\#}P < 0.05$), and between TP1 and TP3 ($^{\$}P < 0.05$, $^{\$\$\$}P < 0.001$), respectively. Abbreviations: FDR, False Discovery rate; ICAM, intracellular adhesion molecule; IGFBP, insulin-like growth factor-binding protein; IQR, interquartile range; LIGHT, tumor necrosis factor superfamily member 14; MMP, matrix metalloproteinase; MPO, myeloperoxidase; NGAL, Neutrophil gelatinase-associated lipocalin; OPN, osteopontin; PlGF, placental growth factor; sCD40L, soluble CD40 Ligand; sRAGE, soluble receptor for advanced glycation end-products; sST, soluble Suppression of Tumorigenicity; TIE, tyrosine kinase with immunoglobulin-like and EGF-like domains; TP, timepoint; VCAM, vascular cell adhesion molecule.

by previous studies. We observed an increase in GFR between TP1 and TP2, followed by a decrease in TP3 (Fig. 3B). Moreover, according to the cut-off values retrieved from the literature, we observed possible stratification potential of GFR. Cardiovascular inflammation marker IGFBP-4 showed persisting high levels during COVID-19 recovery (Fig. 3C). On the other hand, OPN and MPO showed gradual reduction in their levels without any stratification potential (Fig. 3D). Although most cytokines did not change during the progression of COVID-19, IL-18 showed significant reduction in TP2 and TP3 compared to TP1. However, in some patients IL-18 seems to persist in elevated levels even 6 months after COVID-19 (Fig. 3E). Levels of vascular inflammation marker TIE-1 increased in TP2 and TP3 compared to TP1. We observed similar trends in TIE-2 levels (Fig. 3F).

To investigate the possible interconnections between clinical, immunological, and cardiovascular inflammation markers, we performed correlation analyses across all TPs (Fig. 4). During acute COVID-19 (Fig. 4A), we observed positive correlations between most cytokines together with MPO, NGAL, MMP-9 and LIGHT. Majority of cytokines did not correlate with immune cells markers in TP1. Interestingly, in TP2 (Fig. 4B) some cardiovascular inflammation markers correlated with CRP levels and leukocyte counts. Several cardiovascular inflammation markers positively correlated with each other. We found several significant correlations between

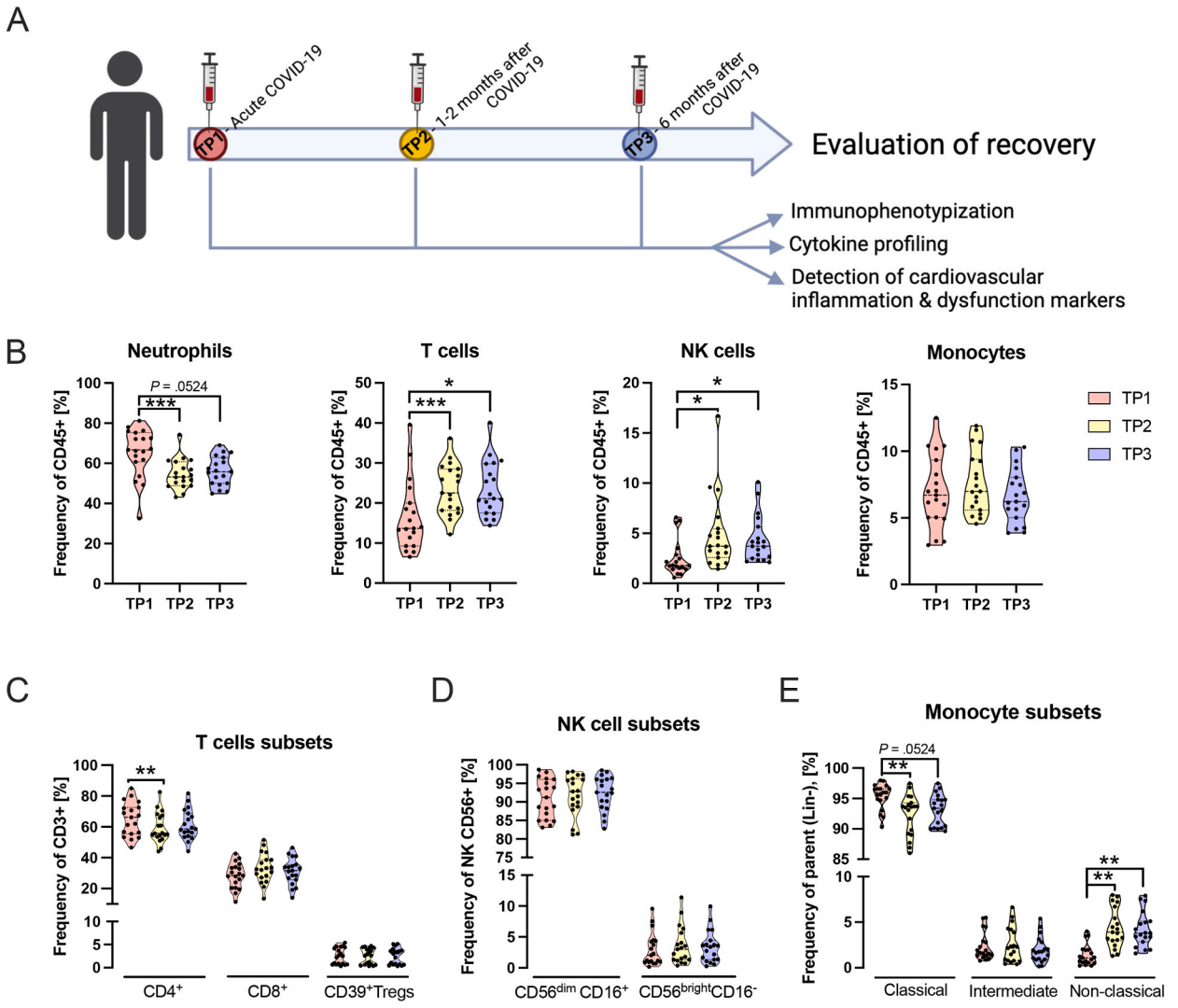


Fig. 1. Changes in the frequency of immune cell types and their subsets during progression of COVID-19 and subsequent recovery. A. Graphical representation of study design. B. Changes in frequency of various leukocytes were associated with COVID-19 progression. C. COVID-19 induced decrease of $CD4^+$ T cells. D. Frequency of NK cell subsets were not affected by COVID-19. E. Frequency of classical and non-classical monocytes was significantly altered during COVID-19 and subsequent recovery. Data were tested using the Friedman test and Dunn's post-hoc test. The Benjamini-Hochberg method was employed to correct for multiple testing errors. FDR-adjusted P values are indicated as follows: $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

