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High Circulating Levels of the Homeostatic Chemokines CCL19 and CCL21 Predict Mortality and Disease Severity in COVID-19

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Background. Immune dysregulation is a major factor in the development of severe coronavirus disease 2019 (COVID-19). The homeostatic chemokines CCL19 and CCL21 have been implicated as mediators of tissue inflammation, but data on their regulation in severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection is limited. We thus investigated the levels of these chemokines in COVID-19 patients.

Methods. Serial blood samples were obtained from patients hospitalized with COVID-19 (n = 414). Circulating CCL19 and CCL21 levels during hospitalization and 3-month follow-up were analyzed. In vitro assays and analysis of RNAseq data from public repositories were performed to further explore possible regulatory mechanisms.

Results. A consistent increase in circulating levels of CCL19 and CCL21 was observed, with high levels correlating with disease severity measures, including respiratory failure, need for intensive care, and 60-day all-cause mortality. High levels of CCL21 at admission were associated with persisting impairment of pulmonary function at the 3-month follow-up.

Conclusions. Our findings highlight CCL19 and CCL21 as markers of immune dysregulation in COVID-19. This may reflect aberrant regulation triggered by tissue inflammation, as observed in other chronic inflammatory and autoimmune conditions. Determination of the source and regulation of these chemokines and their effects on lung tissue is warranted to further clarify their role in COVID-19.

Clinical Trials Registration. NCT04321616 and NCT04381819.

Keywords. SARS-CoV-2; chemokine; predictive markers; respiratory distress syndrome; viral infection.

It is increasingly apparent that the induction of overwhelming systemic inflammatory responses is associated with severe clinical manifestations and unfavorable outcomes in coronavirus disease 2019 (COVID-19). While early triggering of immune defense mechanisms is crucial for an effective elimination of viral particles in the initial stages of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection [1, 2], persistent and dysregulated systemic inflammatory responses are detrimental [3, 4]. Although different forms of immune dysregulation involving both the innate and adaptive immune system have been reported [5–9], the drivers of the extensive and persistent immune activation in severe COVID-19 remain unclear.

Interference with chemokine responses forms the basis of effective immune evasion strategies employed by many viruses. Most studies have focused on inflammatory chemokines, which are recognized as integral parts of the inflammatory signaling cascades triggered by tissue injury and invading pathogens. In contrast, the homeostatic chemokines CCL19 and CCL21

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are constitutively expressed in secondary lymphoid organs. There they promote the homing of T cells and dendritic cells (DCs) that express the common receptor CCR7 and the more recently discovered CCR10 [10]. In addition to this homeostatic function, elevated circulating levels of CCL19 and CCL21 are found in several acute and chronic inflammatory conditions, including autoimmune diseases, atherosclerosis, and various infections [11–14]. Moreover, these chemokines have effects on vascular smooth muscle cells and extracellular matrix remodeling [15], and are associated with pulmonary hypertension in systemic sclerosis [16]. These findings suggest a role of dysregulated CCL19 and CCL21 signaling in inflammatory pulmonary disorders. Studies on CCL19 and CCL21 in COVID-19 are limited, but the ectopic cellular expression of the ORF7a protein encoded by this virus was found to induce secretion of multiple chemokines, including CCL21, in cultured HeLa cells [17].

The present study aimed to investigate the association between CCL19/CCL21 and disease severity, 60-day total mortality, and pulmonary sequelae in COVID-19. CCL19 and CCL21 levels were analyzed in 2 Norwegian prospective cohort studies of hospitalized COVID-19 patients spanning 3 waves of the COVID-19 pandemic.

METHODS

Study Design and Participants

Data from 2 prospective cohort studies were pooled and assessed in our study. A flow chart of the study design is provided in Figure 1. Cohort 1 was the NOR Solidarity trial (NCT04321616), a multicenter, open-label, adaptive randomized controlled trial evaluating the effect of antiviral agents (hydroxychloroquine and remdesivir) in COVID-19 patients admitted to 23 Norwegian hospitals. Study interventions in this trial did not show significant effects on clinical outcomes or viral clearance [18, 19]. The study was approved by the Committee for Medical Research Ethics Region Southeast Norway (approval no. 118684) and the Norwegian Medicines Agency (20/04950-23). Cohort 2 was the Norwegian SARS-CoV-2 study (NCT04381819), an observational study of COVID-19 patients admitted to 5 Norwegian hospitals, conducted as part of an International Severe Acute Respiratory and Emerging Infection Consortium (ISARIC) WHO Clinical Characterization Protocol study [20]. The study was approved by the Regional Committees for Medical Research Ethics Southeast Norway (106624 and 2019/306). All patients aged ≥18 years admitted to the hospital with polymerase chain reaction (PCR)-confirmed SARS-2-CoV-2 infection were eligible for inclusion. Blood samples were obtained from each patient within 48 hours of admission and up to 10 days during hospitalization, as well as at 3-month follow-up in a subset of patients. All participants gave informed consent prior to inclusion, either directly or through a legally authorized representative.

Patients in cohort 1 were included from March to October 2020, while patients in cohort 2 were included from March 2020 to September 2021. The study period thus spanned the first 3 waves of the COVID-19 pandemic in Norway: 18 Mar 2020 to 23 July 2020 (wave 1), 24 July 2020 to 17 February 2021 (wave 2), and 18 February 2021 to 31 July 2021 (wave 3) [21]. From February 2021, Alpha SARS-CoV-2 was the dominating variant, which was superseded by the Delta variant in July 2021 [22, 23].

