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Complex Thrombo-Inflammatory Responses versus Outcomes of Non-COVID-19 Community-Acquired Pneumonia and COVID-19

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Keywords
Community-acquired pneumonia · COVID-19 · Cytokines · Coagulation factors · Hierarchical clustering · Mortality

Abstract
Introduction: The thrombo-inflammatory response and outcomes of community-acquired pneumonia (CAP) due to various organisms (non-COVID-19 CAP) versus CAP due to a single

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virus, SARS-CoV-2 (i.e., COVID-19) may differ. ***Methods:*** Adults hospitalized with non-COVID-19 CAP (December 1, 2021–June 15, 2023) or COVID-19 (March 2, 2020–June 15, 2023) in Canada. We compared non-COVID-19 CAP and COVID-19 baseline, thrombo-inflammatory response, and mortality. We measured plasma cytokine and coagulation factor levels in a sample of patients, did hierarchical clustering, and compared cytokine and coagulation factor levels. ***Results:*** In 2,485 patients (non-COVID-19 CAP, n = 719; COVID-19 patients, n = 2,157), non-COVID-19 CAP patients had significantly lower 28-day mortality (CAP vs. COVID-19 waves 1 and 2; 10% vs. 18% and 16%, respectively), intensive care unit admission (CAP vs. all waves; 15% vs. 39%, 37%, 33%, and 24%, respectively), invasive ventilation (CAP vs. waves 1, 2, and 3 patients; 11% vs. 25%, 20%, and 16%), vasopressor use (CAP 12% vs. 23%, 21%, and 18%), and renal replacement therapy use (CAP 3% vs. Omicron 7%). Complexity of hierarchical clustering aligned directly with mortality: COVID-19 wave 1 and 2 patients had six clusters at admission and higher mortality than non-COVID-19 CAP and Omicron that had three clusters at admission. Pooling all COVID-19 waves increased complexity with seven clusters on admission. ***Conclusion:*** Complex thrombo-inflammatory responses aligned with mortality of CAP. At a fundamental level, the human thrombo-inflammatory response to a brand new virus was "confused" whereas humans had eons of time to develop a more concise efficient thrombo-inflammatory host response to CAP.

Introduction

A variety of respiratory tract pathogens cause community-acquired pneumonia (CAP) and can lead to hospitalization, antimicrobial and corticosteroid [1] treatment [2], intensive care unit (ICU) admission, and death. We recently showed that non-COVID-19 CAP mortality and ICU admission rates increased by 60% and 40% (relatively) during versus prior to the COVID-19 pandemic [3].

Thrombotic coagulation factor and inflammatory cytokine increases are innate host responses in both non-COVID-19 CAP and COVID-19. Furthermore, coagulation and inflammation amplify each other through positive feedback loops between coagulation and inflammation. There is clear evidence of a hypercoagulable state in COVID-19 patients [4–8] such as enhanced thrombin generation, higher plasma von Willebrand factor, soluble thrombomodulin, and coagulation factor VIII (FVIII). Lung pathology in COVID-19 shows progressive diffuse alveolar damage with excessive thrombosis (vascular clotting), impaired fibrinolysis (increased plasma and tissue plasminogen activator inhibitor-1), and increased inflammation with macrophage infiltration [9]. Coagulation is also enhanced in non-COVID-19 CAP with increased coagulation factors and markers of enhanced coagulation. Concomitantly, various immunologic cytokines are increased in non-COVID-19 CAP [10, 11] and COVID-19 [12–15].

However, there may be distinct differences in thrombo-inflammatory responses and outcomes between non-COVID-19 CAP of various causes versus CAP due to SARS-CoV-2. The thrombo-inflammatory responses could inform on distinct immunopathological pathways and guide specific anti-inflammatory and anticoagulant treatment options. To date there are few studies comparing non-COVID-19 CAP and COVID-19 thrombo-inflammatory response and clinical outcomes. One study found that granzyme B, a more viral response cytokine, differed in COVID than non-COVID CAP that IL-6 an integrated pro-inflammatory cytokine was higher in non-COVID CAP, while coagulation markers did not differ [16].

Our hypothesis was that there are differences in patterns of thrombo-inflammatory response between non-COVID-19 CAP and COVID-19 and that these differences in patterns of thrombo-inflammatory response correlate with mortality. Accordingly, we determined *patterns* of thrombo-inflammatory responses by using hierarchical clustering of plasma cytokines and coagulation factors and compared the complexity of thrombo-inflammatory clustering to mortality of non-COVID-19 CAP versus CAP due to a single virus, i.e., SARS-CoV-2.

Methods

This is an independently funded sub-study of ARBs CORONA I which included adults admitted to 14 hospitals in Newfoundland, Ontario, Québec, and British Columbia, Canada for non-COVID-19 CAP (December 1, 2021, to March 31, 2023) or *for* acute COVID-19 (March 2, 2020, to March 31, 2023). We excluded emergency department visits without hospital admission, readmissions, and admissions for other reasons. Using unadjusted and adjusted regression analysis, we compared baseline characteristics, treatments, vital organ support, and mortality of these patients.

Study Design and Setting

ARBs CORONA I [17] is a multicenter Canadian observational cohort study originally designed to examine the use of angiotensin receptor blockers in hospitalized

COVID-19 patients [18] that we amended to an open cohort including non-COVID-19 CAP in order to compare patients with CAP from SARS-CoV-2 to those with another etiology (non-COVID-19 CAP). ARBs CORONA I sites were community and academic hospitals (online suppl. Table 1; for all online suppl. material, see https://doi.org/10.1159/000542420; 14 sites from four provinces [19–23]). Waves of COVID-19 patients were defined by dates of admission [20, 24].

Participants

Non-COVID-19 CAP inclusion criteria were age greater than 18 years; admitted to hospital for acute non-COVID-19 CAP defined by having one of fever, chills, leukocytosis, leukopenia; one of cough, sputum, dyspnea; and new infiltrates on chest X-ray consistent with CAP. COVID-19 inclusion criteria were age greater than 18 years with confirmed SARS-CoV-2 infection (hospital or provincial laboratories clinically approved laboratory SARS-CoV-2 testing) admitted to hospital for acute COVID-19 [25–29]. Site investigators judged that the admitting illness was due to acute COVID-19. We chose patients that had data and plasma available on admission (day 0) and on day 4 of hospitalization. Acute non-COVID-19 CAP and COVID-19 and readmissions, emergency room visits and in COVID-19 patients [20, 24], hospital admissions with positive SARS-CoV-2 test but whose acute illness was not for acute COVID-19 were excluded.

Sites that only enrolled ICU-admitted patients were excluded because crude comparisons between non-COVID-19 CAP and COVID-19 patients would be confounded by the percentage of patients from these sites. Patients were identified prospectively by Research Coordinators at each site and data were collected on electronic Case Report Forms.

Subgroups – ICU-Admitted versus Not ICU-Admitted Patients

To better determine whether severity complexity of the thrombo-inflammatory response is associated with severity of illness, we also evaluated subgroups of patients who were or were not admitted to the ICU during their hospital stay.

Baseline Characteristics

Baseline characteristics included age, sex, and underlying heart failure, hypertension, chronic kidney disease, diabetes, and other comorbidities; heart rate, respiratory rate, temperature, blood pressure, arterial oxygen saturation (SaO_2), serum hemoglobin, creatinine, alanine transaminase, aspartate aminotransferase, bilirubin, D-dimer, troponin, platelet count, white blood cell count, Glasgow Coma Score (GCS), and use of vasopressors, invasive ventilation, and renal replacement therapy (RRT) at admission.

Non-COVID-19 CAP severity was scored using the Modified SMART-CAP score [30]. COVID-19 severity was scored using a modified 4C Mortality Score [31] originally defined by: age, sex, comorbidities, respiratory rate, peripheral pulse oximetry (SpO_2), GCS, urea and C-reactive protein. GCS and C-reactive protein were excluded in our calculation of a modified 4C Mortality Score because they were not consistently recorded. Comorbidities was based on ARBs CORONA I [22] definitions. Blood urea nitrogen was not recorded so serum creatinine level was used as follows: normal: <11 μmol/L; moderate: 110–220 μmol/L; more than moderate: >220 μmol/L.

Treatment

We recorded use of corticosteroids, antiviral agents for COVID-19, antifungal agents, antibiotics, and critical care support (invasive mechanical ventilation, vasopressors, and RRT) while hospitalized.

Outcomes

The primary outcome was 28-day mortality; patients discharged before day 28 and lost to follow up were assumed 28-day survivors [32–34]. Secondary outcomes were hospital mortality, ICU admission rates, organ dysfunction, and ICU and hospital length of stay. Organ dysfunction was scored first, as frequency of invasive ventilation, vasopressors and RRT and second, as days alive and free (DAF) of these therapies within the first 14 days [35] determined by subtracting numbers of days on ventilation, vasopressors or RRT from 14. Deaths within 14 days were assigned 0.

Plasma Cytokine and Coagulation Factor Analyses

We selected convenience samples of our non-COVID-19 CAP and COVID-19 cohorts that were representative of the whole cohort regarding age, sex, and mortality. We combined COVID-19 pandemic waves 1 and 2 and had a separate Omicron wave because we have found similar mortality for waves 1 and 2 and a lower mortality for Omicron [20, 24]. We measured a panel of plasma cytokines and coagulation factors (online suppl. Table 2) on days 0 and 4 in these representative samples of patients by multi-plex Luminex according to the manufacturer's instructions. We used bridging controls to normalize plate-to-plate variability. The effects of corticosteroid-treated versus not corticosteroid-treated on plasma cytokines and coagulation factors was evaluated by

examining the association of initiation of corticosteroids on day 0 versus no corticosteroid use over the first 4 days on the changes in plasma cytokine and coagulation factor levels from day 0 to day 4.

Statistical Analyses

As this was an exploratory sub-study of ARBs CORONA I, no formal sample size calculation was performed. The initial planned sample size of ARBs CORONA I was 497 [17] and enrollment was later continued in an open cohort. Baseline characteristics were compared using Chi-square test, Fisher's exact test (when >20% of cells have expected cell counts <5 or any expected cell count is <1), analysis of variance (ANOVA) or Kruskal-Wallis test. Unadjusted and adjusted regression analyses were done to compare outcomes between CAP and COVID-19 patients. Adjustment factors were age, sex, chronic heart disease, hypertension, chronic kidney disease and diabetes (commonest co-morbidities associated with death in COVID-19 [35–37] and other comorbidities which were significantly different between non-COVID-19 CAP and COVID-19. We accounted for site effect in unadjusted and adjusted comparisons by including a hospital site effect term in the regression model as previously done [24]. Logistic, 0–1 inflated beta and censored quantile [36] regression were used to compare binary outcomes, DAF and length of stay, respectively. Results were expressed as odds ratio (OR), mean difference in DAF and difference in median length of stay (ΔM) with 95% confidence interval (CI). For length of stay analysis, in-hospital deaths were considered as never discharged and censored at the largest observed length of stay [37].

Missing data were minimal (Table 1) and these patients were excluded from the corresponding analysis (5% for the adjusted analysis). For the analyses in which the assay produced upper threshold values that threshold was used. For the analyses in which the assay produced lowest detectable value, we replaced the value with one-half of the lowest detectable limit (online suppl. Table 3).