IL-18 and cardiovascular inflammation markers, including TIE-1. IL-18 levels also correlated with the frequency of monocyte and T cell subsets. In TP3 (Fig. 4C), IL-18 showed strong positive correlation with IL-10 and TNF-α. Additionally, IL-18 showed negative correlations with monocytic CD36. Our results indicate a potential link between IL-18 and increased risk of CVDs development.

4. Discussion

Profound changes of the immune system have been associated with COVID-19 since the early days of the pandemic. COVID-19 has been linked with long-term consequences including worsening of ongoing comorbidities and development of new ones [4,37]. In this study we addressed the interconnection of long-term changes in the inflammatory profile of COVID-19 survivors with possible development of new or exacerbation of pre-existing CVDs.

COVID-19 is associated with much lower levels of circulating cytokines than in other infection-induced inflammatory conditions, such as sepsis [38]. In our cohort, we observed increased IL-6 and IL-18 levels in acute COVID-19 compared to the normal ranges (IL-18 [37–215 pg/mL]; IL-6 [0–43.5 pg/ml]) reported in previous studies [39,40]. While the levels of IL-6 decreased during recovery, elevated IL-18 levels persisted in many patients. Interestingly, plasma levels of most measured inflammatory cytokines were not affected in acute COVID-19 and their levels remained unchanged during recovery. IL-18, a member of the IL-1 family of cytokines, is a

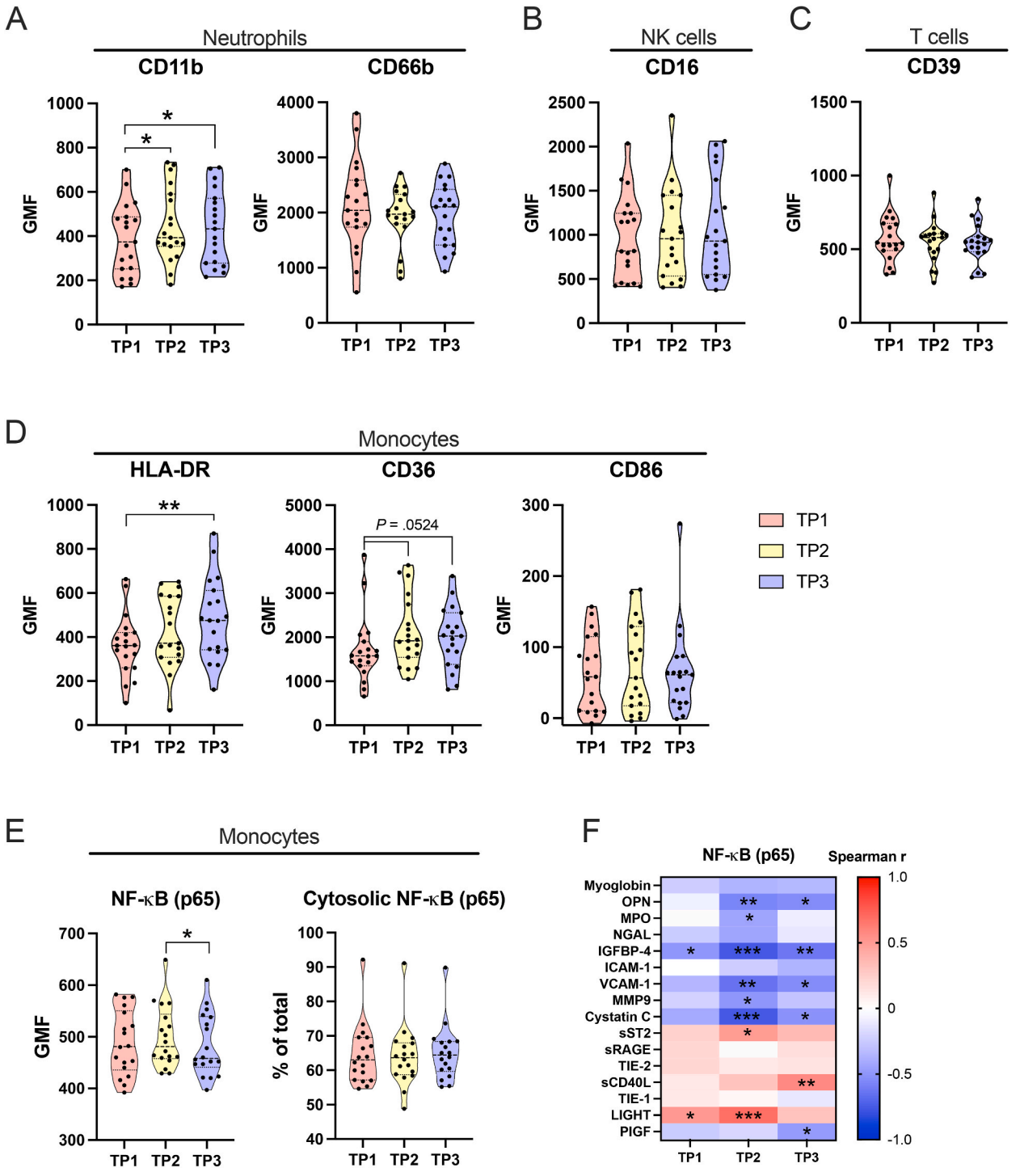


Fig. 2. Alterations of immune cell functionality during progression of COVID-19 and subsequent recovery. A. Expression levels of neutrophil activation markers - CD11b and CD66b during COVID-19 and subsequent recovery. B. Expression levels of NK-cell CD16 was not affected during COVID-19 resolution. C. COVID-19 did not affect expression of CD39 on T cells. D. COVID-19 induced changes in monocyte activation status represented by altered expression of HLA-DR and CD36. There were no changes in CD86 expression on monocytes. E. Alteration of monocyte functionality was also reflected by changes in NF-κB (p65) activity. We observed decrease in NF-κB (p65) activity in TP3. Percentages of cytosolic NF-κB (p65) were not affected. F. Correlation between NF-κB (p65) activity and markers of cardiovascular inflammation showed strong correlations with OPN, IGFBP-4, VCAM-1, MMP-9, Cystatin C, sST2, sCD40L, LIGHT and PIGF. Data were tested using the Friedman test and Dunn's post-hoc test. The Benjamini-Hochberg method was employed to correct for multiple testing errors. FDR-adjusted P values are indicated as follows: $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