Intervention and Outcomes

In cohort 1 (n = 162), participants were randomized to either (1) local standard of care (SoC); (2) SoC plus oral hydroxychloroquine; or (3) SoC plus intravenous remdesivir as described [18]. Previous studies showed no effects of these treatment modalities [18]. Data from the intervention arms in cohort 1 were therefore pooled together with samples from cohort 2 (n = 252) to examine whether levels of CCL19 and CCL21 were associated with disease severity. Severe COVID-19 was defined as 1 or more of the following: (1) development of acute respiratory failure (RF) defined as Po_2/Fio_2 ratio <26.6 kPa (<200 mmHg) during hospitalization; (2) requirement for intensive care unit (ICU) support during hospitalization; and (3) 60-day postadmission mortality. In addition, for cohort 1, patients were evaluated 3 months after inclusion with pulmonary function testing as detailed below.

Blood Sampling Protocol and Biochemical Analyses

Plasma (cohort 1) or serum (cohort 2) was stored at −80°C, and thawed < 3 times. For reference, circulating CCL19 and CCL21 were also analyzed in plasma from 24 age- and sex-matched healthy controls (mean age 55 years [SD 12 years]; 55% men).

Plasma and serum levels of CCL19 and CCL21 were measured by enzyme immunoassays using commercially available antibodies (R&D Systems). Intra-/interassay coefficients of variation were <10%. Comparing within-patient differences in serum versus plasma in 16 healthy controls, we observed no difference for CCL19 ($P = .80$) or CCL21 ($P = .18$).

Three-Month Follow-Up

In total, 257 participants (cohort 1, n = 100; cohort 2, n = 157) attended outpatient follow-up that included blood sampling for routine clinical biochemistry and biobanking. The timing of this 3-month follow-up was different in the 2 cohorts, defined by inclusion date in cohort 1, and date of hospital discharge in cohort 2. In cohort 1, lung function testing (n = 90), consisting of spirometry, and diffusing capacity of the lungs (D_{LCO}) was also performed [24]. The predicted percentage of D_{LCO} and the lower limit of normal were calculated according to the Global Lung Function Initiative Network guidelines [24].

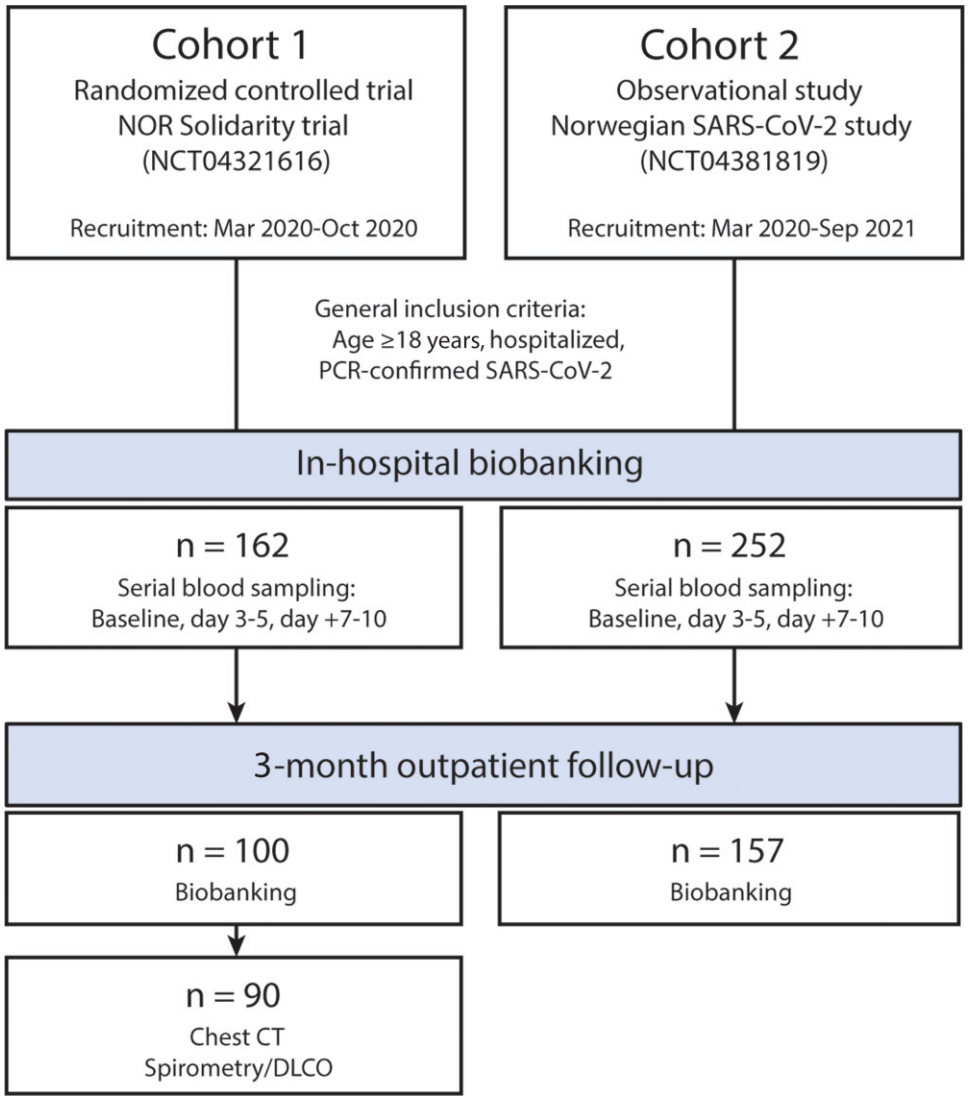

Figure 1. Flow chart showing the study population, with clinical data and blood samples collected from 2 cohort studies. Further details of the 2 trials are provided in the "Methods" section. Blood samples were collected at 3 time points during hospitalization, and at outpatient follow-up after 3 months. A subset of patients from cohort 1 also underwent pulmonary function assessment and chest CT imaging at follow-up. Abbreviations: CT, computed tomography; DLCO, diffusing capacity of the lungs for carbon monoxide; PCR, polymerase chain reaction; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

In Vitro SARS-CoV-2 Stimulation of Dendritic Cells

Human peripheral blood mononuclear cells (PBMC) were isolated from buffy coats of healthy donors, differentiated into DCs, and stimulated with inactivated SARS-CoV-2 as detailed in Supplementary Material.

