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SARS-CoV-2 neutralizing antibodies decline over one year and patients with severe COVID-19 pneumonia display a unique cytokine profile

Vimvara Vacharathit a,1,*, Sirawat Sriratrapimuk b,1,*, Suwimon Manopwisedjaroen a, Suppachok Kirdlarp b, Chanya Srisaowakarn a, Chavachol Setthadum c, Nanthicha Inruengsri a, Praporn Pisitkun d, Mongkol Kunakorn c, Suradej Hongeng e, Somnuek Sungkanuparp b, Arunee Thitithyanont a,1

a Department of Microbiology, Faculty of Science, Mahidol University, Bangkok 10400, Thailand
b Chakri Naruebdindra Medical Institute, Faculty of Medicine Ramathibodi Hospital, Mahidol University, Samut Prakan, Thailand
c Department of Pathology, Faculty of Medicine Ramathibodi Hospital, Mahidol University, Bangkok, Thailand
d Division of Allergy, Immunology, and Rheumatology, Department of Medicine, Faculty of Medicine, Ramathibodi Hospital, Mahidol University, Bangkok, Thailand
e Department of Pediatrics, Faculty of Medicine Ramathibodi Hospital, Mahidol University, Bangkok 10400, Thailand

ABSTRACT

Objectives: As coronavirus disease 2019 (COVID-19) rages on worldwide, there is an urgent need to characterize immune correlates of protection from severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection and to identify immune determinants of COVID-19 severity.

Methods: This study examined the longitudinal profiles of neutralizing antibody (Nab) titers in hospitalized COVID-19 patients clinically diagnosed with mild pneumonia, pneumonia, or severe pneumonia, up to 12 months after illness onset, using live-virus neutralization. Multiplex, correlation, and network analyses were used to characterize serum-derived inflammatory cytokine profiles in all severity groups.

Results: Peak Nab titers correlated with disease severity, and Nab titers declined over the course of 12 months regardless of severity. Multiplex analyses revealed that IP-10, IL-6, IL-7, and VEGF-α were significantly elevated in severe pneumonia cases compared to those with mild symptoms and pneumonia cases. Correlation and network analyses further suggested that cytokine network formation was distinct in different COVID-19 severity groups.

Conclusions: The study findings inform on the long-term kinetics of naturally acquired serological immunity against SARS-CoV-2 and highlight the importance of identifying key cytokine networks for potential therapeutic immunomodulation.

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1. Introduction

The highly transmissible and pathogenic severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the causative agent of coronavirus disease 2019 (COVID-19), has so far infected about 237 million people worldwide, leading to more than 4.8 million deaths within a period of 22 months. In Thailand, approximately 1.7 million cases and over 17,000 deaths have been confirmed at the time of writing [WHO COVID-19 Dashboard]. The ongoing COVID-19 pandemic has taken a significant toll on global public health and economies, calling for a deeper understanding of immune correlates of protection against SARS-CoV-2 that may be vital for the implementation of mitigation strategies and development of treatments and vaccines.

The clinical spectrum of COVID-19 includes asymptomatic infection, a mild to moderate self-limiting disease, and a severe critical illness resulting primarily from pulmonary inflammation and diffuse alveolar damage, which can lead to acute respiratory
distress syndrome (ARDS) requiring invasive mechanical ventilation (Gandhi et al., 2020; Guan et al., 2020; Huang et al., 2020). The mechanisms underlying the severe clinical manifestations of COVID-19 are still unclear, although epidemiological studies suggest that older age, male sex, a high body mass index, comorbidities, and race (particularly Black, Asian, and Minority Ethnic (BAME) individuals) are associated with elevated disease severity and/or an increased risk of in-hospital death from COVID-19 (Li et al., 2020; Tian et al., 2020).