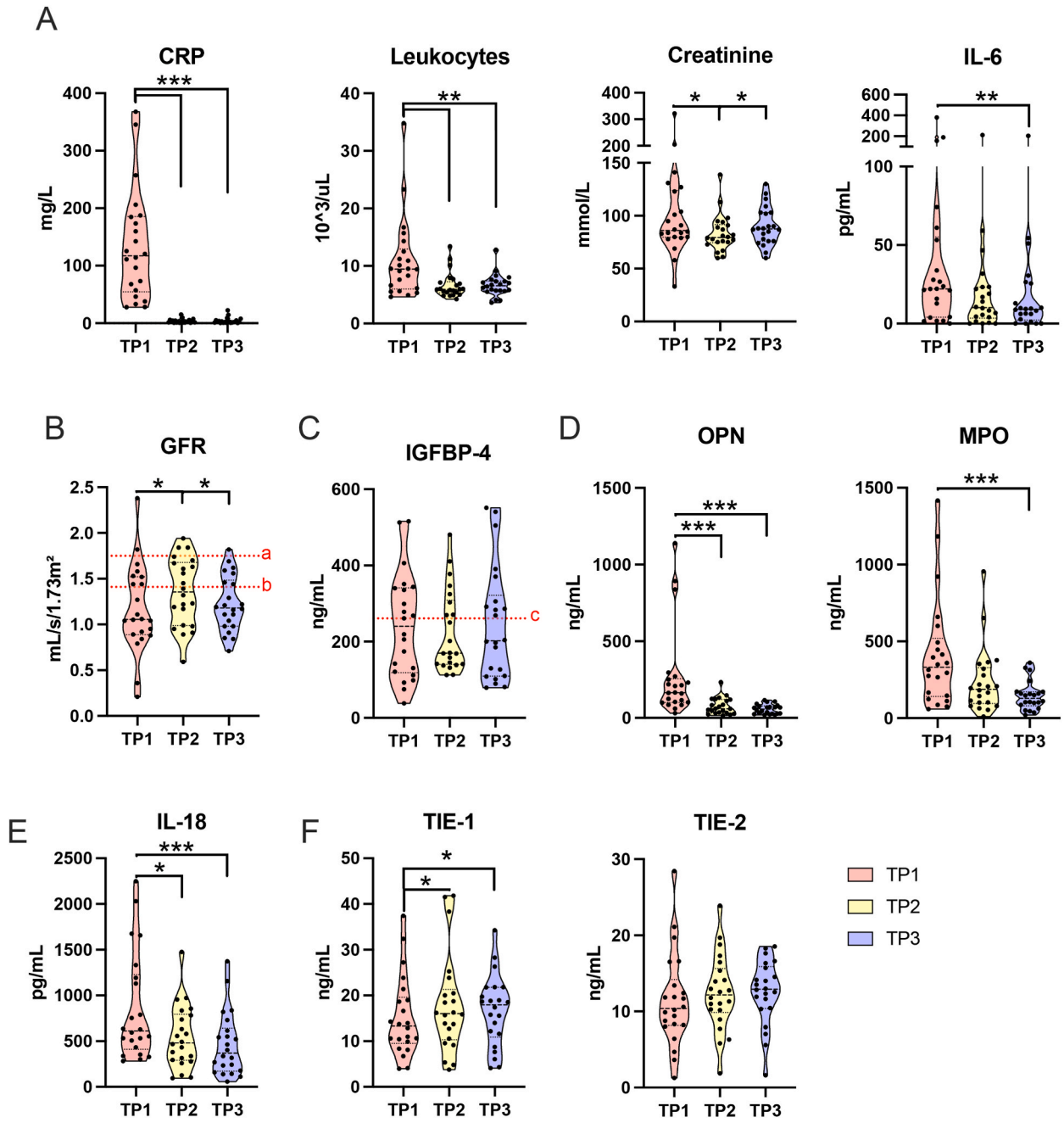


Fig. 3. Mutual interconnection between systemic and cardiovascular inflammation and dysfunction markers during COVID-19 recovery. A. Markers of acute inflammation were diminished during recovery from COVD-19. B. Glomerular filtration rate (GFR) is frequently used marker of CVDs, with defined cut-off values for risk stratification (a.) Korhonen et al. 2016, (b.) Gao et al. 2022C. IGFBP-4 showed persisting levels during COVID-19 recovery - determined cut-off value by Hjortebjerg et al. 2015 (c.). D. Some markers of cardiovascular inflammation – OPN and MPO were significantly reduced during COVID-19 progression. E. IL-18 was reduced during COVID-19 recovery, however in some patients we observed persisting levels of IL-18. F. Circulating levels of TIE-1 were increased and TIE-2 showed increasing trends during recovery. Data were tested using the Friedman test and Dunn's post-hoc test. The Benjamini-Hochberg method was employed to correct for multiple testing errors. FDR-adjusted P values are indicated as follows: $*P < 0.05$, $**P < 0.01$, $***P < 0.001$.

proinflammatory cytokine implicated in a number of pulmonary infections and inflammatory diseases including chronic obstructive pulmonary disease [41], acute respiratory distress syndrome [42] and COVID-19 [43] but also various CVDs [31,44,45]. Increased serum levels of IL-18 have been associated with worse outcomes in patients with documented CVDs [44,46,47]. Some studies have demonstrated the prognostic potential of IL-18 in COVID-19 outcomes [43,48].