Statistics

Clinical characteristics of the participants were compared using Student *t* test or Mann-Whitney *U* test depending on variable distribution, or by χ^2 for continuous and categorical variables, respectively (Table 1). CCL19 and CCL21 were transformed ($\log_{10}$) for temporal comparisons between groups by linear mixed model analysis. Subject was set as random effect, while time, RF, ICU admission, and 60-day mortality were set as fixed effects (independently and also as interactions). In addition, age, sex, estimated glomerular filtration rate and treatment modalities (study drug for cohort 1, and dexamethasone for cohort 2) were included as independent effects. Data are presented as back-transformed estimated marginal means with 95% confidence intervals (CI). Post hoc analysis (Sequential Sidak test) between groups is reported if the group or group* (ie, interaction term) was significant. Similar models were applied to evaluate the effects of randomized treatment and DLCO in cohort 1 and wave or dexamethasone use in cohort 2.

Separate linear mixed models for CCL19 and CCL21, with time treated as a factor variable, were employed to model the

Table 1. Baseline Characteristics and Outcomes in 414 Patients Hospitalized for COVID-19 in Norway, Stratified by 2 Large Multicenter Cohorts and Combined

Parameter	Cohort 1 n = 162	Cohort 2 n = 252	Combined n = 414
Age, y	59.7 ± 15.4	57.0 ± 15.3	58.0 ± 15.4
Male sex, No. (%)	103 (64)	159 (63)	262 (63)
Body mass index, kg/m^2	28.2 ± 4.6	28.8 ± 5.2	28.5 ± 4.9
Treatment group, No. (%)			
SoC	81 (50)	254 (100)	333 (80)
SoC + hydroxychloroquine	43 (27)	0 (0)	43 (10)
SoC + remdesivir	38 (24)	0 (0)	38 (9)
Dexamethasone	2 (1)	134 (53)[a]	136 (33)
Oxygen therapy	91 (56)	194 (77)[a]	285 (69)
Comorbidities, No. (%)			
Chronic cardiac disease	24 (15)	47 (19)	71 (17)
Hypertension	51 (32)	84 (35)	135 (34)
Chronic pulmonary disease	31 (20)	67 (27)	98 (24)
Obesity	43 (29)	70 (28)	113 (28)
Diabetes	27 (17)	58 (25)	85 (22)
Current smoker	5 (4)	16 (7)	21 (6)
Outcomes, No. (%)			
ICU admission	31 (19)	79 (31)[a]	110 (27)
Respiratory failure	50 (31)	75 (30)	125 (31)
Deceased at 60 days	8 (5)	29 (12)	37 (9)
Po_2/Fio_2 ratio at admission, kPa	42.4 (32.4, 49.6)	40.0 (28.1, 48.3)	41.3 (30.0, 49.3)
Laboratory analysis at admission			
Hemoglobin, g/dL	13.2 ± 1.5	12.9 ± 1.8	13.0 ± 1.7
C-reactive protein, mg/L	70 (35, 136)	53 (24, 117)	62 (29, 125)
Ferritin, µg/L	612 (358, 1111)	617 (297, 1146)	615 (322, 1127)
White blood cell count, × 10^9/L	6.5 ± 2.8	6.9 ± 3.2	6.7 ± 3.1
Neutrophils, × 10^9/L	4.8 ± 2.7	5.3 ± 3.1	5.1 ± 3.0
Lymphocytes, × 10^9/L	1.2 ± 0.53	1.1 ± 0.5	1.1 ± 0.5
eGFR, mL/min/1.73m^2	87 ± 25	90 ± 29	89 ± 27

Continuous data are given as mean ± SD or median (25th, 75th) percentile.

Abbreviations: eGFR, estimated glomerular filtration rate; ICU, intensive care unit; SoC, standard of care.

[a] $P < .05$ between cohorts 1 and 2.

association between Po_2/Fio_2 ratio (outcome) and circulating levels of CCL19 and CCL21. A random intercept by subject was used to control for repeated measures, with each subject having between 1 and 3 measured follow-up periods. Associations between admission levels of CCL19 and CCL21 (divided in tertiles) and 60-day mortality were assessed by Kaplan-Meier analysis and Cox regression.

Reanalysis of Public RNAseq Datasets

Publicly available RNASeq datasets were identified via a manual search of the National Center for Biotechnology Information (NCBI) Gene Expression Omnibus (GEO) and details on their analyses are included in the Supplementary Material.

RESULTS

Baseline Characteristics and Outcomes of the Study Population

A flow chart of the study design is given in Figure 1. Demographics and clinical characteristics of the cohorts were quite similar (Table 1), except for the use of antiviral agents in cohort 1, and dexamethasone use in cohort 2 after introduction as SoC in the management of severe COVID-19 (autumn 2020). More patients were admitted to ICU in cohort 2. In the combined cohort, 110 patients (27%) were admitted to ICU, and 125 (31%) patients developed RF the first 10 days after admission (Table 1). Sixty-six patients (22%) with RF did not receive treatment in an ICU.