Neutralizing antibodies (NAbs) are an important immune correlate of protection against SARS-CoV-2. Studies in mouse and non-human primate models suggest that the passive transfer of NAbs against SARS-CoV-2 can help mitigate the disease (Cross et al., 2021; Zost et al., 2020), while clinical trials have shown partial success of early treatment with high-titer convalescent plasma (Chen et al., 2020; Liu and Aberg 2021). Importantly, NAbs titers have been shown to correlate with protection against SARS-CoV-2 reinfection (Addetta et al., 2020; Khoury et al., 2021; Lumley et al., 2021). Thus, an understanding of the long-term kinetics and durability of anti-SARS-CoV-2 NAbs would help inform on the host’s natural response to infection. Several studies have shown that robust NAbs titers persist over at least 5–8 months, although data on the longevity of these protective antibodies beyond 8 months are still scarce, and the majority of these studies did not use live-virus neutralization assays (Dan et al., 2021; Gaebler et al., 2021; Wajnberg et al., 2020).

The role of specific cytokine networks in COVID-19 severity is still unclear. Studies suggest either no correlation (He et al., 2020) or even an inverse correlation (Argyropoulos et al., 2020) between SARS-CoV-2 viral load and COVID-19 severity, suggesting that clinical deterioration may be immune-mediated, independent of viral replication in at least certain subsets of patients. Patients with severe COVID-19 may experience cytokine-release syndrome (CRS), also colloquially referred to as a ‘cytokine storm,’ systemic hyper-inflammation that can lead to ARDS, secondary hemophagocytic lymphohistiocytosis (sHLH) (Mehta et al., 2020), pulmonary edema, multiple organ failure, and death (de la Rica et al., 2020). The use of adjunctive cytokine-targeted therapy, including the US Food and Drug Administration (FDA)-approved monoclonal antibody interleukin 6 (IL-6) receptor antagonist tocilizumab, has been reported to confer clinical improvement in COVID-19 patients (Rubin et al., 2021), suggesting that an intervention in specific inflammatory networks may help attenuate the disease.

This study was performed to investigate the antibody and cytokine profiles of COVID-19 patients clinically diagnosed with mild symptoms, pneumonia, and severe pneumonia. A longitudinal study was conducted of SARS-CoV-2-directed NAbs in these patients up to 12 months post-infection, using in vitro neutralization of live virus, which is the ‘gold standard’ method for NAb assessment that is often circumvented due to its time-consuming nature and the need for a biosafety level 3 (BSL-3) facility (Gundlapalli et al., 2020; Kauffer et al., 2020). A snapshot of inflammatory cytokine profiles in these groups is also provided. Patients with pneumonia and severe pneumonia were profiled separately in acknowledgment of the gamut of COVID-19 clinical manifestations, as there is still a paucity of information on the distinct immune-mediated pathologies underlying various classifications of SARS-CoV-2-induced pneumonia.

2. Methods

All experiments involving live SARS-CoV-2 were performed in a certified BSL-3 facility in the Department of Microbiology, Faculty of Science, Mahidol University. The experimental protocols were approved by Mahidol University, and all methods were performed following standard protocols approved by the institutional review committee.

2.1. Patients and clinical specimen collection

A total of 75 COVID-19 patients hospitalized from March 2020 to May 2020 were enrolled in this study. Sequential serum samples were collected through the Chakri Naruebodindra Medical Institute (CNMI), Faculty of Medicine Ramathibodi Hospital, Mahidol University, Samut Prakan, Thailand between March 2020 and March 2021. Patients were confirmed to be infected with SARS-CoV-2 by RT-PCR on nasopharyngeal and throat swab specimens through amplification of SARS-CoV-2 ORF1AB and N target gene fragments (Sansure Biotech Inc., Changsha, PR China). Sera were stored at −80°C until use.

2.2. Clinical definitions

Pneumonia was defined as clinical symptoms of respiratory tract infection together with abnormal lung imaging compatible with pneumonia. Patients with pneumonia were classified as severe pneumonia based on the following criteria: respiratory rate >30 breaths/min, severe respiratory distress, or an oxygen saturation ≤93% on room air (World Health Organization, “Clinical management of severe acute respiratory infection when novel coronavirus (nCoV) infection is suspected: Interim guidance”, January 28, 2020; https://www.who.int/publications-detail/c clinical-management-of-severe-acute-respiratory-infection-when-novel-coronavirus-(ncov)-infection-is-suspected). Patients who had no symptoms, uncomplicated (mild) symptoms, or non-severe pneumonia were described as having mild to moderate disease.