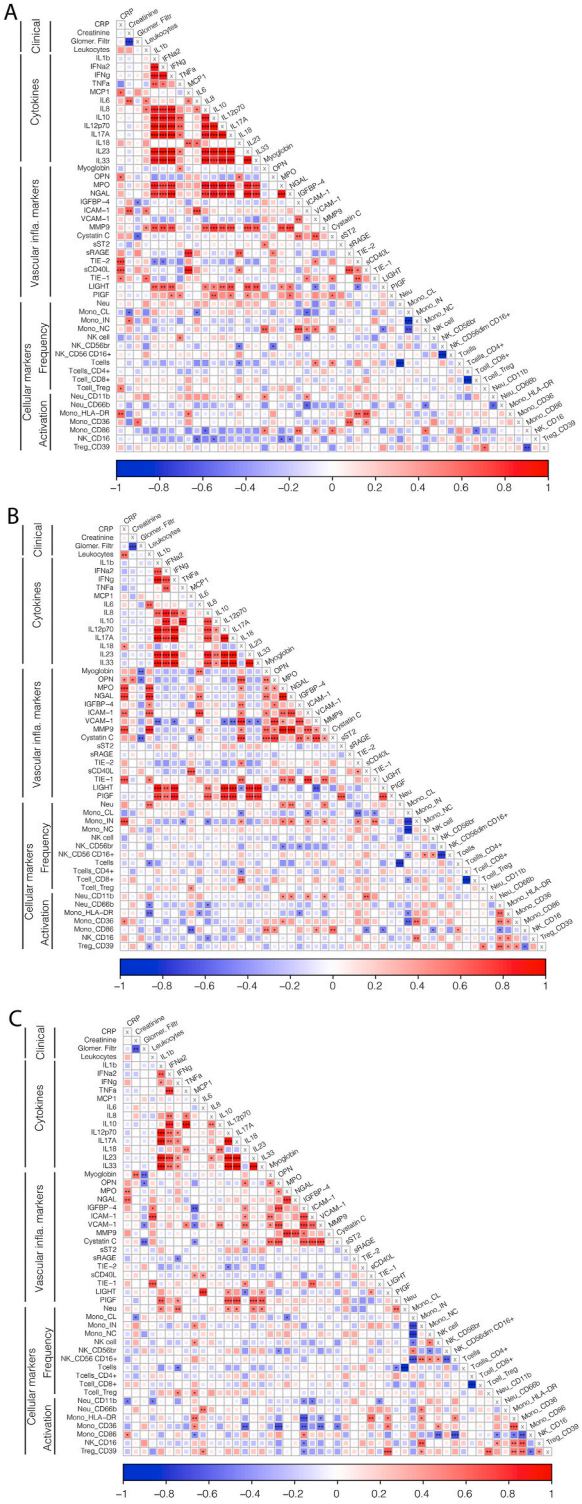


Fig. 4. Multi-parameters correlations within all TPs. Positive correlations are displayed in red and negative correlations in blue. The color intensity and size of the squares is proportional to the Spearman correlation coefficients A. IL-18 levels positively correlated with some vascular inflammation markers – OPN, sRAGE and sCD40L in acute COVID-19 (TP1). Decreased frequency of T cells and CD56^{bright}CD16^{-} NK cells negatively correlated with IL-18. Contrary, positive correlation was found between CD11b expression on neutrophils and IL-18 levels. B. Cardiovascular inflammation markers OPN, MPO, IGFBP-4, ICAM-1, VCAM-1, MMP-9 and Cystatin C positively correlated with IL-18 levels 1–2 months after COVID-19 (TP2). Vascular inflammation markers TIE-1 and TIE-2 also positively correlated with IL-18. Contrary, negative correlation was found

between LIGHT and IL-18. Importantly, the decreased frequency of classical monocytes and $CD4^+$ T cells negatively correlated with IL-18 levels. On the contrary, increased frequency of intermediate monocytes and $CD8^+$ T cells positively correlated with IL-18. C. Correlations between markers OPN, IGFBP-4, ICAM-1, VCAM-1, Cystatin C, TIE-1 and LIGHT and IL-18 levels were still present 6 months after COVID-19 (TP3). Increased CD36 expression on monocytes negatively correlated with IL-18 levels. Visualization was done in R studio using corrplot package. *P < 0.05, **P < 0.01, ***P < 0.001.

The acute phase of COVID-19 was accompanied by early increase in myoglobin, myeloperoxidase (MPO) and osteopontin (OPN). MPO and OPN have been associated with atherosclerosis development and increased risk of coronary artery disease (CAD) [49–51]. While the levels of OPN and myoglobin decreased, elevated MPO persisted during the first recall. Interestingly, our data showed an increase in the circulating receptor tyrosine kinase TIE-1 during recovery. TIE-1 is essential in regulating angiogenesis and vascular remodeling similarly to matrix metalloproteinases [52]. When an inflammation occurs, endothelial TIE-1 is rapidly cleaved, (reflected by increased serum levels) leading to vessel destabilization and subsequent vascular leakage [53,54].