Initial Temporal Profile of Circulating CCL19 and CCL21 and Relation to Respiratory Failure and ICU Admission

As shown in Figure 2*A*, the levels of both markers were higher in COVID-19 patients than in controls, and significantly higher in RF patients compared to patients without RF. CCL19 remained high in RF patients throughout the observation period, while differences in CCL21 were larger at admission and decreased towards the end of observation. The group effect for higher CCL19 in RF patients within cohorts 1 and 2 separately was $P = .051$ and $P = .002$, respectively. Corresponding values

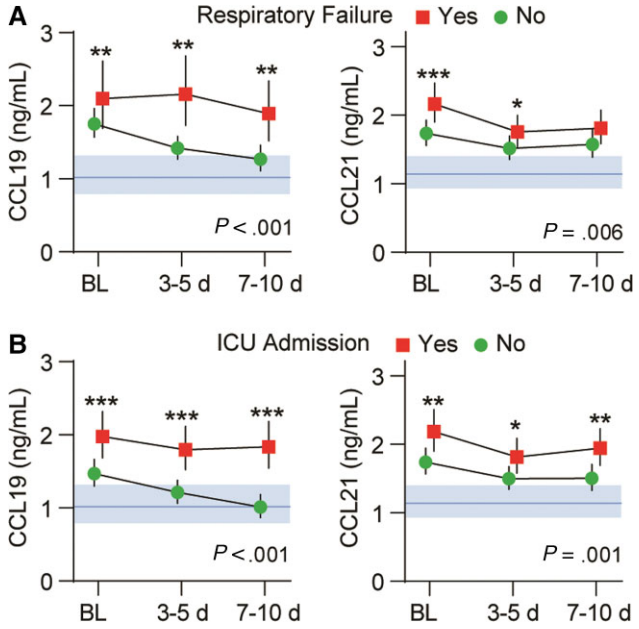

Figure 2. Intrahospital temporal profile of CCL19 and CCL21 in patients hospitalized with COVID-19 (n = 414) according to (*A*) respiratory failure (n = 125) or (*B*) ICU admission (n = 110) during the first 10 days after inclusion. Data is shown as estimated marginal means and 95% CI. The *P* values reflect the group (outcome) effect from the linear mixed models with subject as random effect, and time and respiratory failure or ICU admission as fixed effects (also as interaction) in addition to age, sex, estimated glomerular filtration rate, and treatment modalities. Shaded areas show reference value range from healthy controls. * $P < .05$, ** $P < .01$, *** $P < .001$ between groups. Abbreviations: BL, baseline; ICU, intensive care unit.

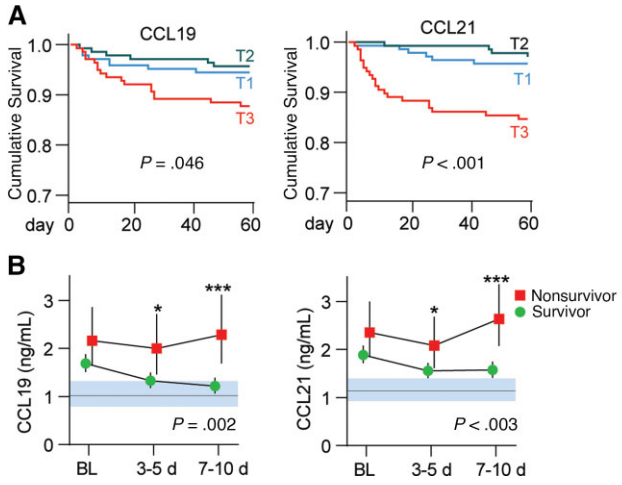

Figure 3. CCL19 and CCL21 and 60-day mortality in patients hospitalized with COVID-19 (n = 414). *A*, Kaplan-Meier analysis 60-day mortality (n = 37) according to tertiles (T) of CCL19 (T1 ≤ 1.27 ng/mL, T2 1.28–2.09 ng/mL, T3 > 2.10 ng/mL) and CCL21 (T1 ≤ 1.41 ng/mL, T2 1.42–2.44 ng/mL, T3 > 2.45 ng/mL). *B*, Temporal profile of CCL19 and CCL21 during the first 10 days after inclusion according to 60-day mortality. Data in B is shown as estimated marginal means and 95% CI. The *P* values reflect the group (outcome) effect from the linear mixed models with subject as random effect, and time and mortality as fixed effects (also as interaction) in addition to age, sex, estimated glomerular filtration rate, and treatment modalities. Shaded areas show reference value range from healthy controls. * $P < .05$, ** $P < .01$, *** $P < .001$ between groups. Abbreviation: BL, baseline.

for CCL21 were $P = .022$ and $P = .020$, respectively. Mixed models regression with Po_2/Fio_2 ratio as dependent and CCL19 or CCL21 and time as covariates revealed a negative correlation with CCL21 (estimate, −0.16; $t = -6.1$; $P < .001$), with a less robust association for CCL19 (estimate, −0.06; $t = -2.3$; $P = .019$).

A similar pattern was observed for ICU admission (Figure 2*B*), with CCL19 and CCL21 remaining elevated throughout the observation period in those admitted to ICU. The overall group effect from the mixed models for higher CCL19 in patients admitted to ICU within cohorts 1 and 2 was $P < .001$ and $P < .001$, respectively. Corresponding values for CCL21 were $P = .001$ and $P = .024$, respectively.

