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# In depth analysis of patients with severe SARS-CoV-2 in sub-Saharan Africa demonstrates distinct clinical and immunological profiles

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## Abstract

Although the COVID-19 pandemic has left no country untouched there has been limited research to understand clinical and immunological responses in African populations. Here we comprehensively characterise patients hospitalised with suspected or confirmed COVID-19, and healthy community controls. PCR-confirmed COVID-19 participants were more likely to receive dexamethasone and a beta-lactam antibiotic, and survive to hospital discharge than PCR-/IgG+ and PCR-/IgG- participants. PCR-/IgG+ participants exhibited a nasal and systemic cytokine signature analogous to PCR-confirmed COVID-19 participants, but increased propensity for *Staphylococcus aureus* and *Streptococcus pneumoniae* colonisation. We did not find evidence that HIV co-infection in COVID-19 participants was associated with mortality or altered cytokine responses. The nasal immune signature in PCR-/IgG+ and PCR-confirmed COVID-19 participants was distinct and predominated by chemokines and neutrophils. In addition, PCR-/IgG+ individuals with high COVID-19 clinical suspicion had inflammatory profiles analogous to PCR-confirmed disease and potentially represent a target population for COVID-19 treatment strategies.

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## Introduction

The coronavirus disease 2019 (COVID-19) pandemic has, to date, been less severe in sub-Saharan Africa compared to Europe and the Americas (1). However, clinical diagnosis, triage and treatment decisions for COVID-19 patients is particularly challenging in resource-poor settings. The increased transmissibility of new severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) variants observed in the second wave of the pandemic has made the need to address these challenges even more urgent (2). Limited access to advanced life-preserving therapies such as mechanical ventilation, and delayed presentation to hospital is common (3). This increases clinical and diagnostic complexity as patients may present when severely unwell, and potentially, without polymerase chain reaction (PCR)-detectable SARS-CoV-2 (4).

Cytokine dysregulation has been consistently observed in COVID-19 patients in multiple settings (5, 6). Consequently, cytokines, including IL-6 and TNF-$\alpha$, have shown potential prognostic value to guide decisions on clinical management of COVID-19 (7, 8) but they lack adequate specificity. For example, IL-6 levels may be 10- to 200- fold higher for patients with the hyperinflammatory phenotype of acute respiratory distress syndrome (ARDS) compared to patients with severe COVID-19 (9). Beyond the use of single markers, systems approaches have demonstrated immune signatures distinct to COVID-19 aligned with disease severity (10-12). They have identified a systemic immune signature showing profoundly altered T-cells, selective cytokine/chemokine upregulation (10) and monocyte and neutrophil activation in hospitalised COVID-19 patients (11, 12). Further, inflammatory dysregulated IL-2, IL-6, IL-10 and IL-15 are associated with COVID-19 related mortality (13). However, there are very few studies that have investigated the respiratory tract, the initial site of infection and disease pathogenesis.

SARS-CoV-2 infection is established when the virus binds to angiotensin-converting enzyme 2 (ACE2)-expressing epithelial cells in the nasal mucosa (14). The few studies reporting mucosal cellular or cytokine responses in COVID-19 have shown cytokine dysregulation and immune cell disruption in the lower airway, correlating with disease severity (15, 16). However, there is a paucity of information on immune responses against SARS-CoV-2 infection in the upper airway (17). Minimally invasive sampling techniques of the nasal mucosa are well-tolerated, and highly useful in human challenge models of respiratory syncytial virus and *Streptococcus pneumoniae* (18, 19). Given importance of the nose in SARS-CoV-2 infection (20), understanding the host–viral interaction at the nasal mucosa could provide additional insights to understand and potentially modulate COVID-19 prognosis.

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Immunological responses against SARS-CoV-2 infection at the nasal mucosa are still poorly understood. Here, we analysed nasal mucosa and peripheral blood samples from a comprehensively characterised cohort of patients admitted to hospital with suspected and/or confirmed COVID-19. These patients were compared with adult healthy community controls. Immunological parameters were studied using 38-multiplex cytokine assays and flow cytometry, while profiling of respiratory pathogens was done using a 33-multiplex polymerase chain reaction (PCR) respiratory pathogen diagnostic panel. Our study provides insights on the cytokine responses in the nasal mucosa following SARS-CoV-2 infection in severe COVID-19 patients and the potential importance of additional confirmatory antibody tests for diagnosis of SARS-CoV-2 PCR-negative patients with high clinical suspicion of COVID-19.