2.3. Immunoglobulin detection

IgG and IgM antibodies directed against SARS-CoV-2 spike (S) and nucleocapsid (N) were detected using the fully automated MAGLUMI analyzers (Shine, Shenzhen, China) according to the manufacturer’s protocols. SARS-CoV-2 S1-targeted IgG and IgA were detected by ELISA (Euroimmun, Medizinische Labordiagnostika AG). The optical density (OD) was detected at 450 nm. A ratio of each sample reading to the calibrator was calculated for each sample (OD ratio).

2.4. Live-virus microneutralization assay

SARS-CoV-2 virus (SARS-CoV-2/01/human/Jan2020/Thailand) isolated from a confirmed COVID-19 patient at Bamrasnaradura Infectious Diseases Institute, Nonthaburi, Thailand, was used for the in vitro experiments. Sera were heat inactivated at 56°C for 30 minutes then two-fold serially diluted starting from 1:10. Equal volumes of SARS-CoV-2 were spiked into the serial dilutions at an infectious dose of 100 TCID50 (50% tissue culture infectious dose) and incubated for 1 hour at 37°C. Vero E6 cells (ATCC USA) were pre-seeded in Dulbecco’s modified Eagle medium (DMEM) supplemented with 2% fetal bovine serum (FBS), 100 U/ml penicillin, and 0.1 mg/ml of streptomycin. One hundred microliters of the virus-serum mixtures at different dilutions were added to 1 × 10^4 pre-seeded Vero E6 cell monolayers in duplicate on a 96-well microtiter plate, then incubated for 2 days at 37°C and 5% CO2. The last two columns contained the virus control, cell control, and virus back-titration. Medium was discarded and cells were fixed and permeabilized with ice-cold 1:1 methanol/aceton fixative for 20 minutes at 4°C. Cells were washed three times with 1 × phosphate buffered saline (PBS) containing 0.05% Tween 20 and then blocked with a blocking buffer consisting of 2% bovine serum albumin (BSA) and 0.1% Tween 20 in 1 × PBS.
for 1 hour. After washing three more times with wash buffer, SARS-CoV/SARS-CoV-2 nucleocapsid mAb (Sino Biological; Cat. No. 40143-R001) diluted 1:5000 in 1× PBS containing 0.5% BSA and 0.1% Tween 20 was added to each well and incubated for 2 hours at 37°C. Detection antibody was removed by washing the plate three more times, then 1:2000 horseradish peroxidase (HRP)-conjugated goat anti-rabbit polyclonal antibody (Dako, Denmark A/S; Cat. No. P0448) was added and the plate incubated at 37°C for 1 hour. Plates were washed three more times, then 3,3',5,5'-Tetramethylbenzidine (TMB) substrate was added (KPL; Cat. No. 5120-0075) for 10 minutes. The reaction was stopped with 1 N HCl. Absorbance was measured at 450 and 620 nm (reference wavelength) with an ELISA plate reader (Tecan Sunrise). The average ODs at 450 and 620 nm were determined for the virus and cell control wells, and the neutralizing endpoint was determined by 50% specific signal calculation. The virus neutralizing endpoint titer of each serum was expressed as the reciprocal of the highest serum dilution with an OD value less than X (World Health Organization, 2011), which was calculated as follows:

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X = \left[ \frac{(\text{average A450} - \text{A620 of 100 × TCID}_{90}\text{ virus control wells})}{(\text{average A450} - \text{A620 of cell control wells})} \right]^{1/2} + (\text{average A450} - \text{A620 of cell control wells})
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Sera that tested negative at 1:10 dilution were assigned a titer of <10. Sera were considered positive if the NAb titer was >20. Live SARS-CoV-2 viruses at passage 3 or 4 and Vero E6 cells at 20 maximum passages were employed.