Analyses were conducted using SAS 9.4 (SAS Institute Inc., Cary, NC) and R 4.0.4 (R Foundation for Statistical Computing, Vienna, Austria). $p < 0.05$ was considered statistically significant. We did not perform viral genotyping to determine SARS-CoV-2 variants, but in Canadian national data, over 90% of COVID-19 were Omicron [38] during the timeframe that we defined herein as Omicron wave. In a British Columbia subset ($n = 141$; December 2021 to April 2022), we confirmed that 94 percent of Omicron wave patients as we defined them herein were Omicron as determined by sequence.

To understand how plasma cytokines and coagulation factors cluster on days 0 and 4, we did hierarchical clustering with the ward agglomeration method and Euclidean distances [39, 40]. We then pooled the COVID-19 waves 1 and 2 and Omicron waves together to examine complexity of clustering in COVID-19 overall.

To better understand how cytokine and coagulation factors were associated with the primary outcome, we did Principal Components Analysis (PCA) [41] using data from all patients on day 0 to create principal component scores. The number of factors retained was chosen to account for 70% of the common variance. Plasma cytokine and coagulation factor levels were log transformed and standardized to have mean 0 and standard deviation of 1 for the PCA. We then compared the PC scores on day 0 by 28-day survival status using Wilcoxon rank-sum test.

The optimal number of clusters was determined by the majority rule in the NbClust function in R. Plasma cytokine levels were log transformed and standardized to have mean 0 and standard deviation of 1 for the hierarchical clustering and heatmap analysis. Plasma cytokine and coagulation factor levels were compared between non-COVID-19 CAP and COVID-19 cohorts by quantile regression. We adjusted our analyses for age, sex, and comorbidities which were different between cohorts ($p < 0.2$; hypertension, liver disease, chronic neurological disorder, chronic hematologic disease, and dementia) and did multiple comparisons adjustment using the False Discovery Rate (FDR) procedure. In order to express the comparison between cohorts as percentage change in median, log transformed data were used in the regression analysis.

Results

Similar proportions of non-COVID-19 CAP and COVID-19 patients were excluded (online suppl. Fig. 1). Non-COVID-19 CAP patients were older than COVID-19 waves 2, 3, and Omicron wave patients and had lower rates of any one of chronic cardiac or kidney disease, hypertension or diabetes than wave 1 and Omicron wave patients (Table 1). Patients in the current study were comparable to the entire cohort (online suppl. Table 4).

Clinical Features

In 2,485 patients, there were non-COVID-19 CAP ($n = 719$) and COVID-19 patients ($n = 2,157$). Non-COVID-19 CAP patients had significantly higher platelet counts, D-dimer, and INR than waves 1 and 2 and Omicron patients (Table 1). In general, non-COVID-19 CAP patients had higher heart and respiratory rates and

Table 1. Baseline characteristics of non-COVID-19 CAP and COVID-19 wave patients admitted to hospital *for* acute non-COVID-19 CAP or COVID-19, respectively

Variable	CAP (n = 719)	COVID-19							
		wave 1 (n = 523)	p value[a]	wave 2 (n = 597)	p value[a]	wave 3 (n = 283)	p value[a]	Omicron (n = 754)	p value[a]
Sex, n (%)			0.806		0.090		0.140		0.100
Male	401 (56.0)	296 (56.7)		362 (60.6)		173 (61.1)		453 (60.2)	
Female	315 (44.0)	226 (43.3)		235 (39.4)		110 (38.9)		299 (39.8)	
Age, mean (SD)	69.6 (16.0)	70.1 (16.3)	0.599	65.9 (17.0)	<0.001	62.9 (16.8)	<0.001	72.4 (14.6)	<0.001
Comorbidities, n (%)									
Chronic cardiac disease	233/718 (32.5)	166/520 (31.9)	0.844	146/590 (24.7)	0.002	49/280 (17.5)	<0.001	297/751 (39.5)	0.005
Chronic kidney disease	119/718 (16.6)	82/522 (15.7)	0.683	96/595 (16.1)	0.830	27/281 (9.6)	0.005	190/747 (25.4)	<0.001
Hypertension	360/718 (50.1)	295/522 (56.5)	0.026	311/594 (52.4)	0.424	128/280 (45.7)	0.209	445/748 (59.5)	<0.001
Diabetes	181/717 (25.2)	171/521 (32.8)	0.004	187/593 (31.5)	0.012	77/282 (27.3)	0.503	250/753 (33.2)	<0.001
Chronic pulmonary disease (not asthma)	233/718 (32.5)	102/522 (19.5)	<0.001	84/591 (14.2)	<0.001	33/278 (11.9)	<0.001	181/748 (24.2)	<0.001
Asthma (physician diagnosed)	68/718 (9.5)	36/521 (6.9)	0.109	54/591 (9.1)	0.836	22/282 (7.8)	0.407	61/744 (8.2)	0.391
Liver disease	38/718 (5.3)	21/520 (4.0)	0.307	21/595 (3.5)	0.125	16/282 (5.7)	0.810	35/738 (4.7)	0.631
Chronic neurological disorder	88/717 (12.3)	68/522 (13.0)	0.693	58/594 (9.8)	0.151	25/280 (8.9)	0.134	125/735 (17.0)	0.011
Malignant neoplasm	110/718 (15.3)	43/520 (8.3)	<0.001	38/589 (6.5)	<0.001	24/281 (8.5)	0.005	110/748 (14.7)	0.742
Chronic hematologic disease	42/717 (5.9)	30/521 (5.8)	0.941	27/594 (4.5)	0.289	6/281 (2.1)	0.013	92/743 (12.4)	<0.001
AIDS/HIV	13/711 (1.8)	4/494 (0.8)	0.140	2/595 (0.3)	0.012	4/282 (1.4)	0.653	8/720 (1.1)	0.259
Obesity (as defined by clinical staff)	51/715 (7.1)	53/485 (10.9)	0.022	57/593 (9.6)	0.105	26/262 (9.9)	0.152	57/719 (7.9)	0.569
Rheumatologic disorder	83/716 (11.6)	41/520 (7.9)	0.032	76/595 (12.8)	0.514	37/281 (13.2)	0.492	107/736 (14.5)	0.096
Dementia	77/717 (10.7)	100/519 (19.3)	<0.001	43/594 (7.2)	0.029	13/281 (4.6)	0.002	70/739 (9.5)	0.422
Malnutrition	9/705 (1.3)	8/520 (1.5)	0.699	3/595 (0.5)	0.147	0/282 (0.0)	0.057	7/719 (1.0)	0.588
Positive culture (blood or sputum) within 48 h before or after hospital admission	160/690 (23.2)	–		–		–		–	
Streptococcus pneumoniae	41/686 (6.0)	–		–		–		–	
Staphylococcus aureus	27/685 (3.9)	–		–		–		–	
Haemophilus influenza	9/686 (1.3)	–		–		–		–	
Klebsiella/enterobacter	11/687 (1.6)	–		–		–		–	
Other	94/688 (13.7)	–		–		–		–	
Influenza, n (%)	71/709 (10.0)	–		–		–		–	–
Received any COVID-19 vaccine prior to admission, n (%)	–	0/523 (0.0)		3/596 (0.5)		22/273 (8.1)		418/592 (70.6)	–
Received at least 2 doses of COVID-19 vaccine prior to admission, n (%)	–	0/523 (0.0)		0/596 (0.0)		0/273 (0.0)		385/585 (65.8)	–
Admitted to ICU on hospital admission day, n (%	62 (8.6)	104 (19.9)	<0.001	85 (14.2)	0.001	36 (12.8)	0.047	94 (12.5)	0.016
Organ support on admission day									
Invasive mechanical ventilation, n (%)	44/719 (6.1)	55/523 (10.5)	0.005	34/597 (5.7)	0.745	16/283 (5.7)	0.780	43/754 (5.7)	0.735
RRT or dialysis, n (%)	5/715 (0.7)	8/514 (1.6)	0.147	7/592 (1.2)	0.362	4/280 (1.4)	0.275	16/749 (2.1)	0.021

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Table 1 (continued)

Variable	CAP (n = 719)	COVID-19							
		wave 1 (n = 523)	p value[a]	wave 2 (n = 597)	p value[a]	wave 3 (n = 283)	p value[a]	Omicron (n = 754)	p value[a]
Vasopressors, n (%)	51/718 (7.1)	35/523 (6.7)	0.778	33/597 (5.5)	0.245	14/283 (4.9)	0.213	36/753 (4.8)	0.059
Temperature (°C), mean (SD)	37.5 (1.4)	37.5 (0.9)	0.998	37.4 (0.9)	0.096	37.4 (0.9)	0.352	37.3 (1.1)	<0.001
Heart rate (beats per minute), mean (SD)	101.2 (22.5)	91.5 (20.6)	<0.001	95.4 (20.0)	<0.001	96.5 (19.1)	0.002	93.4 (21.3)	<0.001
Respiratory rate (breaths per minute), mean (SD)	24.7 (7.8)	22.9 (6.4)	<0.001	24.9 (7.8)	0.711	24.1 (7.4)	0.309	23.7 (7.6)	0.011
Systolic BP, mean (SD)	128.9 (26.1)	128.7 (22.8)	0.916	130.1 (23.2)	0.393	126.9 (20.2)	0.242	132.8 (27.6)	0.006
Diastolic BP, mean (SD)	72.4 (14.0)	73.7 (11.8)	0.085	74.2 (13.1)	0.017	72.7 (12.2)	0.779	73.0 (14.0)	0.385
Oxygen saturation (SaO_2; %), mean (SD)	93.0 (6.1)	93.5 (4.2)	0.098	91.0 (8.4)	<0.001	91.2 (7.6)	<0.001	92.5 (7.5)	0.176
Oxygen status, n (%) Room air Oxygen therapy	 411/715 (57.5) 304/715 (42.5)	 333/519 (64.2) 186/519 (35.8)	0.018	 379/566 (67.0) 187/566 (33.0)	<0.001	 174/271 (64.2) 97/271 (35.8)	0.055	 450/738 (61.0) 288/738 (39.0)	0.175
Leukocyte count, $\times 10^3/\mu L$, median (IQR)	12.9 (8.8, 17.9)	6.5 (4.9, 8.6)	<0.001	6.9 (5.1, 9.2)	<0.001	6.3 (4.6, 8.7)	<0.001	7.6 (5.3, 10.5)	<0.001
Hemoglobin, g/L, median (IQR)	121 (109, 136)	130 (118, 145)	<0.001	132 (118, 145)	<0.001	134 (119, 146)	<0.001	126 (110, 140)	0.011
Creatinine, µmol/L, median (IQR)	88 (66, 125)	85 (68, 114)	0.565	86.5 (70, 118)	0.404	82 (66, 104)	0.065	99 (71, 139)	<0.001
ALT/SGPT, U/L, median (IQR)	24 (15, 37)	29 (17, 52)	<0.001	35 (22, 57)	<0.001	39 (23, 66)	<0.001	25 (17, 43)	0.115
AST/SGOT, U/L, median (IQR)	28 (19, 44)	49 (27, 77)	<0.001	46 (33, 79)	<0.001	50 (39, 75)	<0.001	39 (24, 64)	<0.001
Platelets, $\times 10^9$/L, median (IQR)	236 (177, 312)	199 (156, 252)	<0.001	204 (163, 259)	<0.001	194 (150, 263)	<0.001	199 (151, 259)	<0.001
D-Dimer level, ng/mL, median (IQR)	1,320 (880, 2,690)	967 (585, 1,853)	0.002	935 (604, 1,890)	0.005	861 (621, 1,330)	<0.001	1,095 (582, 1,822)	0.007
Bilirubin, mg/dL, µmol/L, median (IQR)	10.3 (7.0, 16.0)	10.0 (7.2, 13.4)	0.195	9.0 (6.0, 13.0)	0.001	9.0 (7.0, 12.0)	0.064	9.0 (7.0, 14.0)	0.013
INR, median (IQR)	1.20 (1.10, 1.40)	1.10 (1.02, 1.20)	<0.001	1.10 (1.00, 1.20)	<0.001	1.10 (1.00, 1.20)	<0.001	1.17 (1.10, 1.30)	0.139
Troponin, ng/mL, median (IQR)	0.0233 (0.0120, 0.0520)	0.0190 (0.0080, 0.0400)	<0.001	0.0200 (0.0110, 0.0340)	0.021	0.0150 (0.0080, 0.0240)	<0.001	0.0270 (0.0130, 0.0600)	0.030
Glasglow coma scale Unknown 13–15 9–12 8 or less	 405 275 (87.6) 18 (5.7) 21 (6.7)	 319 180 (88.2) 10 (4.9) 14 (6.9)	0.919	 381 195 (90.3) 15 (6.9) 6 (2.8)	0.120	 170 109 (96.5) 3 (2.7) 1 (0.9)	0.021	 480 249 (90.9) 12 (4.4) 13 (4.7)	0.436
Mean arterial pressure (mm Hg), median (IQR)	77.0 (69.0, 86.3)	83.0 (73.0, 93.0)	<0.001	82.5 (73.0, 92.0)	<0.001	81.5 (74.0, 93.0)	0.009	79.0 (69.0, 87.0)	0.305