COVID-19 also affects immune cell numbers and their functions. Severe COVID-19 cases display reduction in lymphocyte counts and increased neutrophil counts in the acute phase [55]. In our cohort, the frequency of neutrophils decreased, while the frequency of T cells and NK cells increased, reflecting recovery. During the first recall, we observed decreased frequency of $CD4^+$ T cells, shifting towards increase in the numbers of $CD8^+$ T cells. We and others have already reported that decreased $CD8^+$ T cell frequencies have been associated with increased mortality of severe COVID-19 cases [56,57] indicating the vital role of $CD8^+$ T cells in COVID-19 resolution. The frequency of monocyte subsets also changed: classical monocytes ($CD14^+CD16^-$) were reduced, whereas the numbers of non-classical monocytes ($CD14^{low}CD16^+$) increased during recovery. Reduction in non-classical ($CD14^{low}CD16^+$) and intermediate monocytes ($CD14^+CD16^+$) reported in acute COVID-19 [20,21] has been associated with selective recruitment to the lungs, possibly contributing to *in situ* differentiation of macrophages [58]. Importantly, macrophage infiltration had been reported as a pathological feature of COVID-19-associated CVDs [59]. Interestingly, macrophage-derived IL-18 can promote NF-κB activation in vascular smooth muscle cells and cardiomyocytes, contributing to the development of CVDs [29,60]. Monocytes of severe COVID-19 cases have been reported to express low levels of HLA-DR [22]. Our data show an increase in HLA-DR expression in the second recall during recovery, suggesting prolonged dysfunction. We also reported that some patients displayed increased monocyte-specific activation of NF-κB. Moreover, we observed increasing trends of CD36 surface expression during recovery, indicating changes in monocyte metabolism [61]. Other studies have reported that neutrophils display upregulation of neutrophil activation markers in acute COVID-19, including CD11b and CD66b [23,24]. In our cohort, expression of CD11b on neutrophils increased, while CD66b expression did not change during recovery.

The perturbation of the immune system is well documented during COVID-19, furthermore the profound changes in the immune function often persist for several months or even years after the recovery from the acute disease, leaving the patient vulnerable to develop or exacerbate other disorders and complications, as documented for e.g. during sepsis [62,63]. Observation of cardiovascular effects was among the first non-immune consequences of the disease during the pandemic's onset [37]. The cardiovascular comorbidities are among the most severe risk factors affecting the patient's survival and long-term health related quality of life [64]. It remains unclear whether the cardiovascular comorbidities after COVID-19 are due to the fact that the heart and vessel cells are the major SARS-CoV-2 virus entry sites [65] or the consequences of inflammation, which induce cardiovascular dysfunction. In the context of COVID-19-induced long-term cardiovascular consequences, we focused on clinically relevant markers reporting health decline with known cut-off value for cardiovascular complications. We observed significant changes in GFR. Interestingly, the detailed analysis of data distribution in our cohort showed part of the levels above the cut-off values suggesting potential diagnostic value in the context of COVID-19 driven CVDs. Some patients were above the cut-off limit established by Gao (2022), suggesting possible development of delayed CVD-related adverse events [66]. In addition to clinically used markers, we reported persisting increased levels of insulin-like growth factor-binding protein (IGFBP)-4 in some patients, indicating stratification potential of this marker. Some patients in our cohort were above the defined cut-off value established by Hjortebjerg (2017) for increased risk of cardiovascular mortality [67].

In conclusion, we believe that this study, albeit exploratory, hinted at the potential link between long-term systemic IL-18 elevation, profound changes in the frequencies and functionality of leukocytes and potential risk of CVDs development or progression in patients who have been hospitalized with moderate to critical COVID-19. Levels of IL-18 correlated with many cardiovascular inflammation/dysfunction markers, especially during COVID-19 recovery. Further research is needed to evaluate the predictive potential of IL-18 in the context of newly developed or worsened CVDs in post-COVID-19 patients.

4.1. Limitations

This single-center, observational study has also some limitations. The major limitation of our study is a relatively small sample size. The limited number of patients did not allow us to stratify our cohort based on the disease severity, nor generalize our findings to a broader range of patients who had recovered from COVID-19. Furthermore, the inclusion/exclusion criteria, low willingness to participate in the study and the drop-out of patients during follow-up contributed to the limited number of subjects in the cohort and may have introduced a selection bias. Patients experiencing CVD symptoms, albeit without any CVD outcome, may have been more likely to attend the follow-up appointments. Second, the longitudinal nature of our study with pre-defined timeframes for patient recalls did not allow us to investigate CVD outcomes that may be occurring in patients later, nor stratify our cohort based on a long-term clinical outcome. Third, the timeframe of patient recruitment, corresponding to the fifth and sixth COVID-19 pandemic wave in

the Czech Republic, may have had an impact on the heterogeneity of the cohort. Further studies evaluating the predictive potential of proposed biomarkers on larger sample sizes are needed.

Funding

The research was supported by the Ministry of Health of the Czech Republic, grant nr. NU22-A-121), all rights reserved and DRO (Institute of Hematology and Blood Transfusion – UHKT, 00023736) and by project nr. LX22NPO5107 (MEYS): Financed by European Union – Next Generation EU.

Ethics declaration

This study was reviewed and approved by the institutional ethics committee of St Anne's University Hospital Brno, with the approval number (6G/2022). All enrolled patients provided written informed consent to participate in the study.

Data availability statement

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

CRediT authorship contribution statement

Ivana Andrejčinová: Writing – review & editing, Writing – original draft, Visualization, Investigation, Formal analysis, Data curation. **Gabriela Blažková:** Writing – review & editing, Investigation, Data curation. **Ioanna Papatheodorou:** Writing – review & editing, Formal analysis. **Kamila Bendíčková:** Writing – review & editing, Investigation. **Veronika Bosáková:** Writing – review & editing, Investigation. **Monika Skotáková:** Writing – review & editing, Formal analysis. **Roman Panovský:** Resources, Investigation, Funding acquisition. **Lukáš Opatřil:** Resources, Investigation. **Ondřej Vymazal:** Writing – review & editing, Investigation. **Petra Kovačovicová:** Writing – review & editing, Formal analysis. **Vladimír Šrámek:** Resources, Investigation. **Martin Helán:** Resources, Investigation. **Marcela Hortová-Kohoutková:** Writing – review & editing, Writing – original draft, Investigation, Formal analysis, Conceptualization. **Jan Frič:** Writing – review & editing, Writing – original draft, Supervision, Project administration, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

We thank Dr. Ondřej Pelák of BD Biosciences Czechia for providing access to the BD FACSymphony A1 analyzer and Dr. Jiří Šinkora for his guidance and expertise with the instrument. We would also like to thank the technical support team of the Center for Translational Medicine for the skillful technical assistance, all of the ICU nurses of St. Anne's University Hospital Brno for help with blood sample collection. This work was supported by COST (European Cooperation in Science and Technology) Action 20117 - Converting molecular profiles of myeloid cells into biomarkers for inflammation and cancer (Mye-InfoBank).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e25938.