2.5. Cytokine measurement

Viruses in serum samples were inactivated with 10% Triton X-100 for 1 hour at room temperature. The concentrations of 25 cytokines, chemokines, and growth factors were measured in duplicate using the Milliplex Human Cytokine/Chemokine/Growth Factor Panel A (HCYT-A60K; Merck Millipore, Burlington, MA, USA) following the manufacturer’s instructions for the following biomarkers: FGF-2, G-CSF, GM-CSF, IFNα2, IFN-γ, IL-1β, IL-1RA, IL-2, IL-3, IL-6, IL-8, IL-9, IL-10, IL-12 p70, IL-17A, IL-22, IP-10, MCP-1, MIP-1α, MIP-1β, PDGF-AA, PDGF-BB, TNF-α, and VEGF-A. The plate was read on a Luminex MAGPIX (Luminex Multiplexing Instrument, Merck Millipore) with a minimum of 50 beads collected per analyte per well. Belysa software was used to analyze the data.

2.6. Statistical analysis

Statistical analyses were done using GraphPad Prism version 9.0.0 (GraphPad Software Inc., La Jolla, CA, USA), IBM SPSS Statistics version 26.0 (IBM Corp., Armonk, NY, USA), and R version 4.0.5 (R Foundation for Statistical Computing, Vienna, Austria). The statistical tests used for each comparison are indicated in the figure legends. Heatmap was performed using the R package ComplexHeatmap. Missing values were imputed with the lower limit of detection (LLD). Data were log-transformed, scaled, and subjected to k-means clustering. The optimal number of clusters was determined using the elbow method and silhouette coefficients. Dendrograms were drawn with the Pearson correlation as a distance metric and linkage was determined by the Ward.D method. Columns were annotated with patient characteristics. Correlation matrix analysis was performed using the R package corrplot. Correlation was determined using Spearman’s test and data were subjected to hierarchical clustering. Only input pairs with \( P \leq 0.05 \) are displayed, with a confidence interval of 95% (95% CI). Network analysis of serum-derived inflammatory mediators was performed using Graphia 2.2 (Freeman et al., 2020). The k-nearest neighbors algorithm (k-NN) classification method, Markov clustering (MCL), and edge reduction with \( k = 3 \) were applied. Edges represent interactions with Spearman’s rank correlation value of >0.2, set to ensure the inclusion of all cytokines.

3. Results

3.1. Patient characteristics

A total of 75 patients with RT-PCR-confirmed COVID-19, hospitalized at the CNMI, Faculty of Medicine Ramathibodi Hospital, were included in the study. Patients were stratified into three clinical groups based on disease severity: mild symptoms, pneumonia, and severe pneumonia. Patient characteristics are presented in Table 1. The median age of the cohort was 39.8 years (interquartile range [IQR] 31.8–49.9 years); 50.7% were female and 49.3% were male. The median age of patients with severe COVID-19 pneumonia was 54.8 years (IQR 50.3–64.0 years), while patients with pneumonia and mild symptoms had median ages of 39.3 (IQR 32.7–48.4) and 34.8 (IQR 27.0–39.9) years, respectively. The median number of days after illness onset at the time of study enrollment was 12 (IQR 7–12.5) for mild cases, 10 (IQR 7–14) for pneumonia cases, and 14 (IQR 9.5–17.5) for severe pneumonia cases. Age was significantly higher in severe pneumonia cases compared to mild symptoms cases and pneumonia cases (Supplementary Material Figure S1).

In this cohort, patients who had severe pneumonia were mostly male (80%), in contrast to those with pneumonia (45.9% male) and with mild symptoms (34.8% male). Within the cohort, 20% of the patients had comorbidities, and severe pneumonia cases were more likely to present with comorbidities (60%) compared to pneumonia cases (16.2%) and mild symptoms cases (0%). The most common comorbidities in this cohort were dyslipidemia (8.0%) and diabetes mellitus (10.7%). The geometric mean peak NAb titer was 3279.1 (95% CI 2108.8–5098.7) in severe pneumonia cases, 581.3 (95% CI 332.8–1015.3) in pneumonia cases, and 197.6 (95% CI 89.7–435.1) in mild symptoms cases.