Table 1 (continued)

Variable	CAP (n = 719)	COVID-19								
		wave 1 (n = 523)	p value[a]	wave 2 (n = 597)	p value[a]	wave 3 (n = 283)	p value[a]	Omicron (n = 754)	p value[a]
FiO_2 (%), median (IQR)	28.0 (21.0, 40.0)	28.0 (21.0, 36.0)	0.121	30.0 (21.0, 45.0)	0.097	31.0 (24.5, 50.0)	0.021	28.0 (21.0, 40.0)	0.200
Modified 4C mortality score, mean (SD)	9.0 (7.0, 11.0)	9.0 (6.0, 11.0)	0.237	9.0 (6.0, 11.0)	0.002	8.0 (5.0, 10.0)	<0.001	10.0 (8.0, 11.0)	<0.001
Modified SMART-COP score[b]			0.386		0.326		0.014		0.022
Unknown	408	327		397		178		486	
0	172 (55.3)	115 (58.7)		112 (56.0)		76 (72.4)		181 (67.5)	
1	77 (24.8)	51 (26.0)		54 (27.0)		17 (16.2)		57 (21.3)	
2	33 (10.6)	21 (10.7)		22 (11.0)		9 (8.6)		17 (6.3)	
3	13 (4.2)	5 (2.6)		9 (4.5)		3 (2.9)		5 (1.9)	
4 or higher	16 (5.1)	4 (2.0)		3 (1.5)		0 (0.0)		8 (3.0)	

ALT, alanine transaminase; AST, aspartate aminotransferase. [a]Compared to CAP; based on Chi-square, Fisher's exact, ANOVA or Kruskal-Wallis test as appropriate. [b]Chest X-ray, albumin, PaO_2/FiO_2, and arterial pH were not included as the score component as they were not consistently captured in the database.

lower mean arterial pressures (but were within normal range) than COVID-19 patients (Table 1). About one quarter of hospitalized non-COVID-19 CAP patients had a positive influenza test or positive sputum or blood culture within 48 h of admission, most commonly for influenza (28%), *Streptococcus pneumoniae, Staphylococcus aureus,* and *Klebsiella/Enterobacter* (Table 1). For the subset of patients included in the hierarchical clustering analysis, characteristics according to COVID wave and CAP are in online supplementary Table 5.

Treatment

Treatment during hospitalization differed between non-COVID-19 CAP and COVID-19. Non-COVID-19 CAP patients had more corticosteroid use than wave 1 (40% vs. 31%), but less than in waves 2, 3, and Omicron wave (40% vs. 88%, 92%, and 76%, respectively) (online suppl. Fig. 2) and more antibiotic use (98%) versus waves 1, 2, 3, and Omicron patients (83%, 84%, 79%, 74%, respectively), but less antiviral agent use (15%) than Omicron wave patients (41%).

Outcomes

Non-COVID-19 CAP had lower 28-day mortality than waves 1 and 2 (10% vs. 18% and 16%, $p \leq 0.001$ and $p = 0.002$ respectively) but similar to Omicron (13%; Fig. 1; Table 2). Kaplan Meier curves showed higher non-COVID-19 CAP survival than COVID-19 waves 1 and 2 (Fig. 2). Non-COVID-19 CAP had lower ICU admission rates than waves 1, 2, 3, and Omicron wave patients (15% vs. 39%, 37%, 33%, and 24% $p < 0.001$, respectively, for all), lower invasive ventilation use than waves 1, 2, and 3 patients (11% vs. 25%, 20%, and 16%, $p < 0.001$, <0.001 and 0.03, respectively) but not Omicron (14%), lower vasopressor use than waves 1, 2, and 3 (12% vs. 23%, 21%, and 18% < 0.001, $p < 0.001$ and $p = 0.010$, respectively) but not Omicron (15%, respectively), and lower RRT use than Omicron (3% vs. 7% $p < 0.001$) (Table 2).

Hierarchical Clustering of Plasma Cytokine and Coagulation Factor Levels

Hierarchical clustering showed that non-COVID-19 CAP and Omicron had three clusters of cytokines and coagulation factors at admission whereas COVID-19 wave 1 and 2 patients had six clusters (Fig. 3). By day four, non-COVID-19 CAP patients simplified to two clusters, the wave 1 and 2 patients simplified to three clusters while the Omicron increased to four clusters.

When we then pooled the COVID-19 waves 1 and 2 and Omicron waves together to examine complexity of

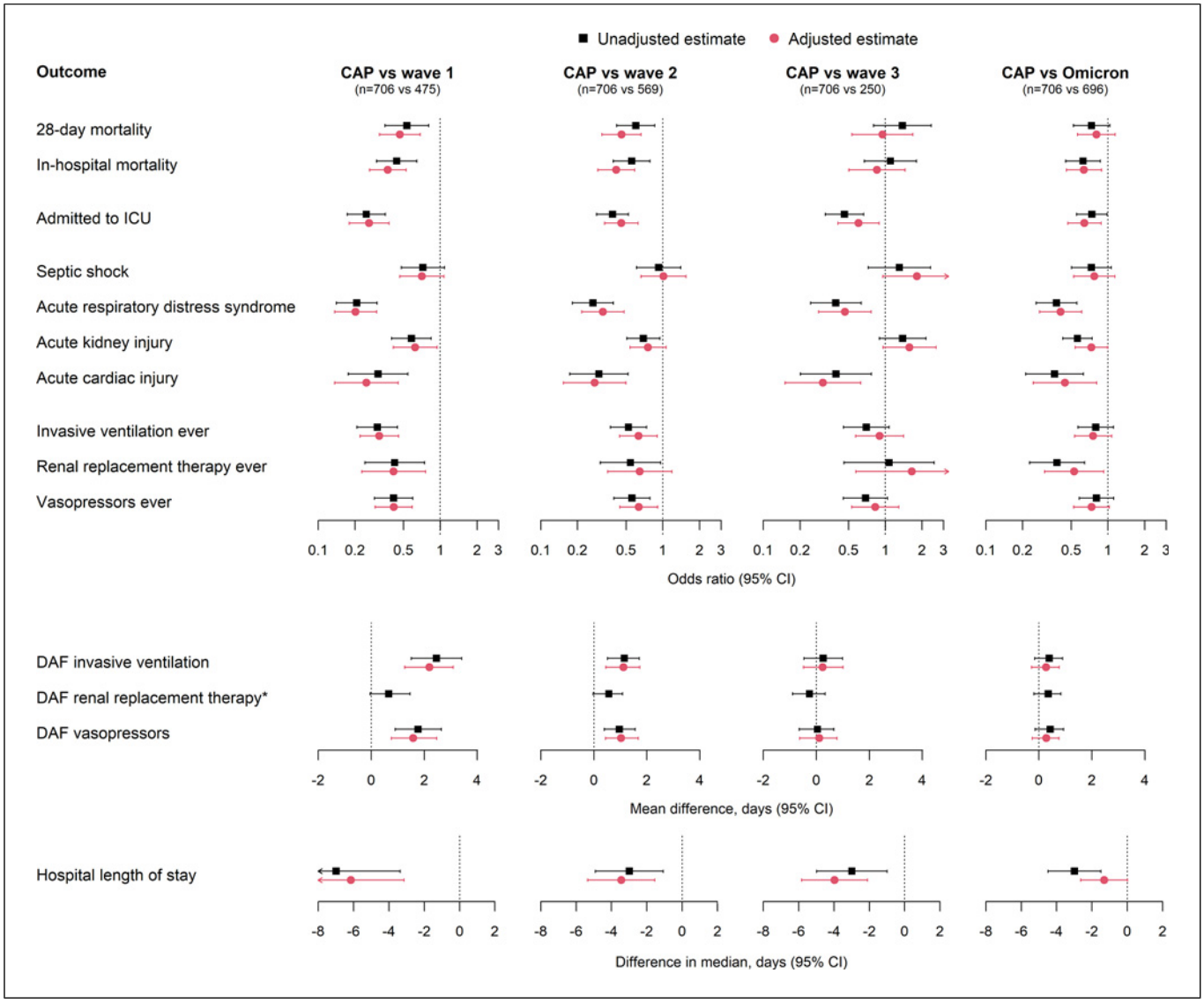


Fig. 1. Comparison of non-COVID-19 CAP and COVID-19 waves 1, 2, 3, and Omicron wave patient outcomes. The following factors were accounted for in the adjusted analysis: age, sex, chronic cardiac disease, chronic kidney disease, hypertension, diabetes, chronic pulmonary disease, liver disease, chronic neurological disorder. Malignant neoplasm, chronic hematologic disease, obesity, rheumatologic disorder, and dementia. The indicated sample size was for the analysis of primary outcome – 28-day outcome. Sample size varied slightly across outcomes due to missing data. *Adjusted regression analysis was not feasible numerically as too few patients received RRT during the first 14 days.

clustering in COVID-19 overall, there was even greater, not less, complexity showing seven clusters on day 0 and four clusters on day 4 (Fig. 3). To dive deeper regarding complexity of thrombo-inflammatory response and severity of illness, we compared the subgroups of ICU-admitted versus not ICU-admitted patients. The results aligned in that there were more cytokine clusters in ICU-admitted than non-ICU-admitted patients (online suppl. Fig. 3). The number of clusters identified in a dataset is, however, influenced by the sample size (there are many more COVID-19 patients): larger datasets might reveal more subtle clusters that smaller datasets could miss.