## Results

### Clinical Overview

Between 21st April 2020 and 25th September 2020, 87 patients (median age 47 years, IQR: 34-62) presenting with severe acute respiratory infection (SARI) were recruited, of whom 60 (69.0%) were male (Table 1). SARS-CoV-2 was confirmed by nucleic acid amplification (NAAT) in 41 participants (classified as 'PCR-confirmed COVID-19") (Figure 1a). The remaining 46 participants with suspected disease were NAAT negative (Figure 1a). Of these, 25 were IgG positive against spike protein 2 and nucleoprotein on serological testing. Using nucleic acid amplification (NAAT) and antibody test results, we reclassified the patient groups into PCR-confirmed COVID-19, PCR-/IgG+ SARI (n=25) and PCR-/IgG- SARI (n=21) participants (Figure 1b). In addition, we recruited 25 ambulatory healthy volunteers. Patient demographics and clinical characteristics are reported in Table 1. Hospitalised individuals with PCR-confirmed COVID-19 were significantly older (median 50 [PCR+] vs. 34 [PCR-/IgG+] vs. 41 [PCR-/IgG-], p=0.017), more likely to have received dexamethasone (63% vs. 4% vs. 0%, p<0.001) and more likely to have received a beta-lactam antibiotic (78% vs. 52% vs.48%, p=0.022) compared to the PCR-/IgG+ and PCR-/IgG- SARI participants. Additionally, PCR-confirmed COVID-19 participants were more likely to survive (93% vs. 80% vs. 57%, p=0.004) with increased hospital length of stay (median days 8 vs. 6 vs. 6, p=0.028) compared to the PCR-/IgG+ and PCR-/IgG- SARI participants. PCR-/IgG+ SARI participants also tended toward longer times from symptom onset to hospital admission compared to PCR-confirmed COVID-19 participants (median 6 vs. 4 days) (Table 1).

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## Distinct cytokine responses induced by SARS-CoV-2 infection in nasal mucosa and systemic circulation

We investigated the cytokine response in nasal lining fluid and serum of our four participant groups. Our analysis was limited to 25 PCR-confirmed COVID-19 participants (who were IgG positive), 16 PCR-/IgG+ SARI participants, 11 PCR-/IgG- SARI participants and 25 healthy controls, from whom we had paired nasal lining fluid and serum samples. We measured the concentration of 37 cytokines (and sCD40L) in paired nasal lining fluid and serum samples of the four study groups (Figure S2 and S3). We found altered cytokine levels in both nasal lining fluid and serum of PCR-confirmed COVID-19 participants, PCR-/IgG+ SARI participants and PCR-/IgG- SARI participants, relative to healthy controls (Figure 2a). Specifically, high concentrations of inflammation-related cytokines were common among all the patient groups compared to healthy controls, including IL-1$\alpha$, IL-1$\beta$, IL-6, MIP-1$\alpha$ and TNF-$\alpha$ in nasal lining fluid, and raised levels of IL-6, IL-10, IP-10 and IL-15 in serum (Figure 2b).

In comparison to PCR-confirmed COVID-19 (nasal, n=7; serum, n=5) and PCR-/IgG+ SARI participants (nasal, n=8; serum, n=8), PCR-/IgG- SARI participants (nasal, n=11; serum, n=14) had an increased number of cytokines with higher concentrations than healthy controls in nasal lining fluid and serum (Figure 2a-b). In comparison to PCR-/IgG- SARI participants (nasal, n=0; serum, n=2), PCR-confirmed COVID-19 (nasal, n=9; serum, n=5) and PCR-/IgG+ SARI participants (nasal, n=5; serum, n=8) had an increased number of cytokines with lower concentrations than healthy controls in nasal lining fluid and serum (Figure 2a-b). Relative to healthy controls, low levels of IL-4, IL-1RA, GRO and VEGF, and high levels of IL-2 in nasal lining fluid, were confined to PCR-confirmed COVID-19 and PCR-/IgG+ SARI participants (Figure 2b). While in serum, low levels of EGF, Flt-3L, MDC and IL-12p70 in serum were distinctive to PCR-confirmed COVID-19 and PCR-/IgG+ SARI participants (Figure 2b). In addition, IL-3 levels were distinctively high in nasal lining fluid and serum of PCR-confirmed COVID-19 and PCR-/IgG+ SARI participants, relative to healthy controls (Figure 2a, S3 and S4). The results demonstrate SARS-CoV-2 infection induces a distinct cytokine response in the nasal mucosa compared to systemic circulation. This also suggests that PCR-confirmed COVID-19 and PCR-/IgG+ SARI participants may be presenting at different stages of the SARS-CoV-2 infection spectrum.