3.2. SARS-CoV-2 neutralizing antibody titers correlate with IgG and IgA titers and wane over the course of 1 year regardless of disease severity

The spike (S) and nucleocapsid (N) proteins are key targets for vaccine design (Ahmed et al., 2020). This study compared the levels of S- and N-specific IgG and IgM, as well as subunit 1 (S1)-IgG and IgA in the three severity groups. Severe pneumonia cases had the highest seropositivity rates across all Ig targets, followed by pneumonia and mild symptoms cases. Levels of all Ig targets were significantly higher in severe pneumonia cases compared to mild symptoms cases. S and N-IgG was also significantly higher in severe cases compared to pneumonia cases (Figure 1A–D). Associations of Ig and NAB titers across clinical disease severity levels were then defined. S and N-IgG, S1-IgG, and S1-IgA showed strong positive correlations with NABs in all severity groups, while S and N-IgM titers were positively correlated with NABs in pneumonia and severe pneumonia cases, but not significantly so in mild cases (Figure 1E–H).

Longitudinal profiling of NAB titers against live SARS-CoV-2 in sera from all three groups was then performed. NAB titers were arbitrarily stratified into low, medium, and high levels based on criteria for COVID-19 convalescent plasma (CCP) donation (Wendel et al. 2021) and it was observed that a majority of the patients’ NAB titers dropped to medium and low titers within 1 year regardless of disease severity (Figure 1I). Patients with severe pneumonia produced significantly higher peak NAB titers (geometric mean 3079.4, 95% CI 1808.4–5244.9) compared to those with
pneumonia (364.8, 95% CI 184.0–723.3) or mild symptoms (62.9, 95% CI 29.7–132.9) within the first month after illness onset. All 3 groups exhibited a decline in NAb titer between 2 and 12 months after illness onset regardless of disease severity. The biggest drop in mean peak NAb titer occurred between 6 and 12 months for all three groups; severe pneumonia cases experienced an approximate 10-fold decrease, pneumonia 5-fold, and mild symptoms cases experienced a 6-fold decline between 6 and 12 months. At the 12-month mark, NAb titers no longer differed significantly between severity groups, and all 3 groups retained lower NAb titers than were produced during the first month post-illness onset, although only those with severe pneumonia experienced a NAb titer decline that was statistically significant in this regard. Compared to the peak geometric mean titers for each group, after 12 months mild symptoms and pneumonia cases retained approximately 20% of antibody concentrations (geometric mean titers of 40 and 116.2, respectively), while those with severe pneumonia retained about 5% (geometric mean titer of 160) (Figure 1J).

3.3. COVID-19 patients with severe pneumonia display distinct inflammatory cytokine profiles

Next, 25 key cytokines associated with inflammation and cytokine storms (Fajgenbaum and June, 2020; Ragab et al., 2020) were assessed in serum from a sub-cohort of 48 patients with mild symptoms, pneumonia, and severe pneumonia using a multiplex assay. The results showed that IP-10, IL-6, IL-7, and VEGF-α concentrations were significantly elevated in severe pneumonia cases compared to mild symptoms and pneumonia cases. Meanwhile, MCP-1, IL-1RA, and IL-8 concentrations were also significantly higher in severe pneumonia cases compared to mild symptoms cases, and TNF-α was significantly upregulated in severe pneumonia cases compared to pneumonia cases. MIP-1α was higher in healthy controls compared to mild and pneumonia cases (Figure 2A). GM-CSF, IL-2, IL-3, IL-10, IL-12 p70, and IL-22 had mostly low or undetectable expression in the majority of patients regardless of disease severity. Levels of other markers including FGF-2, GCSF, IFN-α2, IFN-γ, IL-1β, IL-9, IL-10, IL-17A, MIP-1β, PDGF-AA, and PDGF-BB did not differ significantly between the groups (Supplementary Material Figure S2).