Principal Components Analysis

Comparison of principal component scores by 28-day survival status (online suppl. Table 6) revealed that in COVID-19 wave 1 and 2 patients, all component scores,

Table 2. Outcomes of patients admitted to hospital for acute non-COVID-19 CAP or COVID-19

Variable	CAP (n = 719)	COVID-19							
		wave 1 (n = 523)	p value[a]	wave 2 (n = 597)	p value[a]	wave 3 (n = 283)	p value[a]	Omicron (n = 754)	p value[a]
28-day mortality, n (%)	72/719 (10.0)	95/523 (18.2)	<0.001	93/597 (15.6)	0.002	20/282 (7.1)	0.150	98/754 (13.0)	0.073
In-hospital death, n (%)	72/719 (10.0)	111/523 (21.2)	<0.001	103/597 (17.3)	<0.001	26/283 (9.2)	0.692	117/754 (15.5)	0.002
Admitted to ICU, n (%)	110/717 (15.3)	204/523 (39.0)	<0.001	223/597 (37.4)	<0.001	92/282 (32.6)	<0.001	179/753 (23.8)	<0.001
Septic shock, n (%)	51/706 (7.2)	50/513 (9.7)	0.115	44/568 (7.7)	0.724	15/272 (5.5)	0.340	71/737 (9.6)	0.100
Acute respiratory distress syndrome, n (%)	39/716 (5.4)	108/493 (21.9)	<0.001	100/561 (17.8)	<0.001	35/274 (12.8)	<0.001	99/746 (13.3)	<0.001
Acute kidney injury, n (%)	121/709 (17.1)	131/516 (25.4)	<0.001	133/560 (23.8)	0.003	35/264 (13.3)	0.150	208/731 (28.5)	<0.001
Acute cardiac injury, n (%)	18/695 (2.6)	40/498 (8.0)	<0.001	47/568 (8.3)	<0.001	17/269 (6.3)	0.005	49/711 (6.9)	<0.001
Co-intervention while hospitalized, n (%)									
Antiviral agent	88/717 (12.3)	72/519 (13.9)	0.408	106/594 (17.8)	0.005	47/282 (16.7)	0.068	312/751 (41.5)	<0.001
Antibiotic	708/719 (98.5)	436/523 (83.4)	<0.001	504/597 (84.4)	<0.001	223/283 (78.8)	<0.001	558/754 (74.0)	<0.001
Corticosteroid	290/718 (40.4)	160/523 (30.6)	<0.001	523/597 (87.6)	<0.001	259/283 (91.5)	<0.001	576/754 (76.4)	<0.001
Antifungal agent	34/717 (4.7)	34/523 (6.5)	0.179	26/597 (4.4)	0.738	23/283 (8.1)	0.038	44/752 (5.9)	0.343
Organ support while hospitalized, n (%)									
Invasive mechanical ventilation	76/719 (10.6)	131/523 (25.0)	<0.001	119/597 (19.9)	<0.001	44/283 (15.5)	0.029	104/754 (13.8)	0.059
RRT or dialysis	20/715 (2.8)	33/514 (6.4)	0.002	30/592 (5.1)	0.033	7/280 (2.5)	0.795	53/749 (7.1)	<0.001
Vasopressors	85/718 (11.8)	121/523 (23.1)	<0.001	127/597 (21.3)	<0.001	51/283 (18.0)	0.010	115/753 (15.3)	0.055
Hospital length of stay – decedents (time to death), median (IQR)	7.0 (4.0, 16.5)	11.0 (6.0, 21.0)	0.051	11.0 (7.0, 20.0)	0.004	12.0 (10.0, 19.0)	0.012	14.0 (7.0, 24.0)	0.001
n	72	111		103		25		117	
Hospital length of stay – survivors, median (IQR)	7.0 (4.0, 11.0)	14.5 (7.0, 26.0)	<0.001	8.0 (5.0, 17.0)	<0.001	9.0 (5.0, 17.0)	<0.001	8.0 (4.0, 16.0)	<0.001
n	647	412		493		257		635	
ICU length of stay – decedents (among those who ever admitted to ICU), median (IQR)	7.0 (4.0, 12.0)	16.0 (6.0, 27.0)	0.027	13.0 (8.0, 22.0)	0.007	22.5 (11.0, 46.5)	0.004	15.0 (6.0, 29.0)	0.015
n	26	51		57		12		57	
ICU length of stay – survivors (among those who ever admitted to ICU), median (IQR)	7.0 (4.0, 10.0)	11.0 (5.0, 18.0)	<0.001	8.0 (5.0, 14.0)	0.041	10.0 (4.0, 17.0)	0.052	7.0 (4.0, 12.0)	0.598
n	76	152		165		75		115	

[a]Compared to CAP; based on Chi-square, Fisher's exact, ANOVA, or Kruskal-Wallis test as appropriate.

except for PC2, were significantly different, while no significant difference was observed in Omicron wave patients. For non-COVID-19 CAP patients, only PC3 was significantly different, which was mainly driven by GM-CSF, Granzyme B, IFN-g, IL-10, and IP-10 (online suppl. Tables 6, 7). The PC3 components showed that GM-CS, granzyme B, IFN-g, IL-10, and IP-10 were all lower in CAP survivors (online suppl. Table 8). The magnitude of the difference in PC1 was large, but did not reach statistical significance. For the cytokines with the highest loading in PC1, IL-1ra, IL-8, and MCP-1 were significantly higher in non-survivors (online suppl. Table 8).

Plasma Cytokine and Coagulation Factor Levels

There were no differences in key baseline characteristics or mortality between the sample of non-COVID-19 CAP and COVID-19 patients in whom we measured cytokine and coagulation factors and the whole cohort population (online suppl. Table 4). The thrombo-inflammatory responses at hospital admission day differed significantly between patients with non-COVID-19 CAP versus Omicron wave COVID-19. Notably, there was a broader multi-cytokine and multi-coagulation factor host response in non-COVID-19 CAP than Omicron wave COVID-19. Specifically, at day 0, plasma levels of 11 cytokines and 2 coagulation factors were significantly higher in non-COVID-19 CAP than Omicron COVID-19; at day 4, 14 cytokine levels were significantly higher in non-COVID-19 CAP than Omicron COVID-19 (Table 3B). However, the trajectory of the in-hospital thrombo-inflammatory responses (as assessed by *changes* in cytokine and coagulation factor levels from hospital admission day to day 4) quickly converged; i.e., there were very similar changes over 4 days in cytokine and coagulation factor levels of non-COVID-19 CAP and early and Omicron COVID-19 wave patients (Table 3). The changes in levels from admission day to day 4 differed significantly for only one cytokine – FMS-like tyrosine kinase 3 ligand (Flt3L) – between non-COVID-19 CAP and COVID-19 waves (age-, sex-, and comorbidity-adjusted analyses corrected for multiple comparisons (Table 3). We note that 14 of 50 (28%) of non-COVID CAP patients had influenza and that IP-10 is most commonly associated with acute viral infection. IP-10 differed between early wave COVID-19 and non-COVID CAP on day 0 (Table 3A). In the heat maps, IP-10 was relatively over-expressed in about half the patients on day zero and under-expressed ion nearly all patients on day four (Fig. 3). Surprisingly, corticosteroid treatment had no effects on the change in cytokine levels from day 0 to day 4 but was associated with significant decreases of co-agulation factor XI and prothrombin levels in non-COVID-19 CAP (online suppl. Table 9).

Discussion

In this study, non-COVID-19 CAP had lower ICU admission, organ support, and mortality rates than early COVID-19 waves but comparable to the Omicron wave. There was a broader multi-cytokine and multi-coagulation factor host response in non-COVID-19 CAP than COVID-19. The more complex hierarchical clustering of cytokine and coagulation factor levels of early wave COVID-19 aligned with higher mortality of early wave COVID-19 than non-COVID-19 CAP and Omicron wave COVID-19. Furthermore, there was more cytokine clusters in ICU-admitted than non-ICU-admitted patients. The patterns of more complex hierarchical clustering aligning with worse outcomes are only one possible cause because there are many other differences between COVID-19 and non-COVID-19 CAP such as the proportion of patients admitted to the ICU.

The PCA analyses complement hierarchical clustering by allowing one to see how cytokines gather together. PCA simplifies data into smaller sets of cytokines that have similar quantitative features, as linear combinations of the original variables. PCA illuminates large datasets showing patterns that are otherwise difficult to see. Our PCA showed that PC3 differed in CAP 28-day survival status, and that its components included mainly pro-inflammatory cytokines suggesting perhaps a brisker pro-inflammatory response in CAP than in COVID.

At a fundamental level, the human thrombo-inflammatory response to a brand new virus was "confused" whereas humans had eons of time to develop a more concise efficient thrombo-inflammatory host response to CAP. Patterns of a more complex hierarchical clustering of cytokine and coagulation factor levels aligned with worse clinical outcomes: both non-COVID-19 CAP and Omicron had three clusters at admission and similar outcomes, whereas COVID-19 wave 1 and 2 patients had six clusters and higher mortality than non-COVID-19 CAP and Omicron COVID-19. To further evaluate whether complexity of the thrombo-inflammatory response aligned with severity of illness, we compared ICU-admitted to non-ICU-admitted patients. Indeed, there was a more complex