## Severe COVID-19 is associated with a distinct nasal and serum cytokine profile to non-COVID-19 SARIs

Due to the similarity of the cytokines induced in PCR-/IgG+ SARI participants relative to PCR-confirmed COVID-19 (Figure 2a-b), coupled with clinical manifestations of COVID-19 (Table 1), and

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the positive SARS-CoV-2 IgG serology result (Figure 2b), we explored if clinical reclassification of PCR-/IgG+ SARI participants as COVID-19 disease was warranted. We hypothesised that using an unsupervised analysis the immunological phenotype of PCR-/IgG+ SARI participants would be analogous to PCR-confirmed COVID-19 participants, but distinct from PCR-/IgG- SARI participants and healthy controls. First, we performed a principal-component analysis (PCA) of all the analytes for the patients and controls in either nasal lining fluid and serum. In both compartments, PCA segregated together PCR-confirmed COVID-19 participants and PCR-/IgG+ SARI participants, away from healthy controls, whereas PCR-/IgG- SARI participants appeared as a heterogeneous group (Figure 3a and Figure 3b). Second, we analysed the active cytokine functional families in nasal and serum, to ascertain whether PCR-/IgG+ SARI participants had immunological pathways similar to PCR-confirmed COVID-19 participants. Analysis of the active cytokine functional families has previously been used to define immune signatures unique to COVID-19 (10-12). To aid interpretation of the data, the cytokines were divided into functional groups including growth factors, chemokines, adaptive, pro-inflammatory and anti-inflammatory. In both nasal lining fluid and serum, differential interaction of cytokines was observed among the study groups, with the cytokine interaction profile being generally similar between PCR-/IgG+ SARI participants and PCR-confirmed COVID-19 participants, but distinct from PCR-/IgG- SARI participants and healthy controls (Figure 3c and Figure 3d). Specifically, in the nasal lining fluid, we observed a strong interaction in the chemokine family, including between chemokines and growth factors, in PCR-confirmed COVID-19 participants and PCR-/IgG+ SARI participants (Figure 3c). In the serum, fewer interactions were observed, but still a distinct pattern in the adaptive cytokine family in PCR-confirmed COVID-19 participants and PCR-/IgG+ SARI participants (Figure 3d). Third, in a subset of participants, we explored the nasal immune cellular profile in PCR-confirmed COVID-19 and PCR-/IgG+ SARI participants compared to healthy controls. We observed a higher frequency of neutrophils and lower frequency of $CD3^+$ T cells in the nasal mucosa of PCR-confirmed COVID-19 and PCR-/IgG+ SARI participants compared to healthy controls (Figure 4). Collectively, the findings demonstrate that the PCR-/IgG+ SARI participants induced similar immunological pathways to PCR-confirmed COVID-19 participants but that this was distinct from PCR-/IgG- SARI participants. Taken together, these findings suggest that clinical reclassification of COVID-19 status could be warranted.

### PCR-confirmed COVID-19 participants exhibit reduced propensity for bacterial colonisation

Due to the differential clinical management pathways experienced by the PCR-confirmed COVID-19 and PCR-/IgG+ SARI participants in the study, including beta-lactam antibiotic usage and steroid treatment, we sought to determine the presence of other respiratory pathogens in NP/throat swabs.