The dynamics of oropharyngeal SARS-CoV-2 viral load in cohort 1 has previously been reported [18]. Baseline levels of SARS-CoV-2 in oropharynx did not correlate with baseline levels of CCL19 ($r = -0.00$; $P = .94$) or CCL21 ($r = 0.13$; $P = .18$).

High CCL19 and CCL21 Levels Are Associated With 60-Day Mortality

In the combined cohort, 37 patients died within 60 days of hospital admission (Table 1). Kaplan-Meier analysis of admission levels showed that patients in the upper tertile of CCL19 and CCL21 were at increased risk of death within 60 days (Figure 3*A*). Evaluated as continuous variables, a 1 SD increase in CCL21 was associated with a 2.46 (95% CI, 1.76–3.42; $P < .001$) times higher risk of death, while the association with 60-day mortality was not significant for CCL19 (hazard ratio [HR], 1.27; 95% CI, .91–1.76; $P = .15$). For CCL21, the increased risk of death was present both in patients treated with (HR, 1.93; $P = .004$) and without dexamethasone (HR, 3.30; $P = .001$).

Evaluation of the temporal profile during the first 10 days after inclusion revealed that patients who died had higher levels of CCL19 and CCL21, with the largest differences at the end of the observation period (Figure 3*B*).

Intrahospital Temporal Profile of CCL19 and CCL21 in Relation to Treatment and COVID-19 Waves

As shown in Figure 4*A*, no temporal differences in CCL19 or CCL21 were observed according to treatment with hydroxychloroquine, remdesivir, or SoC within cohort 1 (Figure 4*A*). Dexamethasone use within cohort 2 (Figure 4*B*) was associated with lower levels of CCL19 and a decrease in CCL21 compared to patients who did not receive glucocorticoid treatment. No overall group effects were observed regarding COVID-19 waves and temporal profile of CCL19 or CCL21, but both chemokines showed an interaction between wave and time ($P < .001$; Figure 4*C*). For CCL21 the interaction was driven by higher levels at admission in patients hospitalized during the third wave of the pandemic.

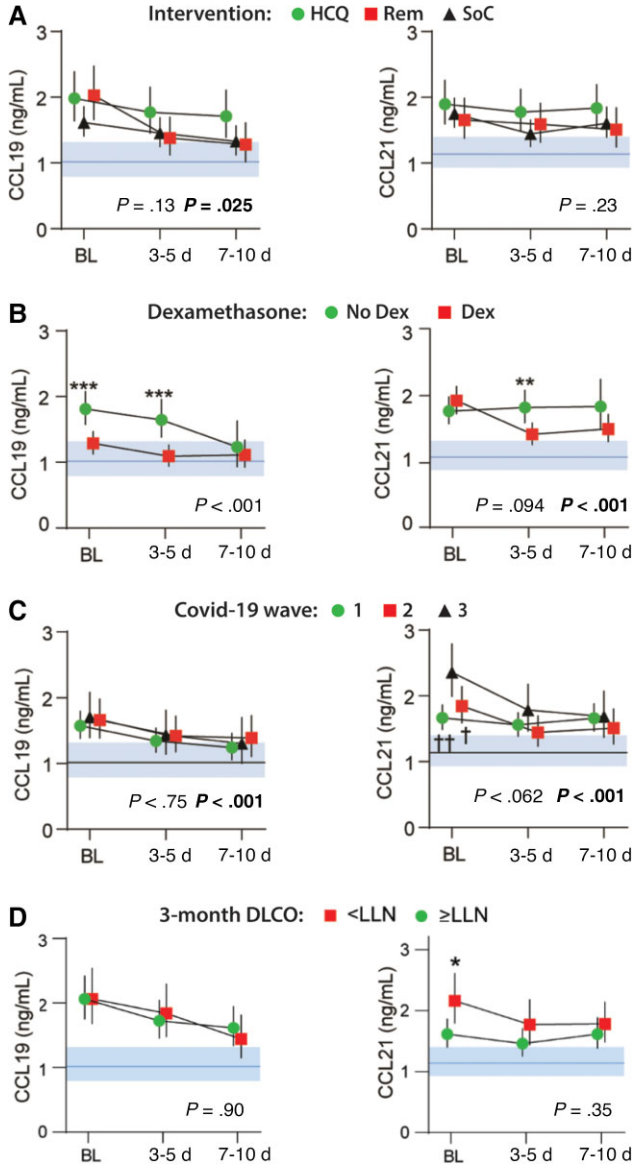

Figure 4. Intrahospital temporal profile of CCL19 and CCL21 according to (*A*) treatment with hydroxychloroquine (n = 43) and remdesivir (n = 38) as compared with their respective SoC (n = 81) in cohort 1 (NOR Solidarity trial); (*B*) dexamethasone treatment; (*C*) COVID-19 wave; and (*D*) DLCO below or above LLN at 3-month follow-up. Data is shown as estimated marginal means and 95% CI. The *P* value indicates group effect, and the bold *P* value indicates the interaction term between time and group from the linear mixed models with subject as random effect, and time and mortality as fixed effects (also as interaction) in addition to age, sex, and estimated glomerular filtration rate. Shaded areas show reference value range from healthy controls. **P* < .01, ****P* < .001 between groups; †*P* < .05, ††*P* < .01 versus wave 3. Abbreviations: DLCO, diffusing capacity of the lungs for carbon monoxide; LLN, lower limit of normal; HCQ, hydroxychloroquine; REM, remdesivir; SoC, standard of care.