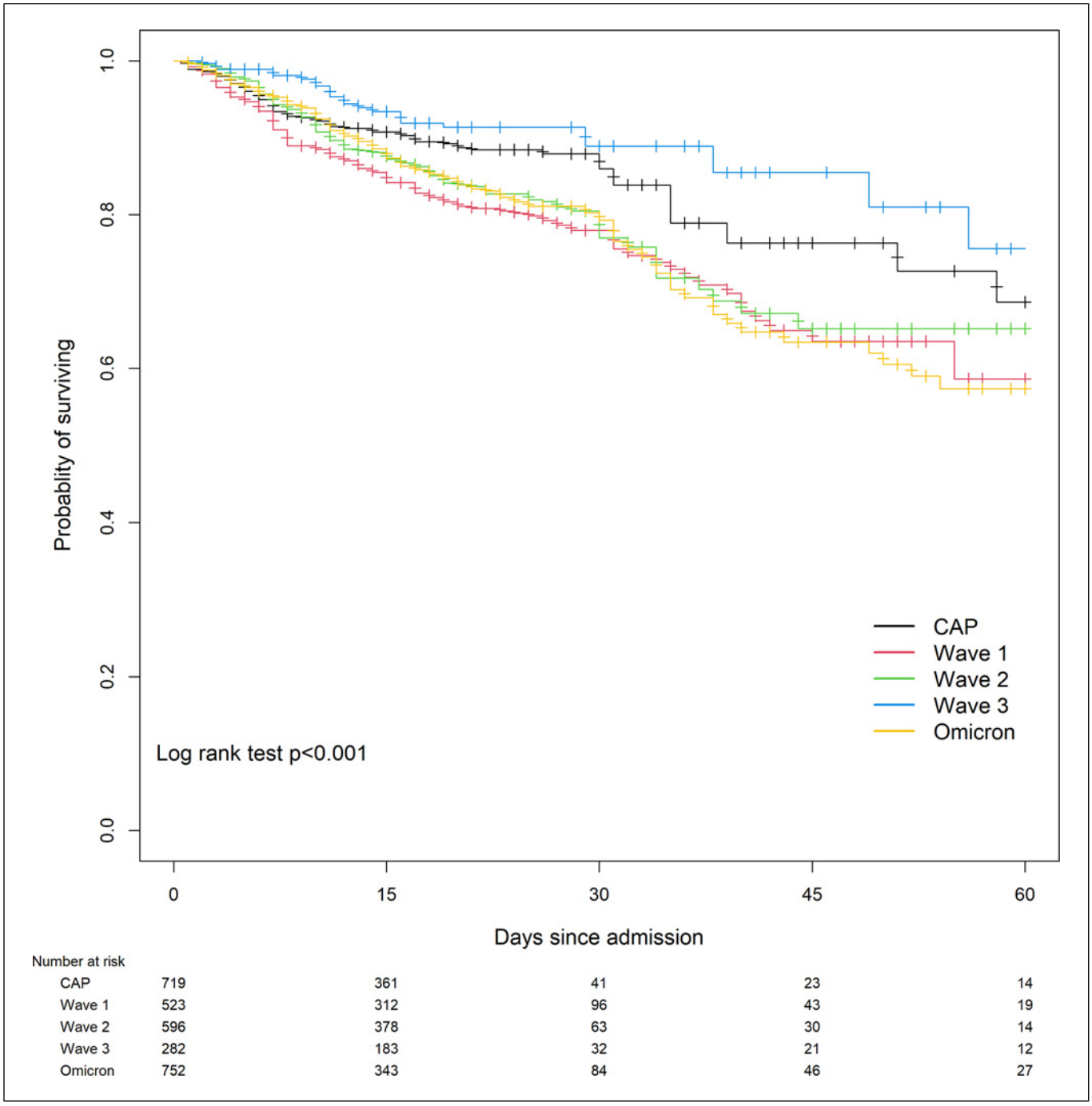
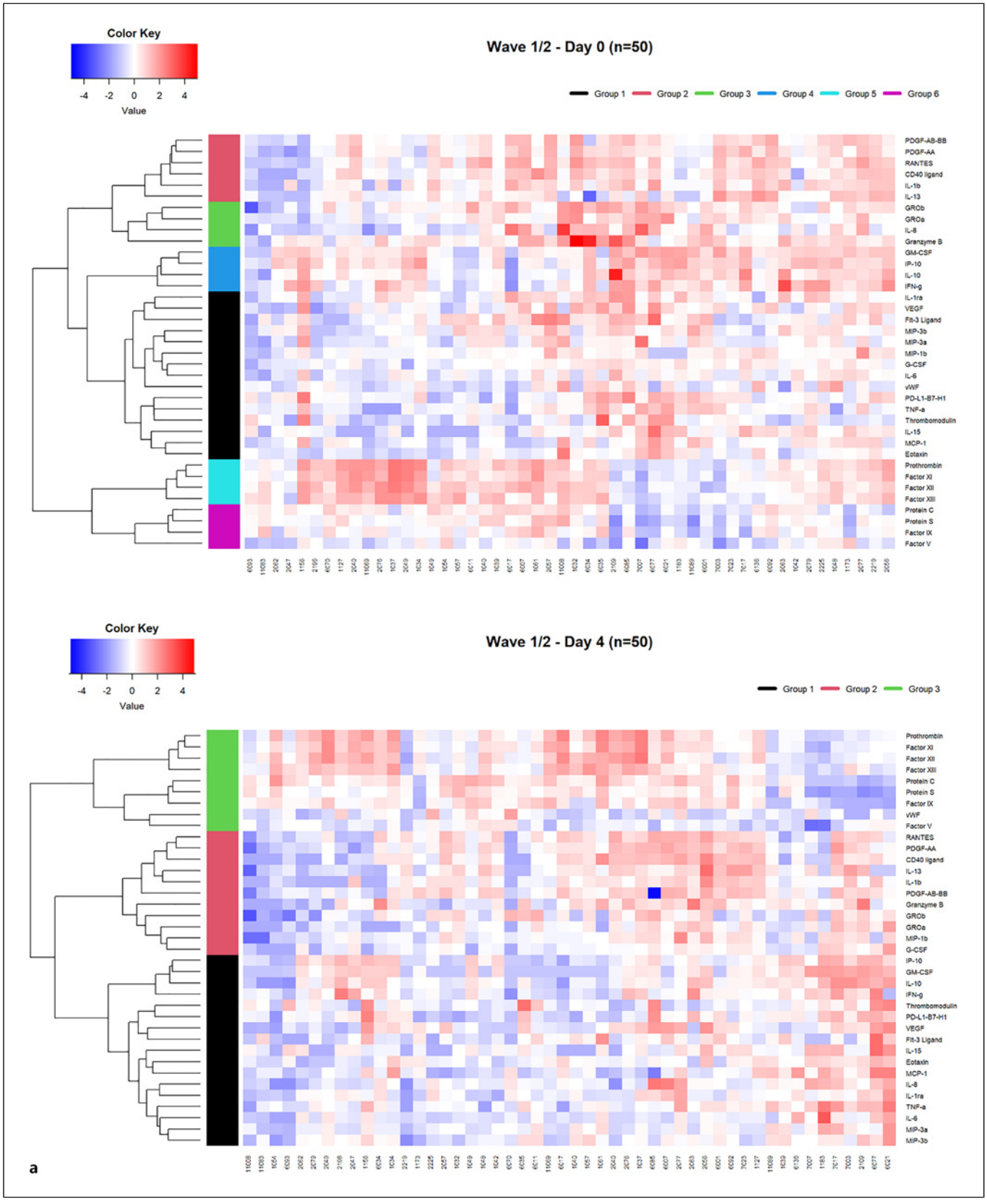


Fig. 2. Kaplan-Meier curves of non-COVID-19 CAP and COVID-19 waves 1, 2, 3, and Omicron wave patients by log-rank test. The numbers at risk table was provided beneath the Kaplan Meier curves.

inflammatory response, i.e., more clusters was present in ICU-admitted than non-ICU-admitted patients.

We speculate that the thrombo-inflammatory host response was more complex in early COVID-19 waves because there was no prior history of human exposure to SARS-CoV-2. The more complex thrombo-inflammatory response may have contributed more organ injury and higher mortality or may simply mark poor prognosis.



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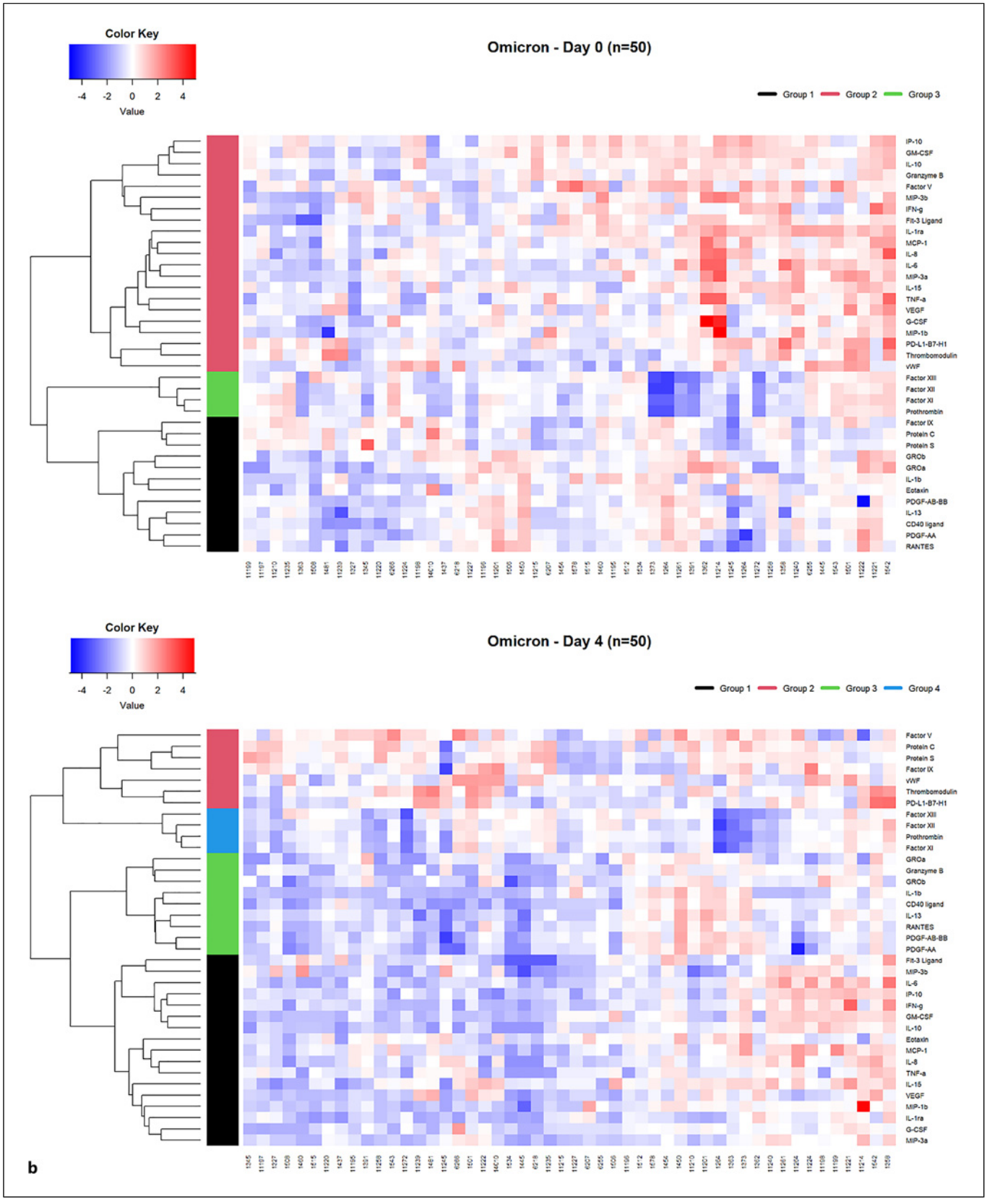