References

[1] D. Wang, et al., Clinical characteristics of 138 hospitalized patients with 2019 novel coronavirus–infected pneumonia in Wuhan, China, JAMA 323 (11) (Mar. 2020) 1061, https://doi.org/10.1001/jama.2020.1585.
[2] Z. Wu, J.M. McGoogan, Characteristics of and important lessons from the coronavirus disease 2019 (COVID-19) outbreak in China: summary of a report of 72 314 cases from the Chinese center for disease control and prevention, JAMA 323 (13) (Apr. 2020) 1239, https://doi.org/10.1001/jama.2020.2648.
[3] T. Guo, et al., Cardiovascular implications of fatal outcomes of patients with coronavirus disease 2019 (COVID-19), JAMA Cardiol 5 (7) (Jul. 2020) 811, https://doi.org/10.1001/jamacardio.2020.1017.
[4] T. Kotecha, et al., Patterns of myocardial injury in recovered troponin-positive COVID-19 patients assessed by cardiovascular magnetic resonance, Eur. Heart J. 42 (19) (May 2021) 1866–1878, https://doi.org/10.1093/eurheartj/ehab075.
[5] C. Bavishi, R.O. Bonow, V. Trivedi, J.D. Abbott, F.H. Messerli, D.L. Bhatt, Special Article - acute myocardial injury in patients hospitalized with COVID-19 infection: a review, Prog. Cardiovasc. Dis. 63 (5) (Sep. 2020) 682–689, https://doi.org/10.1016/j.pcad.2020.05.013.