The relationship between cytokine concentrations and patient characteristics were then assessed through a clustered heatmap. Data were annotated with the patients’ disease severity (labeled ‘Severity’), NAb antibody titer at the time of sampling (‘Titer’), number of days from disease onset at the time of sampling (‘Onset’), age, and sex. The analysis resulted in two patient clusters and three cytokine clusters. Cytokine cluster 1 consisted of PDGF-AA and PDGF-BB, cluster 2 comprised GCSF, MCP-1, IL-8, IL-6, IP-10, IL-1RA, IL-10, MIP1β, IL-7, and VEGF-α, while cluster 3 included GM-CSF, IL-3, IL-2, IL-12 p70, IL-22, IFN-γ, MIP1α, TNF-α, IL-1β, FGF-2, IL-17A, IFN-α2, and IL-9. Patient cluster A (n = 42), exhibited mixed cytokine profiles, while cluster B (n = 6) comprised 100% of severe pneumonia cases; these patients generally displayed upregulation of cytokine cluster 2. Severe pneumonia cases in cluster A had a median age of 54.8 years and 62.5% were male, while those in cluster B had a median age of 54.7 years and 100% were male (Figure 2B).

Although patients with severe pneumonia were significantly associated with older age compared to the other groups (Supplementary Material Figure S1), and the majority of the patients with severe pneumonia were male (12/15), there was no statistically significant correlation between target cytokine levels and patient age or sex within each severity group. Cytokine levels and days after illness onset were also not significantly correlated, with the exception of VEGF-α, which displayed a positive correlation (data not shown).

3.4. Cytokines form tight-knit inflammatory networks associated with COVID-19 severity

To determine the strength of association between target cytokines in the context of clinical disease severity, separate hierarchical correlation matrix analyses were performed for each group. All groups displayed differential patterns of pairwise positive and negative correlations (Figure 3A–C).

Next, the relationship between pairs of target cytokines in different severity groups was visualized using network analyses. The three groups were found to have distinct cytokine network topologies characterized by differential groups of closely associated inflammatory mediators (Figure 3D–F). Notably, there was a significantly positively correlated group comprising IL-6, IL-1RA, MIP-
1/β, TNF-α, MCP-1, GCSF, and IP-10 in severe pneumonia cases (Figure 3F), some of which were significantly upregulated in severe cases compared to mild and pneumonia cases (Figure 2A).

4. Discussion

The kinetics and duration of NAB titers in response to viral infection are not always accurately predictable from the early phases of infection (Sallusto et al., 2010), although many valuable studies have extrapolated the trajectory of SARS-CoV-2 NAB production through applications of machine learning algorithms (Legros et al., 2021; Wheatley et al., 2021; Chia et al. 2021). Moreover, most longitudinal studies have not used live SARS-CoV-2 clinical isolates in neutralization tests, which require BSL-3 certified laboratories, and have often opted for safer alternatives such as pseudotyped SARS-CoV-2-based neutralization assays instead (Dan et al., 2021; Gaebler et al., 2021; Nie et al., 2020). In the present study, temporal changes in NAB titers were monitored in hospitalized COVID-19 patients with varying disease severity over a period of up to 1 year after illness onset using live-virus neutralization.