A High Admission Level of CCL21 Is Associated With Impaired Lung Function After 3 Months

At 3-month follow-up, CCL19 and CCL21 levels in the 257 patients assayed were comparable to those of healthy controls (Supplementary Figure 1). However, for the 90 patients who performed pulmonary testing, a high baseline level of CCL21 during hospitalization was associated with impaired DLCO at 3-month follow-up (Figure 4*D*).

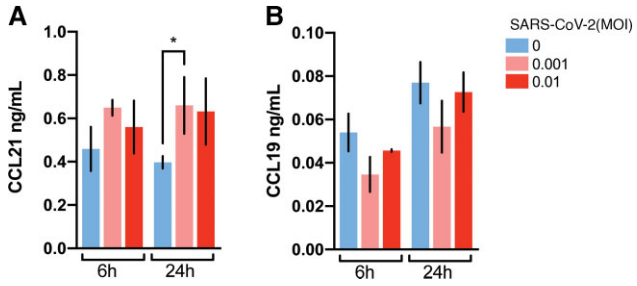

Figure 5. In vitro secretion of homeostatic chemokines in SARS-CoV-2–exposed monocyte-derived dendritic cells. Quantitation of secreted CCL21 (*A*) and CCL19 (*B*) in cultures of monocyte-derived dendritic cells after exposure to inactivated SARS-CoV-2 viral particles (0.001 or 0.01 multiplicity of infection [MOI]) for 6 and 24 hours. Results are shown as mean ± SD (n = 3 per treatment condition). **P* < .05, independent samples *t* test.

Effect of Inactivated SARS-CoV-2 on the Release of CCL21 and CCL19 in Dendritic Cells

Previous data have suggested that SARS-CoV-2 may trigger immunomodulatory signaling responses in monocyte-derived cells [25]. To determine whether the presence of SARS-CoV-2 particles themselves could induce secretion of homoeostatic chemokines, in vitro differentiated monocyte-derived dendritic cells (moDC) were cultured in the presence of inactivated SARS-CoV-2 virus. Whereas there was a significant increase in the release of CCL21 in the presence of viral particles (Figure 5*A*), CCL19 secretion from moDC was low and was not significantly affected by the presence of SARS-CoV-2 (Figure 5*B*).

Reanalysis of CCR7/CCL19/CCL21 mRNA Expression From Public RNAseq Data Repositories

To obtain further insight into the regulation of homeostatic chemokines in COVID-19, we reanalyzed the expression of CCL19, CCL21, CCR7, and CCR10 in lung tissue and peripheral blood specimens isolated from COVID-19 patients in RNAseq datasets deposited in public repositories (Supplementary Table 1).

In the first autopsy study (L1) [26], CCL19 and CCL21 mRNA expression levels were modestly increased in lung tissue from COVID-19 patients compared to healthy controls, with particularly high CCL21 expression levels in biopsies containing arterial tissue (Figure 6*A*). The second autopsy study (L2) [27] showed higher CCL21 mRNA levels in the lungs of patients with high SARS CoV-2 viral load within the lung parenchyma, with lower expression of CCR7 and CCR10 in this subset (Figure 6*B*). A third autopsy study (L3) detected no differences in CCR7 and CCL21 expression levels compared to healthy lungs [28].