[6] W.E. Moody, et al., Persisting adverse ventricular remodeling in COVID-19 survivors: a longitudinal echocardiographic study, J. Am. Soc. Echocardiogr. 34 (5) (May 2021) 562–566, https://doi.org/10.1016/j.echo.2021.01.020.
[7] T. Sonnweber, et al., Cardiopulmonary recovery after COVID-19: an observational prospective multicentre trial, Eur. Respir. J. 57 (4) (Apr. 2021) 2003481, https://doi.org/10.1183/13993003.03481-2020.
[8] V.O. Puntmann, et al., Outcomes of cardiovascular magnetic resonance imaging in patients recently recovered from coronavirus disease 2019 (COVID-19), JAMA Cardiol 5 (11) (Nov. 2020) 1265, https://doi.org/10.1001/jamacardio.2020.3557.
[9] R. Eiros, et al., Pericardial and myocardial involvement after SARS-CoV-2 infection: a cross-sectional descriptive study in healthcare workers, Rev. Esp. Cardiol. Engl. Ed. 75 (9) (Sep. 2022) 734–746, https://doi.org/10.1016/j.rec.2021.11.001.
[10] D. Patoulias, et al., Inflammatory markers in cardiovascular disease; lessons learned and future perspectives, Curr. Vasc. Pharmacol. 19 (3) (Dec. 2020) 323–342, https://doi.org/10.2174/1570161118666200318104434.
[11] S.D. Anker, Inflammatory mediators in chronic heart failure: an overview, Heart 90 (4) (Apr. 2004) 464–470, https://doi.org/10.1136/hrt.2002.007005.
[12] S. Van Linthout, C. Tschöpe, Inflammation – cause or consequence of heart failure or both? Curr. Heart Fail. Rep. 14 (4) (Aug. 2017) 251–265, https://doi.org/10.1007/s11897-017-0337-9.
[13] N. Glezeva, J.A. Baugh, Role of inflammation in the pathogenesis of heart failure with preserved ejection fraction and its potential as a therapeutic target, Heart Fail. Rev. 19 (5) (Sep. 2014) 681–694, https://doi.org/10.1007/s10741-013-9405-8.
[14] S. Khan, M.S. Shafiei, C. Longoria, J.W. Schoggins, R.C. Savani, H. Zaki, SARS-CoV-2 spike protein induces inflammation via TLR2-dependent activation of the NF-κB pathway, Elife 10 (Dec. 2021) e68563, https://doi.org/10.7554/eLife.68563.
[15] Y. Zhao, et al., SARS-CoV-2 spike protein interacts with and activates TLR41, Cell Res. 31 (7) (Jul. 2021) 818–820, https://doi.org/10.1038/s41422-021-00495-9.
[16] W. Guan, et al., Clinical characteristics of coronavirus disease 2019 in China, N. Engl. J. Med. 382 (18) (Apr. 2020) 1708–1720, https://doi.org/10.1056/NEJMoa2002032.
[17] C. Qin, et al., Dysregulation of immune response in patients with coronavirus 2019 (COVID-19) in Wuhan, China, Clin. Infect. Dis. 71 (15) (Jul. 2020) 762–768, https://doi.org/10.1093/cid/ciaa248.
[18] L. Yang, X. Xie, Z. Tu, J. Fu, D. Xu, Y. Zhou, The signal pathways and treatment of cytokine storm in COVID-19, Signal Transduct. Targeted Ther. 6 (1) (Jul. 2021) 255, https://doi.org/10.1038/s41392-021-00679-0.
[19] A. Rajamanickam, et al., Dynamic alterations in monocyte numbers, subset frequencies and activation markers in acute and convalescent COVID-19 individuals, Sci. Rep. 11 (1) (Oct. 2021) 20254, https://doi.org/10.1038/s41598-021-99705-y.
[20] A. Gatti, D. Radrizzani, P. Viganò, A. Mazzone, B. Brando, Decrease of non-classical and intermediate monocyte subsets in severe acute SARS-CoV -2 infection, Cytometry 97 (9) (Sep. 2020) 887–890, https://doi.org/10.1002/cyto.a.24188.
[21] J. Hadjadj, et al., Impaired type I interferon activity and inflammatory responses in severe COVID-19 patients, Science 369 (6504) (Aug. 2020) 718–724, https://doi.org/10.1126/science.abc6027.
[22] A. Silvin, et al., Elevated calprotectin and abnormal myeloid cell subsets discriminate severe from mild COVID-19, Cell 182 (6) (Sep. 2020) 1401, https://doi.org/10.1016/j.cell.2020.08.002, 1418.e18.
[23] G. Carissimo, et al., Whole blood immunophenotyping uncovers immature neutrophil-to-VD2 T-cell ratio as an early marker for severe COVID-19, Nat. Commun. 11 (1) (Oct. 2020) 5243, https://doi.org/10.1038/s41467-020-19080-6.
[24] B. Peruzzi, et al., Quantitative and qualitative alterations of circulating myeloid cells and plasmacytoid DC in SARS-CoV-2 infection, Immunology 161 (4) (Dec. 2020) 345–353, https://doi.org/10.1111/imm.13254.
[25] J. Schulte-Schrepping, et al., Severe COVID-19 is marked by a dysregulated myeloid cell compartment, Cell 182 (6) (Sep. 2020) 1419–1440, https://doi.org/10.1016/j.cell.2020.08.001, e23.
[26] S. Bhaskar, et al., Cytokine storm in COVID-19—immunopathological mechanisms, clinical considerations, and therapeutic approaches: the REPROGRAM consortium position paper, Front. Immunol. 11 (Jul. 2020) 1648, https://doi.org/10.3389/fimmu.2020.01648.
[27] A. Blum, H. Miller, Pathophysiological role of cytokines in congestive heart failure, Annu. Rev. Med. 52 (1) (Feb. 2001) 15–27, https://doi.org/10.1146/annurev.med.52.1.15.
[28] J.W. Williams, L. Huang, G.J. Randolph, Cytokine circuits in cardiovascular disease, Immunity 50 (4) (Apr. 2019) 941–954, https://doi.org/10.1016/j.immuni.2019.03.007.
[29] S. Liang, et al., SARS-CoV-2 spike protein induces IL-18-mediated cardiopulmonary inflammation via reduced mitophagy, Signal Transduct. Targeted Ther. 8 (1) (Mar. 2023) 108, https://doi.org/10.1038/s41392-023-01368-w.
[30] A. Saxena, I. Russo, N.G. Frangogiannis, Inflammation as a therapeutic target in myocardial infarction: learning from past failures to meet future challenges, Transl. Res. 167 (1) (Jan. 2016) 152–166, https://doi.org/10.1016/j.trsl.2015.07.002.
[31] Y. Seta, Interleukin 18 in acute myocardial infarction, Heart 84 (6) (Dec. 2000) 668–669, https://doi.org/10.1136/heart.84.6.668.
[32] L. Lyakh, G. Trinchieri, L. Provezza, G. Carra, F. Gerosa, Regulation of interleukin-12/interleukin-23 production and the T-helper 17 response in humans, Immunol. Rev. 226 (1) (Dec. 2008) 112–131, https://doi.org/10.1111/j.1600-065X.2008.00700.x.
[33] M.V. Zykov, O.L. Barbarash, V.V. Kashtalap, A.G. Kutikhin, L.S. Barbarash, Interleukin-12 serum level has prognostic value in patients with ST-segment elevation myocardial infarction, Heart Lung 45 (4) (Jul. 2016) 336–340, https://doi.org/10.1016/j.hrtlng.2016.03.007.
[34] A. Abbas, et al., Interleukin 23 levels are increased in carotid atherosclerosis: possible role for the interleukin 23/interleukin 17 Axis, Stroke 46 (3) (Mar. 2015) 793–799, https://doi.org/10.1161/STROKEAHA.114.006516.
[35] G. Li, et al., Coronavirus infections and immune responses, J. Med. Virol. 92 (4) (Apr. 2020) 424–432, https://doi.org/10.1002/jmv.25685.
[36] The National Institute of Health Coronavirus Disease 2019 (COVID-19) treatment guidelines. http://www.covid19treatmentguidelines.nih.gov, 2024. (Accessed 25 January 2024).
[37] T. Guo, et al., Cardiovascular implications of fatal outcomes of patients with coronavirus disease 2019 (COVID-19), JAMA Cardiol 5 (7) (Jul. 2020) 811, https://doi.org/10.1001/jamacardio.2020.1017.
[38] G. Lippi, M. Plebani, Cytokine 'storm', cytokine 'breeze', or both in COVID-19? Clin. Chem. Lab. Med. CCLM 59 (4) (Mar. 2021) 637–639, https://doi.org/10.1515/cclm-2020-1761.
[39] L.M.C. Jacobs, et al., Biomarkers of sustained systemic inflammation and microvascular dysfunction associated with post-COVID-19 condition symptoms at 24 months after SARS-CoV-2-infection, Front. Immunol. 14 (Oct. 2023) 1182182, https://doi.org/10.3389/fimmu.2023.1182182.
[40] E.A. Said, et al., Defining IL-6 levels in healthy individuals: a meta-analysis, J. Med. Virol. 93 (6) (Jun. 2021) 3915–3924, https://doi.org/10.1002/jmv.26654.
[41] H. Imaoka, et al., Interleukin-18 production and pulmonary function in COPD, Eur. Respir. J. 31 (2) (Feb. 2008) 287–297, https://doi.org/10.1183/09031936.00019207.
[42] T. Dolinay, et al., Inflammasome-regulated cytokines are critical mediators of acute lung injury, Am. J. Respir. Crit. Care Med. 185 (11) (Jun. 2012) 1225–1234, https://doi.org/10.1164/rccm.201201-0003OC.
[43] H. Satış, et al., Prognostic value of interleukin-18 and its association with other inflammatory markers and disease severity in COVID-19, Cytokine 137 (Jan. 2021) 155302, https://doi.org/10.1016/j.cyto.2020.155302.
[44] Z. Mallat, et al., Evidence for altered interleukin (IL)-18 pathway in human heart failure, Faseb. J. 18 (14) (Nov. 2004) 1752–1754, https://doi.org/10.1096/fj.04-2426fje.
[45] H. Suchanek, et al., High serum interleukin-18 concentrations in patients with coronary artery disease and type 2 diabetes mellitus, Eur. Cytokine Netw. 16 (3) (Sep. 2005) 177–185.
[46] S. Blankenberg, et al., Interleukin-18 is a strong predictor of cardiovascular death in stable and unstable angina, Circulation 106 (1) (Jul. 2002) 24–30, https://doi.org/10.1161/01.CIR.0000020546.30940.92.