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Bacterial co-infection with SARS-CoV-2 is associated with adverse outcomes (21, 22). A total of 80 participants from the patient groups (from whom we had complete clinical and antibody data) were tested for presence of respiratory pathogens using the fast-track diagnostics (FTD)-33 respiratory panel. The median time from admission to NP/throat swab collection for the respiratory panel testing was three days (IQR 1-5). Using the FTD-33 panel we identified 12 other respiratory pathogens present in our patient population, in addition to SARS-CoV-2 and HIV (Figure 4a). Overall, 74% (59/80) of participants had one or more pathogen(s) (Figure 5a). Presence of bacterial pathogens was common, identified in 74% (59/80), but viral pathogens (excluding HIV) was only present in 14% (11/80) of participants (Figure 5a and S4a). The most common respiratory pathogen across all study groups was *Klebsiella pneumoniae* (Figure S4b). A total of 27/44 of the participants with a *K. pneumoniae* had received ceftriaxone or amoxicillin within 24 hours of admission, suggesting that this was likely a hospital-acquired multidrug resistant (MDR) *K. pneumoniae.* MDR *K. pneumoniae is* known to be endemic at Queen Elizabeth Central Hospital (23). Furthermore, we observed differences between PCR-confirmed COVID-19 and PCR-/IgG+ SARI participants. The PCR-confirmed COVID-19 participants had lower prevalence rates of *Staphylococcus aureus* (3/38 [8%] vs. 6/24 [25%], p=0.018) and tended toward lower *Streptococcus pneumoniae* (8/38 [21%] vs. 10/24 [42%], p=0.071) than PCR-/IgG+ SARI participants (Figure 5b-c). These findings show that presence of bacterial pathogens in the upper respiratory tract was very common in our hospitalised patient cohort. They also show that PCR-confirmed COVID-19 patients had lower proclivity for *S. aureus* and *S. pneumoniae* colonisation than PCR-/IgG+ SARI participants, potentially impacted by increased use of beta-lactam antibiotic usage in this study group.

## Association of HIV co-infection with cytokine responses, respiratory bacterial prevalence and clinical outcomes in COVID-19 patients

In our cohort, people living with HIV were distributed across the patient groups (Table 1). We therefore explored the influence of HIV co-infection on the cytokine responses, presence of respiratory pathogens, and mortality. We reclassified the PCR-/IgG+ SARI participants as COVID-19 and combined them with the PCR-confirmed COVID-19 to form a single COVID-19 patient group. There were no statistically significant differences in the levels of cytokines in nasal lining fluid and serum between HIV-infected and HIV-uninfected COVID-19 participants (Figure 6a-b). Furthermore, we did not observe increased frequency of respiratory pathogens in NP/throat swab nor higher mortality (Fisher's exact test, p=0.277) in HIV-infected compared to HIV-uninfected COVID-19 participants (Figure 5b-c and S4b). Together, in our cohort, we did not find evidence suggestive that

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HIV co-infection in COVID-19 participants was associated with altered cytokine responses, increased prevalence of respiratory pathogens nor increased mortality.

## Discussion

We provide the first clinical and immunological analysis to our knowledge of suspected or confirmed COVID-19 patients admitted to hospital in a low-income sub-Saharan African country. We identified individuals with manifestations of COVID-19 who were SARS-CoV-2 RT-qPCR negative but IgG positive that showed immunological profile analogous to PCR-confirmed COVID-19 participants. This was distinct from PCR-/IgG- SARI participants and healthy controls. The PCR-/IgG+ SARI subgroup experienced poorer clinical outcomes compared to PCR-confirmed participants, potentially due to later hospital presentation or failure to access effective treatment for COVID-19. In our cohort, access to drugs such as dexamethasone in patients suspected of COVID-19 was determined by RT-qPCR test positivity and not SARS-CoV-2 IgG antibody status. Identification of this subgroup of SARI patients highlights the importance for further optimisation of triage and clinical treatment pathways in the era of COVID-19, especially in low-resource settings.

Low cost, effective and pragmatic interventions such as dexamethasone have been widely adopted and incorporated into treatment pathways (24). The COVID-19 treatment guidelines recommend against dexamethasone administration for patients who do not require supplemental oxygen (25) but there are no specific recommendations for how COVID-19 should be diagnosed. The RECOVERY dexamethasone trial (24) that showed efficacy in hospitalised COVID-19 patients, included patients with “clinically suspected” or laboratory confirmed SARS-CoV-2 infection. The PCR-/IgG+ SARI subgroup could potentially fit into this “clinically suspected” COVID-19 classification. Empirical antibiotics are recommended for severe COVID-19 patients if co-existing bacterial pneumonia cannot be excluded (26). Consistent with this recommendation, there was increased beta-lactam antibiotic usage in PCR-confirmed COVID-19 participants who demonstrated a lower prevalence of *S. pneumoniae* and *S. aureus* colonisation. The PCR-/IgG+ participants did not access these standardised clinical management strategies and may be at a disadvantage compared to PCR-confirmed patients, with whom they share analogous immunological profiles, potentially putting the patients at increased risk of poor prognosis. Well conducted interventional trials are required to determine if this important subgroup could benefit from diagnostic reclassification and treatment as COVID-19.