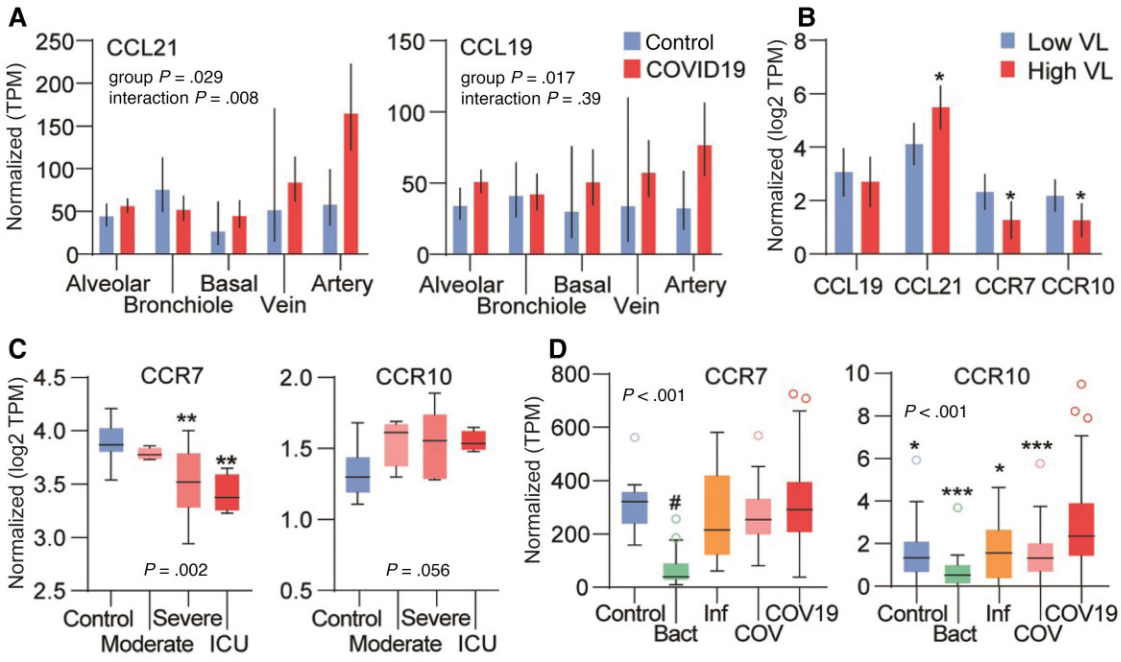

Figure 6. Regulation of CCR7, CCR10, CCL19, and CCL21 in public RNAseq analysis data of tissues from COVID-19 patients. *A*, Differences in CCL19 and CCL21 mRNA expression in lung tissue from COVID-19 patients (n = 19) and controls (n = 3). Source data GSE163529. *B*, mRNA expression in relation to virus load (VL) in COVID-19 patients (GSE150316, n = 15). *C*, mRNA expression of CCR7 and CCR10 in peripheral blood mononuclear cells isolated from COVID-19 patients (n = 16) grouped by clinical disease severity (moderate, severe disease, and requiring ICU treatment) and age-/sex-matched healthy controls. Source dataset GSE152418. *D*, Whole blood leukocytes isolated from patients with COVID-19 (COV19), seasonal coronavirus infection (COV; n = 19), influenza (Inf; n = 17), bacterial pneumonia (Bact; n = 20) and matched healthy controls (n = 19). Source dataset GSE161731. Normalized gene expression quantified as transcripts per million (TPM). *A* and *B*, *P* values are from the group and group*tissue location effects from the mixed model analysis (see description of statistics in Supplementary Material). *C* and *D*, *P* values are from the Kruskal-Wallis test with asterisks reflecting the results of the post hoc test. $*P < .05$, $**P < .01$, $***P < .001$; $\#P < .01$ versus other groups.

In studies comparing mRNA expression in PBMCs and whole blood isolated from patients and healthy controls, CCL19 and CCL21 were not detectable or showed very low mRNA counts (Supplementary Table 1, studies PB1-3, WB1–5). CCR7 in PBMCs was lower, while CCR10 tended to be higher in COVID-19 patients compared to controls in study PB1 (Figure 6*C*) [29]. In another PBMC study (PB2) [30], there was no change in CCR7 expression, while CCR10 was increased in COVID-19 (Supplementary Table 1). In the third PBMC study (PB3) [31] CCR7 expression levels were higher at recovery compared to earlier stages of the disease (Supplementary Table 1). In leukocytes from whole blood, CCR7 was lower in COVID-19 patients compared to controls in one study (WB1) [32] but showed no signs of differential regulation in 4 other studies (WB2–5) (Figure 6*D*) [33–36]. Two studies (WB3, WB5) [34, 36] reported increased CCR10 levels in COVID-19 patients compared to healthy controls, also compared to patients infected with seasonal coronavirus or with bacterial pneumonia (Figure 6*D*).

DISCUSSION

Combining data from 2 independent Norwegian multicenter cohorts, we here report high circulating levels of CCL19 and CCL21 on hospital admission and during the in-hospital course of 414 patients with COVID-19, with similar results within each cohort. High levels of both chemokines were associated with adverse outcomes, that is, the degree of RF, need for ICU support, and 60-day all-cause mortality. Finally, high CCL21 on admission correlated with persistent impairment of pulmonary function at 3-month follow-up. Our findings suggest the homeostatic chemokines, particularly CCL21, could be involved in the pathogenesis of COVID-19–related pulmonary pathology and might provide independent prognostic information in hospitalized COVID-19 patients.

High levels of inflammatory cytokines and chemokines and associations with poor outcomes have consistently been reported in hospitalized COVID-19 patients [37], but data on the homeostatic chemokines are scarce. In agreement with our findings, CCL21 was upregulated in patients with thrombotic complications and ranked third as a predictor of mortality amongst 71 cytokines/chemokines assayed [3]. Elevated CCL19 and CCL21 levels in COVID-19 patients could reflect several scenarios: (1) a general increase in homeostatic chemokine secretion in spleen/secondary lymphoid tissue, possibly accentuated by ongoing systemic inflammation or induced by viral antigens; (2) impaired chemokine clearance due to

decreased turnover, for example, via CCR10 that has been found upregulated in PBMCs in COVID-19 patients [29, 30]; or (3) increased/ectopic secretion within nonlymphoid tissue (eg, lung). The lack of expression of CCL19 and CCL21 in PBMCs points to affected organs or associated lymphoid tissue as likely sources of increased circulating levels in our COVID-19 cohorts. Supporting a link between pulmonary CCL21 production and impaired lung function, a recent preprint report demonstrated the formation of perivascular foci with strong expression of CCL21 in lung tissue from patients with severe COVID-19 [38]. These areas showed high expression of fibrosis-associated markers, and accumulation of immune cell aggregates with features consistent with tertiary lymphoid structures. CCL19 expression was also elevated in these areas [38].

In agreement with a report showing an early rise in CCL21 [3], peak levels of CCL19 and CCL21 in our study were observed in samples collected within the first 48 hours of hospital admission, suggesting early perturbations in homeostatic chemokines during infection, possibly related to delayed viral clearance or high viral load. Interestingly, in the patients who died within 60 days, the largest difference in CCL21 levels compared with those who survived was seen at the last blood sample taken 7 to 10 days after admission, indicating persistent and increasing CCL21 activation in these patients. Reanalysis of publicly available gene expression datasets revealed indications of differential regulation of CCL21 expression in pulmonary tissue, while CCR7 and CCR10 receptor levels appeared unchanged. In the material from Desai et al [27], higher CCL21 mRNA levels were seen in lungs of patients with high SARS CoV-2 viral load within the lung parenchyma. While we in the present study found no correlation between circulating CCL19 and CCL21 levels and SARS-CoV-2 viral load in oropharyngeal/nasal samples at hospital admission, the upper airway viral load does not necessarily reflect levels of ongoing viral shedding within the lung parenchyma, given considerable topographical differences in viral shedding [39]. Hence, the possibility of a direct role of SARS-CoV-2 in eliciting CCL19 and CCL21 signaling remains unresolved.