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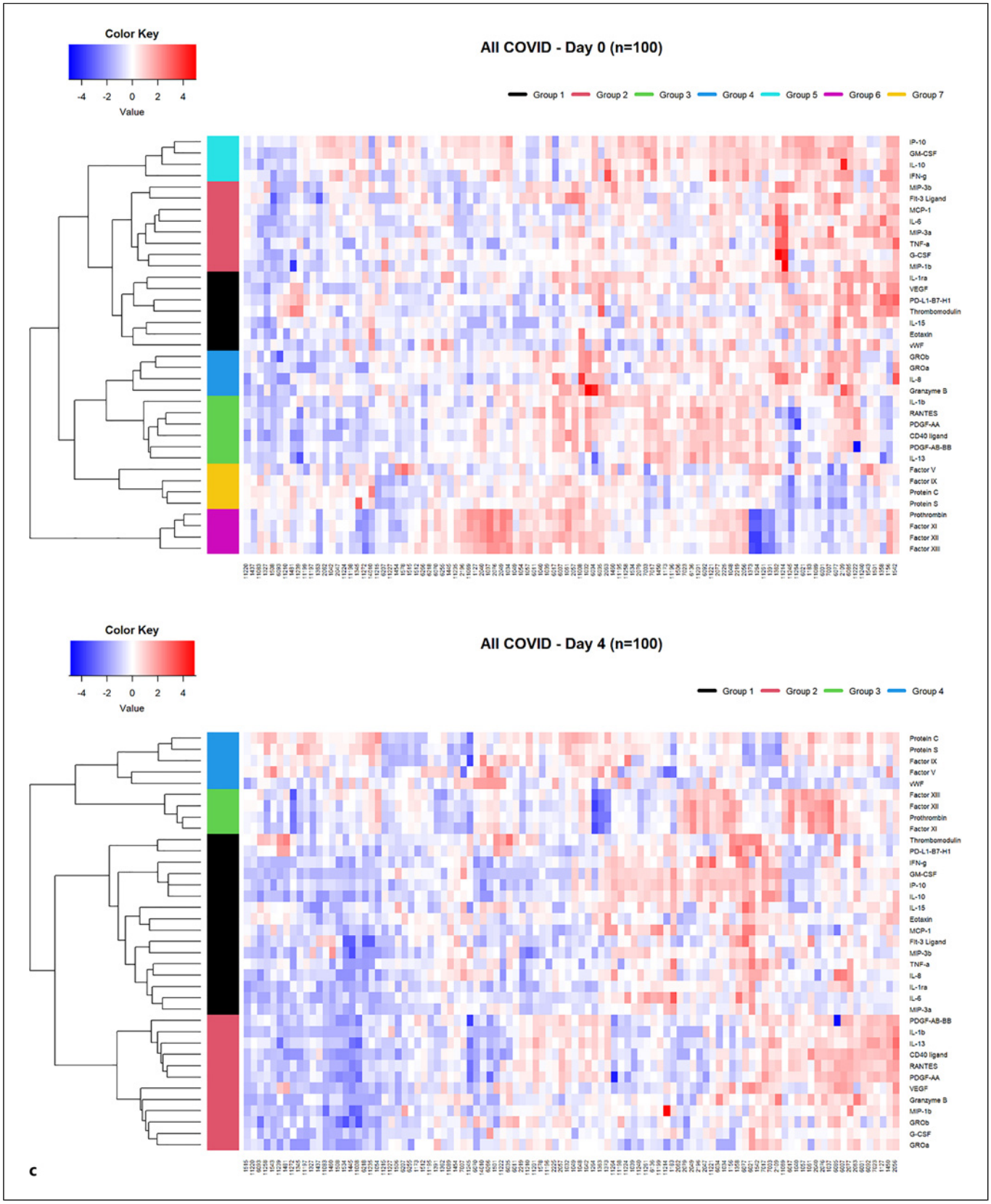

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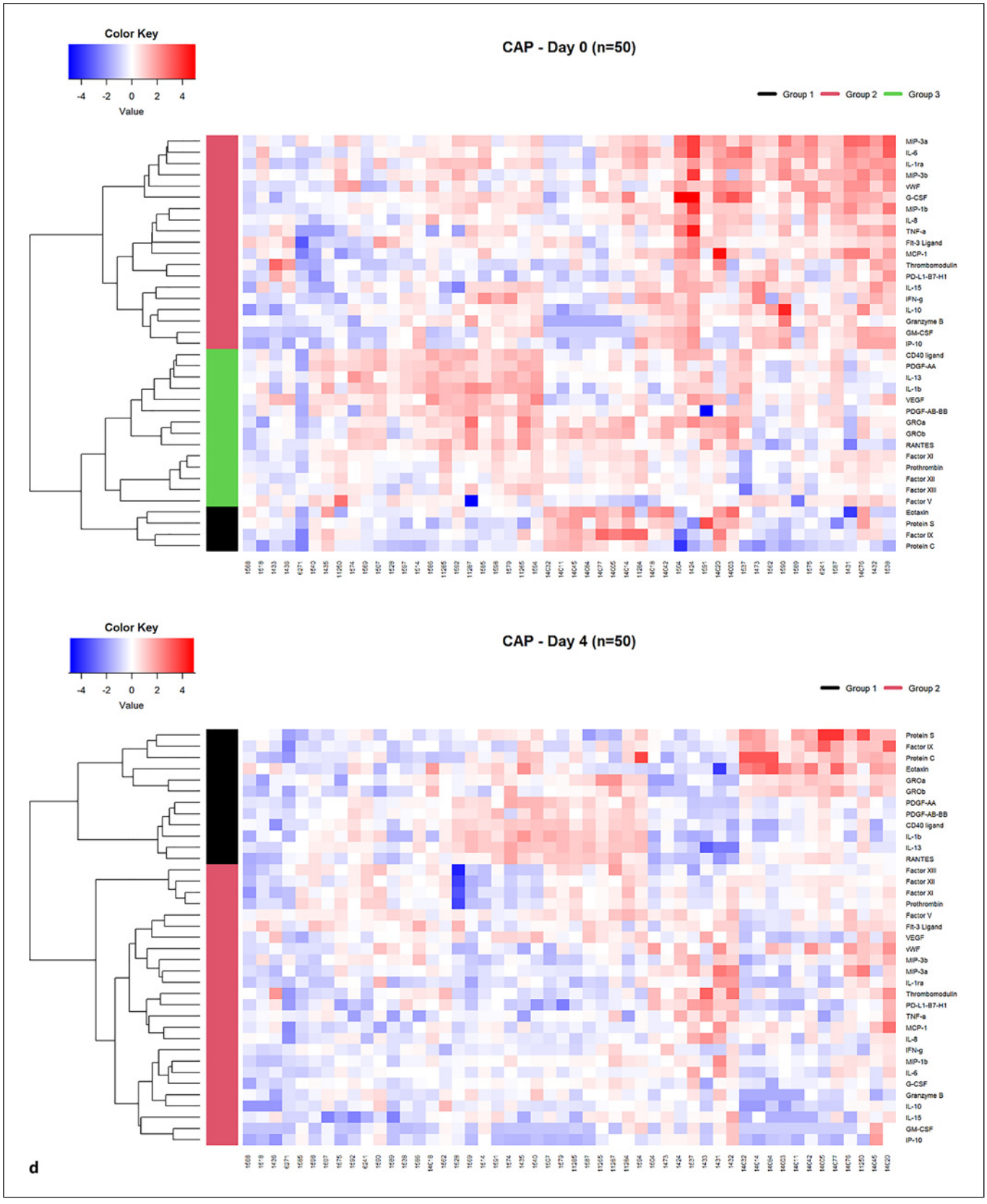

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When we pooled COVID-19 waves 1 and 2 with Omicron wave, the clustering was even more complex with 7 clusters on the hospital admission day, emphasizing the complex innate thrombo-inflammatory response to SARS-CoV-2.

We chose to do hierarchical clustering of cytokine and coagulation factor levels rather than directly on patient data to determine relationships between clusters of biomarkers in CAP. The hierarchical clustering identifies pathways that are at play in non-COVID-19 and non-COVID-19 CAP that may be susceptible to immunomodulation (precision medicine). Patient endotypes in acute respiratory distress syndrome [42, 43] and sepsis [44] defined enhanced response to corticosteroids [45] and activated protein C [44], respectively. We have not done these more common patient-based clustering approach that others have done to discover endotypes based on patient clustering. Endotyping helps define potential treatment personalization while hierarchical clustering reveals how certain biomarkers group together, potentially indicating shared biological pathways or responses to CAP.

The heat maps reveal that pro-inflammatory cytokines behave similarly in all patients and are highly correlated (generally high in about 25% of patients and low in about 75% of patients) and that non-pro-inflammatory cytokines behave similarly in patients. These are not endotypes and are not comparable to patient endotypes.

There were significant differences in almost all the principal component scores derived from day 0 cytokines and coagulation factor levels between survivor and non-survivor in COVID-19 wave 1 and 2 patients, but little to no differences were observed in Omicron wave and non-COVID-19 CAP patients. The number of patients who died in our samples were limited, which might result in low statistical power to detect a significant difference in the latter two cohorts.

The levels of only one cytokine – Flt3L – increased more in non-COVID-19 CAP patients than COVID-19 patients. Flt3L is a hematopoietic cell cytokine that links the innate and adaptive immune systems by increasing monocyte-derived myeloid dendritic cells in the lung in pneumonia [46] and in lipopolysaccharides-induced acute respiratory distress syndrome [47]. Flt3L mitigates lung injury in some pneumonia models [48] but worsens it in others [49]. Flt3L is also an adjuvant that enhances pneumococcal vaccine [50, 51].

Early wave COVID-19 patients had more severe disease than non-COVID-19 CAP perhaps because of the nature of wild type SARS-CoV-2 and lack of COVID-19 vaccines or effective drugs then. Later SARS-CoV-2 variants and use of vaccines and drugs may explain why mortality was similar for non-COVID-19 CAP and COVID-19 wave 3 and Omicron wave. Non-COVID-19 CAP patients had much higher use of antibiotics and lower use of antiviral agents than COVID-19 wave patients suggesting good compliance with non-COVID-19 CAP guidelines [2]. Corticosteroids were used in about three quarters of later wave COVID-19 patients likely because corticosteroids decreased ventilation and mortality rates in acute COVID-19 [32, 34, 52, 53]. Corticosteroids were also used frequently – about half – in non-COVID-19 CAP perhaps because of efficacy in a severe CAP trial [1]. Very recent international guidelines recommend corticosteroids in severe CAP [54].

Another study [55] compared COVID to non-COVID CAP and also found that markers of coagulation activation, including D-dimer, were not different between COVID and non-COVID CAP. Corticosteroids blunt the pro-inflammatory response in non-COVID-19 CAP [10, 11, 56] and COVID-19 [57]. However, corticosteroids can also be procoagulant [58] and increase risk of thrombosis in some studies [59, 60]. There may be procoagulant effects of corticosteroid treatment in non-COVID-19 CAP. In our study, perhaps surprisingly corticosteroids did not change cytokine levels in early hospitalized CAP but did decrease coagulation factor XI and prothrombin. Non-COVID-19 CAP patients also had a more pro-coagulant profile than COVID-19; higher platelet count, D-dimer and INR. Our study differs from other studies that found that corticosteroids mitigated the cytokine response in non-COVID-19 CAP [10, 56] and COVID-19 CAP [57], perhaps related to differences between studies in timing and doses of corticosteroid treatment, timing of plasma collection and assay methods.

We could not evaluate cytokine clustering of survivors versus non-survivors within each wave or whether the endotypes that we found identified differences in mortality or responses to corticosteroids in non-COVID-19 CAP

Fig. 3. Hierarchical clustering and heat maps of plasma cytokines and coagulation factors on day 0 and 4 in (**a**) COVID-19 waves 1 and 2, (**b**) Omicron wave, (**c**) pooled COVID-19-19 overall, and (**d**) non-COVID-19 CAP. Red is higher expression while blue is lower expression. Individual patients are the columns and individual rows are the cytokines and coagulation factors. Colors on the left indicate the individual clusters as determined by hierarchical clustering.