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Cytokine dysregulation is a hallmark of severe COVID-19 (27). Consistent with published studies (5, 6), we observed high concentrations of inflammation-associated cytokines, including IL-6, TNF-α, IP-10, IL-10, IL-1α and IL-1β in serum and nasal lining fluid from PCR-confirmed COVID-19 patients. However, elevation of these cytokines was also observed in serum from non-COVID SARI participants. Instead, the induction of the chemokine family in the nasal mucosa was distinctive to severe COVID-19 and was distinct from systemic circulation. The predominance of the chemokine family was in line with the infiltration of neutrophils in the upper airway observed in this cohort and others (28, 29). A study that performed transcriptomic analysis on paired upper and lower respiratory tract samples from two COVID-19 patients and showed very high congruency in the majority of the cell types between the sites (28), suggesting a shared immune response between the sites. Consistent with this suggestion, the chemokine-dominated signature and neutrophil infiltration observed in the upper airway of severe COVID-19 patients in our study was similar to that reported in the lower airway (15, 16, 30). Neutrophils promote inflammation and play a pathogenic role in COVID-19 (31). Circulating neutrophils from severe COVID-19 patients show exaggerated oxidative burst, NETosis and phagocytosis relative to healthy controls (32). Measurement of dysregulated neutrophil function in the nose was not possible in this study, but presence of NETs has been reported in the lungs of deceased severe COVID-19 patients with acute respiratory distress syndrome (ARDS) (33), where they are thought to drive severe pulmonary complications of COVID-19. In ARDS patients, low levels of VEGF in the lower airway are a marker of acute lung injury (34), and in our study, severe COVID-19 patients exhibited distinctively low concentrations of VEGF in nasal lining fluid. Collectively, this suggests that the nasal mucosa could provide a snapshot of immunological activity in the lung in patients with COVID-19. This warrants further investigation as nasal sampling is well tolerated and more easily accessible than lower airway sampling.

Some of the major features of severe COVID-19 are lymphopenia and neutrophilia in the systemic circulation (35, 36). As such, the neutrophil to lymphocyte ratio has been shown to have prognostic value, predicting those at high risk of severe disease or death (37). However, the mechanisms behind lymphopenia and neutrophilia during COVID-19 are still not well understood. Interestingly, in our study, concentrations of IL-3 and Flt-3L were distinctively altered in severe COVID-19 participants compared to non-COVID-19 SARI participants. Specifically, in severe COVID-19 participants, IL-3 was detected at very high levels in the nasal lining fluid, while Flt-3L was repressed in serum. IL-3 and Flt-3L are key cytokines involved in haematopoiesis of leukocytes (38, 39). IL-3 induces expansion and generation of myeloid cells (38, 39), while Flt-3L binds to Flt-3 and activates common lymphoid progenitor cells to increase the number of lymphocytes (40, 41). Alterations in these cytokines in

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mouse models are associated with dysregulated haematopoiesis and altered leukocyte cellularity (39, 41). Therefore, it is plausible that high levels of IL-3 and repressed levels of Flt-3L could contribute to the high neutrophil to lymphocyte ratio and altered leukocyte numbers observed in severe COVID-19 patients.

Due to the high HIV prevalence in our setting, we were able to explore the impact of HIV co-infection on COVID-19. While data from the United Kingdom and South Africa suggests an increased risk of mortality in HIV-infected COVID-19 patients (42-44), a systematic review and data from the United States did not demonstrate this association (45-47). In our cohort, we did not find statistically significant differences in the cytokine responses, prevalence of respiratory pathogens nor mortality between HIV-infected and HIV-uninfected COVID-19 participants. The small sample size of this study limits our ability to adjust for the multiple potential confounders that could impact on clinical outcomes, including age, gender, co-morbidities, antiretroviral therapy status and HIV viral load. Larger cohort studies with comprehensive immunological data and sufficient power to adjust for multimorbid diseases are required to provide further clarity on this issue.