The decline in NAB titers over time, regardless of COVID-19 severity, may be due to transient plasmablast expansion, which show signs of decay less than 10 days after the onset of COVID-19 symptoms (Laing et al., 2020). NAB decline may also stem from a biphasic shift between antibodies produced by short-lived plasma cells during the acute phase to those subsequently generated by the 10–20% that differentiate into long-lived memory plasma cells (Turner et al., 2021). It is still unclear why antibody levels correlate with COVID-19 severity; high viral loads may result in higher disease severity in some patients (Fajnzylber et al., 2020), which in turn may result in a robust production of antibodies in response to extended antigen exposure. Unfortunately, cycle threshold (CT) values, which semi-quantitatively assess the SARS-CoV-2 viral load, were not recorded for the present cohort, but correlations between viral load and cytokine levels would be an important issue to address in the future. Alternatively, theories of antibody-dependent enhancement (ADE) in COVID-19 are emerging, but so far no definitive role for ADE in human coronaviruses has been established (Lee et al., 2020). Nevertheless, a recent study suggests that NABs may expand coronavirus cell tropism by binding to Fc receptor-expressing immune cells and guiding viral entry
Figure 2. Cytokine expression in COVID-19 cases with mild symptoms, pneumonia, and severe pneumonia. (A) Multiplex analysis of target serum-derived inflammatory cytokines in COVID-19 patients with mild symptoms ($n = 12$), pneumonia ($n = 22$), and severe pneumonia ($n = 14$), and in healthy controls ($n = 3$) at the time of study enrollment. Healthy control blood was collected pre-pandemic. The non-parametric Kruskal–Wallis test with Dunn’s multiple comparisons test was used to determine the statistical significance of mediator levels between all three groups at the time of study enrollment. $^*$ $P \leq 0.05$, $^{**} P \leq 0.01$, $^{***} P \leq 0.001$. Dashed horizontal lines represent the lower limit of detection for each target. Only targets with significantly different levels between groups are shown here; the rest can be found in Supplementary Material Figure S2. (B) Clustered heatmap of 25 inflammatory cytokines from hospitalized COVID-19 patients at the time of study enrollment. Values below the lower limit of detection were imputed for each target, and cytokine data were log-transformed, scaled, and both rows and columns were subjected to the k-means clustering algorithm. Positive z-score values are red and negative values are blue. Columns were annotated with patient characteristics including disease severity (‘Severity’), NAb titer (‘Titer’), number of days from illness onset at the time of study enrollment (‘Onset’), age, and sex.
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(NRCT)
(čvšככ½(ה)ןבכ½)332/2564,
the
Ramathibodi
Foundation,
and
the
National
Science
and
Technology
Development
Agency
(NSTDA)
(grant
number
P-20-50640).
The
funding
sources
had
no
involvement
in
the
study
design,
the
collection,
analysis,
and
interpretation
of
the
data,
in
the
writing
of
this
manuscript,
or
in
the
decision
to
submit
the
manuscript
for
publication.

Ethical
approval:
The
experimental
protocols
were
approved
by
Mahidol
University,
and
all
methods
were
performed
following
standard
protocols
approved
by
the
institutional
review
committee.

Figure
3. Correlograms
and
network
analyses
of
serum-derived
inflammatory
cytokines
across
COVID-19
disease
severity
groups.
Correlation
matrices
of
cytokine
expression
in
hospitalized
COVID-19
patients
with
(A) mild
symptoms
(n = 12),
(B) pneumonia
(n = 22),
and
(C) severe
pneumonia
(n = 14)
at
the
time
of
study
enrollment.
Only
significantly

(P < 0.05)
mediator
interactions
are
shown.
Positive
and
negative

correlations
are
shown
in
blue
and
red,
respectively.
The
size
and
color
intensity
of
the
dots
are
proportional

to
the
Spearman

correlation
coefficients
(r_S).
Pairwise
correlation
networks
showing
positive
relationships
between
cytokines
in
hospitalized
COVID-19
patients
with
(D) mild
symptoms,
(E) pneumonia,
and
(F) severe
pneumonia.
Nodes
represent
cytokines/chemokines/growth
factors
and
edges
represent
positive
correlations.
Node
color
represents
clusters
based
on
the
Markov
cluster

algorithm.
Edge
color
represents
the

Spearman
rank

correlation

coefficient
value
between

connecting
nodes.

The
results
of
this
study
highlight
the
need
to
maintain
protective

measures
in
the
face
of
a
potentially
 transient
serological
immunity
against
SARS-CoV-2
and
the
emergence
of
unusually
divergent

viral
strains
(Cohen
and
Burbeiro,
2020;
Walker
et
al.,
2020;
Frampton
et
al.
2021).
There
is
also
a
need
for
further
in-depth
studies
of
inflammatory
cytokine
networks
linked
to
COVID-19
severity,
which
may
lead
to
new
prognostic
and/or
therapeutic
avenues.

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Written informed consent was obtained and studies were approved by the Institutional Review Board, Faculty of Medicine Ramathibodi Hospital, Mahidol University (Approval No. MURA2021/447).

Conflict of interest: All authors declare no conflict of interest.

Supplementary materials

Supplementary material associated with this article can be found in the online version, at doi:10.1016/j.ijid.2021.09.021.

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