Table 3. Plasma cytokine and coagulation factor levels in non-COVID-19 CAP and COVID-19 wave 1 and 2 and Omicron wave

Variable	CAP vs. wave 1/2			CAP vs. Omicron		
	percentage change in median (95% CI)	*p* value	p^{FDR} value	percentage change in median (95% CI)	*p* value	p^{FDR} value
3A. Day 0 adjusted analyses						
Eotaxin	27.9 (−2.2, 67.2)	0.072	0.157	**39.8 (10.5, 76.9)**	**0.006**	**0.021**
MIP-3b	17.1 (−18.1, 67.7)	0.384	0.490	19.2 (−26.2, 92.3)	0.470	0.527
MCP-1	15.7 (−11.0, 50.4)	0.275	0.442	15.7 (−12.8, 53.5)	0.311	0.383
MIP-3a	**174.9 (69.9, 344.9)**	**<0.001**	**0.002**	**190.7 (70.8, 394.6)**	**<0.001**	**0.002**
PD-L1-B7-H1	−16.6 (−33.3, 4.2)	0.109	0.201	−22.0 (−38.1, −1.8)	0.035	0.076
MIP-1b	20.6 (1.6, 43.2)	0.032	0.092	**44.1 (13.7, 82.6)**	**0.003**	**0.014**
CD40 ligand	23.0 (−19.0, 86.9)	0.329	0.487	**102.3 (33.8, 205.9)**	**<0.001**	**0.007**
GROa	37.0 (−6.4, 100.6)	0.104	0.201	**108.0 (38.3, 212.9)**	**<0.001**	**0.005**
IP-10	**−66.4 (−82.3, −36.2)**	**<0.001**	**0.016**	−46.9 (−71.4, −1.6)	0.045	0.087
GROb	−14.1 (−51.6, 52.5)	0.601	0.742	**118.4 (23.1, 287.5)**	**0.008**	**0.027**
IL-8	27.8 (−26.1, 120.9)	0.377	0.490	20.5 (−14.7, 70.4)	0.288	0.367
IFN-g	−51.8 (−80.2, 17.0)	0.106	0.201	21.9 (−48.0, 185.7)	0.647	0.684
Flt3 ligand	−0.6 (−22.2, 26.9)	0.959	1.000	22.7 (1.2, 48.9)	0.038	0.078
G-CSF	51.8 (−3.4, 138.6)	0.070	0.157	**113.6 (28.9, 253.9)**	**0.003**	**0.014**
GM-CSF	−48.9 (−70.1, −12.4)	0.015	0.061	−32.7 (−61.3, 17.2)	0.160	0.225
Granzyme B	**−37.8 (−55.6, −12.9)**	**0.006**	**0.032**	15.4 (−16.1, 58.9)	0.376	0.447
IL-10	−7.2 (−34.0, 30.4)	0.666	0.746	23.3 (−8.3, 65.9)	0.165	0.225
IL-13	12.9 (−9.2, 40.4)	0.274	0.442	19.3 (−2.3, 45.7)	0.082	0.133
IL-15	29.4 (−0.6, 68.5)	0.055	0.143	36.8 (0.2, 86.8)	0.048	0.087
IL-1b	3.5 (−30.7, 54.6)	0.865	0.942	22.5 (−22.8, 94.5)	0.386	0.447
IL-1ra	31.6 (−14.9, 103.4)	0.215	0.379	9.4 (−32.0, 75.9)	0.709	0.729
IL-6	134.2 (15.3, 375.7)	0.019	0.070	77.2 (−12.8, 260.0)	0.113	0.174
PDGF-AA	18.9 (−15.8, 67.8)	0.323	0.487	**112.4 (45.3, 210.6)**	**<0.001**	**0.002**
PDGF-AB-BB	13.5 (−32.0, 89.3)	0.626	0.746	**130.7 (32.9, 300.6)**	**0.003**	**0.014**
RANTES	−10.7 (−45.2, 45.4)	0.647	0.746	**133.4 (51.1, 260.7)**	**<0.001**	**0.002**
TNF-a	26.4 (−0.8, 61.0)	0.058	0.143	**40.1 (7.0, 83.5)**	**0.015**	**0.039**
VEGF	23.6 (−21.6, 94.8)	0.359	0.490	53.3 (7.0, 119.7)	0.020	0.050
Factor XI	−28.0 (−46.1, −3.8)	0.027	0.090	**53.0 (10.1, 112.5)**	**0.012**	**0.033**

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Table 3 (continued)

Variable	CAP vs. wave 1/2			CAP vs. Omicron		
	percentage change in median (95% CI)	*p* value	p^{FDR} value	percentage change in median (95% CI)	*p* value	p^{FDR} value
Factor XII	**−44.4 (−62.1, −18.5)**	**0.003**	**0.027**	26.9 (−7.3, 73.8)	0.136	0.202
Factor XIII	**−34.5 (−51.3, −11.7)**	**0.006**	**0.032**	32.3 (3.0, 69.9)	0.029	0.066
Prothrombin	**−39.0 (−58.0, −11.3)**	**0.010**	**0.047**	**37.5 (8.0, 75.1)**	**0.010**	**0.031**
Factor IX	0.2 (−23.0, 30.4)	0.990	1.000	−0.7 (−18.6, 21.1)	0.941	0.941
Protein C	**−40.3 (−56.2, −18.5)**	**0.001**	**0.016**	−28.0 (−48.1, −0.1)	0.049	0.087
Protein S	−0.0 (−20.6, 25.9)	1.000	1.000	14.9 (−10.5, 47.5)	0.274	0.363
vWF	71.1 (5.3, 178.1)	0.030	0.092	61.2 (−1.7, 164.3)	0.058	0.098
Factor V	**45.4 (12.7, 87.6)**	**0.004**	**0.032**	**−37.8 (−53.3, −17.0)**	**0.001**	**0.009**
Thrombomodulin	8.3 (−9.5, 29.6)	0.379	0.490	6.8 (−11.5, 29.0)	0.487	0.530
3B. Day 4 adjusted analyses						
Eotaxin	16.8 (−26.3, 85.2)	0.506	0.711	28.0 (−15.7, 94.5)	0.244	0.348
MIP-3b	−11.3 (−39.5, 30.1)	0.538	0.711	**87.4 (20.6, 191.4)**	**0.006**	**0.017**
MCP-1	5.4 (−22.3, 42.9)	0.735	0.859	26.0 (−6.9, 70.5)	0.133	0.246
MIP-3a	−1.5 (−24.6, 28.6)	0.909	0.961	**79.7 (24.7, 159.0)**	**0.002**	**0.010**
PD-L1-B7-H1	−10.1 (−32.8, 20.2)	0.469	0.694	11.1 (−15.7, 46.3)	0.452	0.523
MIP-1b	8.6 (−6.4, 26.0)	0.272	0.504	**30.1 (13.1, 49.6)**	**<0.001**	**0.003**
CD40 ligand	−10.9 (−49.2, 56.3)	0.685	0.845	**112.8 (29.2, 250.6)**	**0.003**	**0.014**
GROa	62.6 (16.5, 126.9)	0.005	0.056	**127.8 (68.7, 207.7)**	**<0.001**	**<0.001**
IP-10	−57.5 (−77.1, −21.2)	0.007	0.065	−21.4 (−53.6, 33.2)	0.369	0.470
GROb	51.6 (−24.3, 203.4)	0.238	0.472	**120.1 (33.7, 262.3)**	**0.002**	**0.010**
IL-8	−8.9 (−36.7, 31.1)	0.614	0.783	48.3 (−10.6, 145.9)	0.126	0.245
IFN-g	−28.7 (−56.3, 16.3)	0.174	0.415	23.7 (−30.6, 120.8)	0.468	0.525
Flt3 ligand	**17.6 (−7.3, 49.1)**	**0.180**	**0.415**	**44.4 (14.5, 82.1)**	**0.002**	**0.010**
G-CSF	16.2 (−14.1, 57.1)	0.327	0.550	**46.0 (10.1, 93.7)**	**0.009**	**0.024**
GM-CSF	**−61.7 (−77.5, −34.9)**	**<0.001**	**0.009**	3.3 (−25.0, 42.3)	0.840	0.888
Granzyme B	−33.1 (−54.6, −1.4)	0.042	0.174	2.7 (−27.4, 45.3)	0.878	0.902
IL-10	−39.6 (−60.3, −8.0)	0.019	0.115	28.1 (−8.7, 79.7)	0.151	0.266
IL-13	2.7 (−19.7, 31.3)	0.830	0.904	**37.8 (10.5, 71.9)**	**0.005**	**0.016**
IL-15	−15.3 (−39.2, 17.9)	0.322	0.550	−24.1 (−48.6, 12.1)	0.164	0.276

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Table 3 (continued)

Variable	CAP vs. wave 1/2			CAP vs. Omicron		
	percentage change in median (95% CI)	*p* value	p^{FDR} value	percentage change in median (95% CI)	*p* value	p^{FDR} value
IL-1b	7.1 (−34.1, 73.9)	0.781	0.875	**92.2 (18.9, 210.8)**	**0.008**	**0.023**
IL-1ra	−33.7 (−55.9, −0.5)	0.047	0.174	31.1 (−11.6, 94.5)	0.176	0.284
IL-6	44.6 (−2.1, 113.5)	0.064	0.207	66.3 (1.2, 173.3)	0.045	0.098
PDGF-AA	0.4 (−37.2, 60.6)	0.985	0.985	**84.1 (36.2, 148.8)**	**<0.001**	**0.002**
PDGF-AB-BB	−24.1 (−52.2, 20.7)	0.242	0.472	**60.4 (11.3, 131.2)**	**0.012**	**0.029**
RANTES	−33.8 (−63.6, 20.5)	0.176	0.415	**124.8 (44.9, 248.7)**	**<0.001**	**0.003**
TNF-a	−10.5 (−28.9, 12.7)	0.342	0.550	13.9 (−8.4, 41.7)	0.240	0.348
VEGF	1.0 (−33.8, 54.0)	0.963	0.985	**87.2 (32.4, 164.8)**	**<0.001**	**0.004**
Factor XI	−30.4 (−48.6, −5.8)	0.019	0.115	**57.4 (15.4, 114.9)**	**0.005**	**0.016**
Factor XII	−22.9 (−48.8, 16.0)	0.211	0.458	38.0 (−0.5, 91.4)	0.054	0.111
Factor XIII	−13.9 (−39.4, 22.2)	0.398	0.614	13.4 (−6.2, 37.1)	0.192	0.295
Prothrombin	−28.9 (−50.7, 2.5)	0.067	0.207	20.7 (2.8, 41.7)	0.022	0.052
Factor IX	−20.5 (−38.5, 2.6)	0.078	0.221	−13.0 (−32.9, 12.6)	0.288	0.394
Protein C	−28.8 (−48.4, −1.6)	0.040	0.174	−13.1 (−35.6, 17.2)	0.355	0.469
Protein S	−8.0 (−29.3, 19.7)	0.532	0.711	−9.0 (−28.6, 15.9)	0.443	0.523
vWF	49.6 (6.2, 110.8)	0.022	0.115	15.9 (−26.5, 82.7)	0.522	0.568
Factor V	**75.0 (46.8, 108.5)**	**<0.001**	**<0.001**	−8.9 (−28.7, 16.3)	0.451	0.523
Thrombomodulin	−3.0 (−19.3, 16.5)	0.743	0.859	0.8 (−15.8, 20.7)	0.932	0.932
3C. Adjusted analyses for change from day 0 to day 4						
Eotaxin	16.2 (2.8, 31.3)	0.016	0.102	21.2 (2.5, 43.2)	0.024	0.190
MIP-3b	−24.6 (−42.5, −1.3)	0.040	0.177	23.8 (−9.3, 68.9)	0.177	0.381
MCP-1	0.5 (−22.0, 29.5)	0.968	0.999	23.7 (−7.5, 65.4)	0.149	0.381
MIP-3a	**−35.0 (−48.1, −18.6)**	**<0.001**	**0.004**	3.5 (−24.7, 42.3)	0.831	0.905
PD-L1-B7-H1	4.2 (−10.7, 21.6)	0.595	0.744	9.1 (−2.7, 22.4)	0.136	0.381
MIP-1b	−7.0 (−21.0, 9.5)	0.383	0.567	12.3 (−1.9, 28.6)	0.091	0.306
CD40 ligand	−9.5 (−36.2, 28.4)	0.573	0.744	31.0 (−9.9, 90.5)	0.157	0.381
GROa	33.7 (−0.3, 79.2)	0.053	0.177	42.6 (8.6, 87.2)	0.011	0.137
IP-10	−17.6 (−43.5, 20.3)	0.313	0.552	15.5 (−16.2, 59.1)	0.376	0.547
GROb	19.3 (−21.8, 81.9)	0.410	0.584	49.2 (1.3, 119.7)	0.043	0.228