Despite the strengths of this study including use of an internationally recognised protocol with standardised data collection tools, well-characterised clinical cohort and paired nasal and systemic immune responses, our study had some limitations. Due to programmatic constraints, it was not feasible to conduct longitudinal sampling among our participants to monitor recovery and response to therapy. In addition, whilst the majority of participants were recruited within the first 72 hours of admission, a proportion of our participants were recruited later in their hospital admission. Lack of critical care facilities precluded universal recruitment and sampling in the most severe cases, and our patient population may therefore not be entirely representative of all participants with SARS-CoV-2 induced SARI. Furthermore, we cannot exclude secondary bacterial infections as potential contributors to increased mortality in the PCR-/IgG+ SARI participants, as we did not have access to blood culture or autopsy results.

We have demonstrated that SARS-CoV-2 infection induces a chemokine and neutrophil-dominated profile in the nasal mucosa different from systemic circulation, and distinct from non-COVID-19 SARI. We have identified a subgroup of SARS-CoV-2 PCR-negative IgG positive individuals with clinical and immunological manifestations of COVID-19, who may benefit from standardised COVID-19 clinical management protocols (including use of steroids and beta-lactam antibiotics). Further, operationalisation of sensitive and specific antibody assays for SARS-CoV-2 IgG detection to support

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clinical diagnosis needs to be considered in resource-limited settings. We recommend that interventional trials should target this clinically important subgroup of patients to determine if treatment pathways applied for PCR-confirmed COVID-19 can be implemented safely for PCR-/IgG+ SARI patients to improve clinical outcomes.

## Methods

### Study design and recruitment

We prospectively recruited patients using the tier one sampling strategy from the International Severe Acute Respiratory and Emerging Infection Consortium (ISARIC) Clinical Characterisation Protocol (CCP) (48). Briefly, the CCP is a standardised protocol that enables data and biological samples to be collected rapidly in a globally harmonised manner for any severe respiratory infection of public health interest (48). Patients over 18 years old were approached for informed written consent if they met inclusion criteria: severe acute respiratory infection (SARI) with suspected or confirmed SARS-CoV-2. For patients who lacked capacity, assent was sought from a proxy as per our ethical approvals. Subsequently, informed consent was obtained retrospectively from these patients, where possible. Patients were excluded from recruitment if they or their proxy declined to participate. We aimed to recruit within 72hrs of hospital admission. Nasopharyngeal airway, nasosorption, nasal biopsy and peripheral blood samples were collected at the point of patient recruitment (Figure S1). Thereafter, patients were followed up until hospital discharge or death.

All participants were recruited at Queen Elizabeth Central Hospital (QECH), Blantyre, Malawi. QECH is a government hospital of 1000 beds, providing free inpatient medical care to the city of Blantyre (population 800,264), and tertiary care to those referred from the Southern region (population 7,750,629, 2018). Most adults present directly to the hospital or are referred from community healthcare facilities. After triage and testing in dedicated areas, patients with confirmed SARS-CoV-2 were treated in a cohort ward, including a high dependency area. Additionally, patients with SARI were screened for SARS-CoV-2 in two separate adult medical wards. Context-sensitive standard operating procedures were used to treat SARS-CoV-2 as detailed in a separate publication (49). Invasive mechanical ventilation, continuous positive airways pressure and high flow oxygen were not available for SARS-CoV-2 at this institution during this period.

During the period November 2019 to October 2020, we recruited healthy participants with no acute intercurrent or chronic illness as a healthy control group. All healthy participants, recruited immediately before (November 2019-March 2020) and after (September 2020-October 2020) the

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first peak in Malawi's reported COVID case count (Figure 1a), were confirmed as seronegative for HIV-infection and had no known medical conditions. We incorporated baseline samples for healthy participants who had volunteered to participate in another study in this analysis (50).

The two study protocols were approved by the Malawi National Health Science Research Committee (NHSRC, 20/02/2518 and 19/08/2246) and Liverpool School of Tropical Medicine (study sponsor) Research Ethics Committee (LSTM REC, 20/026 and 19/017). Patient and health participant samples were anonymised at the point of sample collection by the research nurses using unique participant identification barcodes. Study activities were monitored by the Malawi-Liverpool-Wellcome Trust Clinical Research programme (MLW)'s Clinical Research Support Unit and we complied with all relevant ethical regulations.