Strong, systemic immune responses manifested by increased inflammatory cytokine and chemokine levels are commonly observed in severe cases of acute viral diseases. In severe dengue fever, a secreted form of the viral nonstructural antigen NS1 potentially triggers key aspects of the pathogenesis of severe disease manifestations (dengue hemorrhagic fever) [40]. By triggering leukocyte inflammatory responses and interfering with the integrity of endothelial glycocalyx, NS1 released by infected cells may explain both the endothelial dysfunction and the systemic inflammatory response observed in severe dengue [41]. Understanding of the impact of SARS-CoV-2 antigens on host cells is still evolving, but in vitro studies have identified a multitude of interactions of potential relevance to clinical disease manifestations [42], including disruption of endothelial barrier function [43] and aberrations in immune signaling [44, 45]. Conceivably, persistence or high levels of particular viral antigens in affected tissues could directly impact the pathophysiology of severe disease manifestation in COVID-19. Of particular relevance to the present work, the ORF7a protein of SARS-CoV-2 has been reported to induce the expression of CCL19 and CCL21 in HeLa cells [17]. In our experiments, PBMC-derived DCs exposed to inactivated SARS-CoV-2 viral particles produced significant amounts of CCL21 but not CCL19. Transcriptional changes involving alterations in cytokine release have been observed in monocytes/macrophages exposed to SARS-CoV-2 particles [25]. Although the physiological relevance of these findings remains uncertain, the SARS-CoV-2 virus may trigger distinct inflammatory signaling in monocyte-derived cells that potentially could impact the ensuing immune response. Moreover, our findings may suggest that CCL21 could be directly induced by SARS-CoV-2 and not only be a secondary phenomenon to a general state of systemic inflammation, potentially contributing to the formation of lymphoid and inflammatory tissue within the lungs.

Current knowledge about the direct impact of dexamethasone treatment on the CCR7/CCL19/CCL21 axis is limited. Herein we found that dexamethasone use within cohort 2 was associated with lower levels of CCL19 and CCL21 as compared with nonusers. However, the study was not designed to evaluate the effects of dexamethasone on these markers, and the data should be interpreted with caution. Regarding hydroxychloroquine/remdesivir, we found no effects on CCL19/CCL21 levels. There are no published data to suggest a direct impact of these agents on homeostatic chemokine signaling, although such effects cannot be excluded. It is possible that the inclusion of more targeted immunomodulatory agents in COVID-19 treatment will provide more clarity regarding CCL19/CCL21 regulation. Levels of these chemokines have, to the best of our knowledge, not been reported in published results from clinical trials. The Janus kinase (JAK) pathway inhibitor baricitinib has been approved for use in patients hospitalized with severe COVID-19. Other JAK inhibitors have been shown to inhibit CCR7/CCL19-mediated migration of DCs [46, 47]. It would therefore be of interest to examine if JAK inhibitors such as baricitinib affect the levels and/or function of CCL19/CCL21 in COVID-19.

The present study has some limitations. As mentioned above, the present study was not designed to evaluate the effects of therapeutics used in COVID-19 management, and the impact of such interventions on chemokine levels therefore cannot be excluded. The regulation of secretion of CCL19 and CCL21 under inflammatory conditions is largely unknown. Moreover, the cellular source(s) of CCl19/CCL21 in severe COVID-19 are still uncertain.

In summary, we report a striking increase in the circulating levels of the homeostatic chemokines CCL19 and CCL21 in hospitalized COVID-19 patients, with high levels correlating with progression to severe pulmonary disease, need for ICU support, and 60-day mortality. Furthermore, high admission levels of CCL21 were associated with prolonged impaired pulmonary function 3 months after hospital discharge. Given the key role of these chemokines in lymphoid tissue homeostasis and regulation of adaptive immune responses, potentially promoting lymphoid tissue within the SARS-CoV-2 infected lungs, these findings warrant further investigations to determine the drivers, source, and functional impact of increased levels of CCL19 and CCL21 in COVID-19.

Supplementary Data

Supplementary materials are available at *The Journal of Infectious Diseases* online (http://jid.oxfordjournals.org/). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

Notes

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Author contributions. T. U., T. B. D., B. H., S. L. M., P. A., and A. T. were responsible for the study conception and execution of the present substudy. A. B. D., A. M. D. R., K. N. H., A. M., A. K. F., P. A., and M. T. were responsible for the management, coordination, research activity planning, and execution of the NOR-Solidarity trial. J. C. H., A. M. D. R., L. H., A. B. K., A. T., A. L., K. T., A. R. H., K. E. M., S. G. D., B. F., F. M., and S. B. were responsible for the management, coordination, research activity, planning, and execution of the Norwegian SARS-CoV-2 study. T. V. L. and O. H. S. were responsible for the 3-month follow-up protocol for pulmonary function testing. A. M. D. R., T. B. D., A. B. D., B. H., P. A., B. K. G., and J. C. H. coordinated the collection and storage of the biobank material. T. B. D., T. U., A. E. M., M. K., A. L., S. L. M., and B. H. were responsible for the biochemical analyses and in vitro studies. All authors revised and approved the final version of the manuscript.

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Data availability. Anonymized patient-level data for participants in this study, statistical analysis plan, and statistical coding can be made available after the approval of the institutional review board. Requests should be made to the corresponding author.

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