[47] D. Formanowicz, M. Wanic-Kossowska, E. Pawliczak, M. Radom, P. Formanowicz, Usefulness of serum interleukin-18 in predicting cardiovascular mortality in patients with chronic kidney disease – systems and clinical approach, Sci. Rep. 5 (1) (Dec. 2015) 18332, https://doi.org/10.1038/srep18332.
[48] S.M.T. Nasser, A.A. Rana, R. Doffinger, A. Kafizas, T.A. Khan, S. Nasser, Elevated free interleukin-18 associated with severity and mortality in prospective cohort study of 206 hospitalised COVID-19 patients, Intensive Care Med. Exp. 11 (1) (Feb. 2023) 9, https://doi.org/10.1186/s40635-022-00488-x.
[49] A.K. Thukkani, J. McHowat, F.-F. Hsu, M.-L. Brennan, S.L. Hazen, D.A. Ford, Identification of α-chloro fatty aldehydes and unsaturated lysophosphatidylcholine molecular species in human atherosclerotic lesions, Circulation 108 (25) (Dec. 2003) 3128–3133, https://doi.org/10.1161/01.CIR.0000104564.01539.6A.
[50] R. Zhang, Association between myeloperoxidase levels and risk of coronary artery disease, JAMA 286 (17) (Nov. 2001) 2136, https://doi.org/10.1001/jama.286.17.2136.
[51] H.A.-H. Abdel-Azeez, M. Al-Zaky, Plasma osteopontin as a predictor of coronary artery disease: association with echocardiographic characteristics of atherosclerosis, J. Clin. Lab. Anal. 24 (3) (2010) 201–206, https://doi.org/10.1002/jcla.20378.
[52] E.A. Korhonen, et al., Tie1 controls angiopoietin function in vascular remodeling and inflammation, J. Clin. Invest. 126 (9) (Aug. 2016) 3495–3510, https://doi.org/10.1172/JCI84923.
[53] H.G. Augustin, G. Young Koh, G. Thurston, K. Alitalo, Control of vascular morphogenesis and homeostasis through the angiopoietin–Tie system, Nat. Rev. Mol. Cell Biol. 10 (3) (Mar. 2009) 165–177, https://doi.org/10.1038/nrm2639.
[54] S. Savant, et al., The orphan receptor Tie1 controls angiogenesis and vascular remodeling by differentially regulating Tie2 in tip and stalk cells, Cell Rep. 12 (11) (Sep. 2015) 1761–1773, https://doi.org/10.1016/j.celrep.2015.08.024.
[55] C. Huang, et al., Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China, Lancet 395 (10223) (Feb. 2020) 497–506, https://doi.org/10.1016/S0140-6736(20)30183-5.
[56] M. De Zuani, et al., High CD4-to-CD8 ratio identifies an at-risk population susceptible to lethal COVID-19, Scand. J. Immunol. 95 (3) (Mar. 2022) e13125, https://doi.org/10.1111/sji.13125.
[57] R.-H. Du, et al., Predictors of mortality for patients with COVID-19 pneumonia caused by SARS-CoV-2: a prospective cohort study, Eur. Respir. J. 55 (5) (May 2020) 2000524, https://doi.org/10.1183/13993003.00524-2020.
[58] I. Sánchez-Cerrillo, et al., COVID-19 severity associates with pulmonary redistribution of CD1c+ DCs and inflammatory transitional and nonclassical monocytes, J. Clin. Invest. 130 (12) (Oct. 2020) 6290–6300, https://doi.org/10.1172/JCI140335.
[59] C. Basso, et al., Pathological features of COVID-19-associated myocardial injury: a multicentre cardiovascular pathology study, Eur. Heart J. 41 (39) (Oct. 2020) 3827–3835, https://doi.org/10.1093/eurheartj/ehaa664.
[60] L. Zhang, et al., Cardiovascular risk after SARS-CoV-2 infection is mediated by IL18/IL18R1/HIF-1 signaling pathway Axis, Front. Immunol. 12 (Jan. 2022) 780804, https://doi.org/10.3389/fimmu.2021.780804.
[61] L. Zhao, Z. Varghese, J.F. Moorhead, Y. Chen, X.Z. Ruan, CD36 and lipid metabolism in the evolution of atherosclerosis, Br. Med. Bull. 126 (1) (Jun. 2018) 101–112, https://doi.org/10.1093/bmb/ldy006.
[62] H.C. Prescott, D.C. Angus, Enhancing recovery from sepsis: a review, JAMA 319 (1) (Jan. 2018) 62, https://doi.org/10.1001/jama.2017.17687.
[63] Z. Mostel, et al., Post-sepsis syndrome – an evolving entity that afflicts survivors of sepsis, Mol. Med. 26 (1) (Dec. 2020) 6, https://doi.org/10.1186/s10020-019-0132-z.
[64] M. Patel, O. Uthman, Risk factors for newly-developed cardiovascular disease and quality of life during the COVID − 19 pandemic: an analysis of the English longitudinal study of ageing, BMC Publ. Health 23 (1) (Jul. 2023) 1294, https://doi.org/10.1186/s12889-023-16135-3.
[65] D. Lindner, et al., Association of cardiac infection with SARS-CoV-2 in confirmed COVID-19 autopsy cases, JAMA Cardiol 5 (11) (Nov. 2020) 1281, https://doi.org/10.1001/jamacardio.2020.3551.
[66] Z. Gao, et al., Establishment and validation of the cut-off values of estimated glomerular filtration rate and urinary albumin-to-creatinine ratio for diabetic kidney disease: a multi-center, prospective cohort study, Front. Endocrinol. 13 (Dec. 2022) 1064665, https://doi.org/10.3389/fendo.2022.1064665.
[67] R. Hjortebjerg, et al., Insulin-like growth factor binding protein 4 fragments provide incremental prognostic information on cardiovascular events in patients with ST-segment elevation myocardial infarction, J. Am. Heart Assoc. 6 (3) (Mar. 2017) e005358, https://doi.org/10.1161/JAHA.116.005358.