### SARS-CoV-2 PCR diagnostic testing

After collection, nasopharyngeal swabs in Universal Transport Medium (UTM) (Copan, Brescia, Italy) were stored at 7°C and processed for 2019-nCOV RNA testing within 48 hours, using the CDC 2019-nCoV RNA real time reverse transcriptase PCR diagnostic panel (Integrated DNA Technologies, Iowa, USA) or the Da An-RT-PCR reagent set for 2019-nCoV RNA detection (Da An Gene Co., Ltd of Sun Yat-Sen University, Guangdong, P.R. China). A cycle threshold (Ct) value of <40 was considered positive for both assays based on CDC and Da An guidelines. Both assays utilize an internal control to identify presence of human RNA (CDC - ribonuclease Protein, Da An internal control is not published). A negative extraction control and a PCR no-template control were also performed with every test.

### Respiratory fast track diagnostic panel

Aliquots of UTM were stored at -80°C and tested in batches using the FTD® Respiratory Pathogens 33 kit (Fast track Diagnostics Ltd., Luxembourg) as per the manufacturer's instructions. In brief, samples were extracted using the QIAamp UCP Pathogen Mini Kit (Qiagen Ltd., UK) for both DNA and RNA. Each sample was then tested using the RT-qPCR based FTD panel. This panel includes the following pathogens: parainfluenza viruses 1, 2, 3 and 4; human coronaviruses NL63, 229E, OC43 and HKU1; human metapneumoviruses A/B; human rhinovirus; human respiratory syncytial viruses A/B; human adenovirus; enterovirus; human parechovirus; human bocavirus; *Pneumocystis jirovecii; Mycoplasma pneumoniae; Chlamydophila pneumoniae; Streptococcus pneumoniae; Haemophilus influenzae* B; *Staphylococcus aureus; Moraxella catarrhalis; Bordetella* spp.; *Klebsiella pneumoniae; Legionella pneumophila/longbeachae; Salmonella* spp.; *Haemophilus influenzae* and internal control. Ct values <40 were considered positive.

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### SARS-CoV-2 IgG Enzyme Linked-Immunosorbent Assay

Peripheral blood collected in serum separation tubes underwent centrifugation at 500g for 8 mins to isolate serum. Serum was stored at -80°C. To measure SARS-CoV-2 antibodies, we used a commercial enzyme linked-immunosorbent assay (ELISA) targeting Spike (S2) and Nucleoprotein (NP) from SARS-CoV-2 (Omega diagnostics, UK; ODL 150/10; Lot #103183). The assay was performed as per the manufacturer's instructions. In brief, participant serum was diluted (1:200) in sample diluent (150mM Tris-buffered saline, pH 7.2 with antimicrobial agent). The diluted samples, diluent alone (negative control), manufacturer's cut-off control and positive control were added at 100µl per well to a plate pre-coated with S2 and NP. The plate was incubated at room temperature for 30 mins. After incubation, the plate was washed three times with a wash buffer (100mM Tris-buffered saline with detergent, pH 7.2) using a plate washer (Asys Atlantis, Biochrom Ltd, UK). 100µl anti-human IgG conjugated to horseradish peroxidase was then added to each well and incubated for 30 minutes at room temperature. After incubation, the plate was washed four times with a wash buffer, and 100µl of TMB (3,3',5,5'-Tetramethylbenzidine) Substrate (aqueous solution of TMB and hydrogen peroxide) was added. The plate was incubated for 10 minutes at room temperature, before addition of 100µl of Stop Solution (0.25M sulphuric acid). The optical density (OD) of each well was read at 450nm in a microplate reader (BioTek ELx808, UK) within 10 minutes of adding the Stop Solution. The ratio of OD in the test samples to the assay threshold control was calculated. The assay interpretation was as follows; positive result (ratio ≥1) and negative result (ratio <1).

### Flow cytometry analysis

For immunophenotyping, nasal cells were dislodged from curettes by pipetting and stained with an antibody cocktail containing anti-human CD3 APC, anti-human CD14 PE-Cy7, anti-human CD66b PE, anti-human CD19 Brilliant Violet 510, and anti-human CD45 Alexa Fluor 700 (all BioLegend, UK). Samples were acquired on an LSR FORTESSA flow cytometer (BD Biosciences, UK) and analyzed using Flowjo v10.5.3 (BD Biosciences, USA).