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Table 3 (continued)

Variable	CAP vs. wave 1/2			CAP vs. Omicron		
	percentage change in median (95% CI)	*p* value	p^{FDR} value	percentage change in median (95% CI)	*p* value	p^{FDR} value
IL-8	−26.2 (−47.5, 3.6)	0.079	0.224	0.1 (−29.7, 42.6)	0.995	0.995
IFN-g	−4.0 (−35.7, 43.4)	0.842	0.951	6.7 (−37.1, 80.8)	0.809	0.905
Flt3 ligand	**30.8 (10.1, 55.4)**	**0.002**	**0.030**	**40.1 (14.7, 71.0)**	**0.001**	**0.040**
G-CSF	1.8 (−21.5, 31.9)	0.895	0.974	12.2 (−13.0, 44.7)	0.373	0.547
GM-CSF	−17.8 (−32.2, −0.3)	0.047	0.177	16.1 (−14.1, 56.8)	0.329	0.547
Granzyme B	−19.6 (−45.1, 17.7)	0.260	0.552	2.5 (−22.8, 36.1)	0.862	0.911
IL-10	−25.0 (−43.7, −0.1)	0.049	0.177	20.5 (−9.9, 61.3)	0.207	0.383
IL-13	4.4 (−11.2, 22.7)	0.603	0.744	10.5 (−4.3, 27.6)	0.174	0.381
IL-15	−35.3 (−54.7, −7.8)	0.017	0.102	−27.7 (−49.8, 4.2)	0.082	0.304
IL-1b	−0.4 (−27.6, 37.1)	0.982	0.999	51.9 (−5.2, 143.4)	0.082	0.304
IL-1ra	−29.0 (−49.4, −0.2)	0.049	0.177	30.5 (−1.5, 72.9)	0.063	0.292
IL-6	3.9 (−27.4, 48.9)	0.832	0.951	19.6 (−20.3, 79.3)	0.385	0.547
PDGF-AA	−13.3 (−35.2, 16.1)	0.335	0.563	29.5 (−12.6, 91.9)	0.195	0.381
PDGF-AB-BB	−21.5 (−39.7, 2.3)	0.073	0.224	33.7 (3.6, 72.4)	0.026	0.190
RANTES	−22.3 (−45.2, 10.2)	0.156	0.411	14.9 (−15.8, 56.8)	0.380	0.547
TNF-a	−9.9 (−26.2, 10.0)	0.304	0.552	17.9 (−7.1, 49.7)	0.174	0.381
VEGF	−11.6 (−29.4, 10.8)	0.284	0.552	22.8 (1.5, 48.5)	0.034	0.212
Factor XI	−7.0 (−27.4, 19.3)	0.566	0.744	13.9 (−12.6, 48.5)	0.334	0.547
Factor XII	−8.4 (−24.2, 10.8)	0.366	0.567	4.5 (−14.5, 27.8)	0.664	0.834
Factor XIII	1.7 (−14.5, 21.0)	0.848	0.951	−2.7 (−16.1, 12.9)	0.718	0.834
Prothrombin	−11.9 (−30.3, 11.5)	0.290	0.552	4.0 (−15.2, 27.7)	0.704	0.834
Factor IX	−19.6 (−32.1, −4.8)	0.012	0.102	−10.3 (−23.9, 5.8)	0.194	0.381
Protein C	−0.0 (−24.1, 31.7)	0.999	0.999	0.5 (−26.0, 36.4)	0.976	0.995
Protein S	7.4 (−4.1, 20.4)	0.216	0.532	−2.8 (−17.1, 13.9)	0.721	0.834
vWF	10.0 (−11.2, 36.3)	0.381	0.567	−29.4 (−45.7, −8.3)	0.010	0.137
Factor V	**76.9 (45.3, 115.3)**	**<0.001**	**<0.001**	8.3 (−19.5, 45.8)	0.597	0.789
Thrombomodulin	−5.4 (−13.8, 3.9)	0.245	0.552	−3.0 (−10.1, 4.8)	0.440	0.602

Results are expressed as percentage change in median and were based on quantile regression. In the adjusted analysis, we adjusted for age, sex, and comorbidities that were different between groups ($p < 0.2$). pFDR accounted for multiple comparisons through false discovery rate. VWF, von Willebrand factor; CI, confidence interval.

because our sample size was too small for such analysis. Our study strengths include recruitment of patients hospitalized for non-COVID-19 CAP and COVID-19 in a multicenter Canadian cohort in which we detailed phenotyping using a standardized case report form, compared mortality, organ dysfunction and the uses of mechanical ventilation, vasopressors and RRT [20, 35] and measured plasma cytokines and coagulation factors in a representative subgroup of patients. We chose patients such that all were still alive on day 4 and suggest that that eliminates concerns regarding mortality competing risks. Limitations of our observational study are that we could not determine causation. We did not record some COVID-19 therapies (e.g., anticoagulants, immunomodulatory drugs) or non-COVID-19 CAP vaccines (i.e., influenza and pneumococcal vaccines) that could have altered outcomes. The number of clusters identified in a dataset is, however, influenced by the sample size (n = 50 in early wave, n = 50 Omicron and n = 50 non-COVID CAP); larger datasets might reveal more subtle clusters that smaller datasets miss. This issue could cause misleading conclusions about the inherent complexity of the disease states using number of clusters alone. One potential limitation is that the evaluation of changes in plasma cytokine, chemokine, and coagulation factors over time is that day four samples are only available in survivors still in hospital on day four. So, for example, by day four, that non-COVID-19 CAP patients simplified to two clusters may be due to a specific pattern being present only in those who were discharged or who died.

Conclusions

The greater complexity of the thrombo-inflammatory response of early wave COVID-19 compared with non-COVID-19 CAP aligned with higher mortality of early wave COVID-19 than non-COVID-19 CAP. Mortality, ICU admission, invasive ventilation and vasopressor rates of non-COVID-19 CAP were less than early COVID-19 waves but comparable to Omicron COVID-19 wave.

Statement of Ethics

This study protocol was reviewed and approved by Providence Health Care and University of British Columbia Human Research Committee (#H20-00600-AO36) and Research Ethics Boards at each of the participating sites. This full list of participating site and Ethics Committees can be found at Mohammed Y., Goodlett D.R., Cheng M., Vinh D.C., Lee T.C., McGeer A., Sweet D., Tran K., Lee T., Murthy S., Boyd J.H., Singer J., Walley K.R., Patrick D.M., Quan C., Ismail S., Amar L., Pal A., Bassawon R., Fesdekjian L., Gou K., Lamontagne F., Marshall J., Haljan G., Fowler R., Winston B.W., Levin A., and Russell J.A. for ARBs CORONA l. Evolution of proteomics from acute to post-COVID-19 condition. *J Proteome Research*. 2023;23(1):52–70. https://doi.org/10.1021/acs.jproteome.3c00324). De-identified clinical data were deemed low risk and informed consent was not required. The need for informed consent was waived by the Providence Health Care and University of British Columbia Human Research Committee and Research Ethics Boards at each of the participating sites.

Conflict of Interest Statement

Dr. Russell reports patents owned by the University of British Columbia (UBC) that are related to (1) the use of PCSK9 inhibitor(s) in sepsis, (2) the use of vasopressin in septic shock and (3) a patent owned by Ferring for use of selepressin in septic shock. Dr. Russell is an inventor on these patents. Dr. Russell was a founder, Director and shareholder in Cyon Therapeutics Inc. (now closed) and is a shareholder in Molecular You Corp. Dr. Russell is Senior Research Advisor of the British Columbia, Canada Post COVID – Interdisciplinary Clinical Care Network (PC-ICCN). Dr. Russell is no longer actively consulting for any industry. Dr. Russell reports receiving consulting fees in the last 3 years. Dr. Russell was a funded member of the Data and Safety Monitoring Board (DSMB) of an NIH-sponsored trial of plasma in COVID-19 (PASS-IT-ON) (2020–2021). Dr. Russell has received grants for COVID-19 and for pneumonia research: 4 from the Canadian Institutes of Health Research (CIHR) and 3 from the St. Paul's Foundation (SPF). Dr. Russell was a non-funded Science Advisor and member, Government of Canada COVID-19 Therapeutics Task Force (June 2020–2021).

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Author Contributions

J.A.R. designed the study, obtained funding, interpreted the data and led the manuscript. T.L. did the statistical analyses. H.J.K. did the cytokine and coagulation factor assays. All other authors recruited patients and contributed to the editing of the manuscript. Greg Haljan, Anne McCarthy, Juthaporn Cowan, Jennifer Tsang, Francois Lelouche, Alexis F. Turgeon, Patrick Archambault, Francois Lamontagne, Robert Fowler, Jennifer Yoon, Peter Daley, Matthew P. Cheng, Donald C. Vinh, Todd C. Lee, Karen C. Tran, Brent W. Winston, John H. Boyd, Keith R. Walley, Allison McGeer, David M. Maslove, and John C. Marshall recruited patients and contributed to the editing of the manuscript. Joel Singer supervised statistical analyses and contributed to the editing of the manuscript. Fagun Jain was Project Manager facilitated patient recruitment and contributed to the editing of the manuscript. Francois Lelouche was not available to confirm co-authorship, but

the corresponding author James A. Russellaffirms that author Francois Lelouche contributed to the paper, had the opportunity to review the final version to be published and guarantees author Francois Lelouche co-authorship status and the accuracy of the author contribution and conflict of interest statements.

Data Availability Statement

Datasets on which the conclusions of the paper rely available to editors and reviewers but not readers because of ethics restrictions. Further inquiries can be directed to the corresponding author.

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