### Luminex analysis of nasal lining fluid

Cytokines were eluted from stored nasosorption filters (Mucosal Diagnostics, Hunt Developments (UK) Ltd., Midhurst, UK) using 200 µl of elution buffer (Millipore) by centrifugation at 1500g, then the eluate was cleared by further centrifugation at 1595*g*. The samples were acquired on a MAGPIX (Luminex, UK) using a 38-plex magnetic human cytokine kit (Millipore) and analyzed with xPONENT software following the manufacturer's instructions. The analytes included sCD40L, EGF, Eotaxin/CCL11, FGF-2, Flt-3 ligand, Fractalkine, G-CSF, GM-CSF, GRO, IFN-α2, IFN-γ, IL-1α, IL-1β, IL-

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1RA, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-12 (p40), IL-12 (p70), IL-13, IL-15, IL-17A, IP-10, MCP-1, MCP-3, MDC (CCL22), MIP-1α, MIP-1β, TGF-α, TNF-α, TNF-β and VEGF.

### Statistical analysis

Clinical data were analysed using Stata V15.1 (StataCorp, Stata Statistical Software: Release 15, College Station, Texas, USA). Categorical variables were compared using the Fisher's exact test. Continuous variables were tested for normality and appropriate statistical tests applied. Non-normally distributed measurements are expressed as the median and were analysed by the Kruskal-Wallis test. A p-value of $< 0.05$ was considered statistically significant. Immunological and diagnostic data and all figures were produced using R v.3.5.1 (R Development Core Team, Vienna, Austria) and GraphPad Prism v9.0.0 (GraphPad Software, San Diego, California, USA).

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## Author contributions

The author contributions were as follows. Methodology: BM, KCJ, KBG, JC, CVDV, JR and KJ. Investigation: CA, CVDV, RK, PM, CB, JN, NB, TP, SC, AA, OK, VN, EC, AZ, BD, ES, NB, DM, AC, AC, CN, LM, PM, CM, AL, DT, MC, LM and JM. Data analysis: KCJ, JJ, BM, JC and MYRH. Interpretation: KCJ, BM, KBG, JC, JJ and SBG. Data curation: PK, JG and CM. Project administration: KSM, KM, CP, JM, MN, GK, JP, RK, MG, VK, SL, PM, TS and PI. Sample collection: MM, SK, HT, SN, EN, LK, PM and BC. Writing: BM, KCJ, KGB, JJ, JC and SBG. Conceptualization and supervision: BM, KCJ, KBG and JC. All authors read and approved the final manuscript.

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## Declaration of Interests

The authors declare no competing interests.

## References

1. Government SA. Update on Covid-19 2020 [Available from: https://sacoronavirus.co.za.
2. Kuehn BM. Africa Succeeded Against COVID-19's First Wave, but the Second Wave Brings New Challenges. Jama. 2021;325(4):327-8.
3. Andrews B, Semler MW, Muchemwa L, Kelly P, Lakhi S, Heimburger DC, et al. Effect of an Early Resuscitation Protocol on In-hospital Mortality Among Adults With Sepsis and Hypotension: A Randomized Clinical Trial. JAMA. 2017;318(13):1233-40.
4. Mallett S, Allen AJ, Graziadio S, Taylor SA, Sakai NS, Green K, et al. At what times during infection is SARS-CoV-2 detectable and no longer detectable using RT-PCR-based tests? A systematic review of individual participant data. BMC Med. 2020;18(1):346.
5. Chi Y, Ge Y, Wu B, Zhang W, Wu T, Wen T, et al. Serum Cytokine and Chemokine Profile in Relation to the Severity of Coronavirus Disease 2019 in China. J Infect Dis. 2020;222(5):746-54.
6. Mudd PA, Crawford JC, Turner JS, Souquette A, Reynolds D, Bender D, et al. Distinct inflammatory profiles distinguish COVID-19 from influenza with limited contributions from cytokine storm. Sci Adv. 2020;6(50).
7. Chen LYC, Hoiland RL, Stukas S, Wellington CL, Sekhon MS. Confronting the controversy: interleukin-6 and the COVID-19 cytokine storm syndrome. Eur Respir J. 2020;56(4).
8. Del Valle DM, Kim-Schulze S, Huang HH, Beckmann ND, Nirenberg S, Wang B, et al. An inflammatory cytokine signature predicts COVID-19 severity and survival. Nat Med. 2020;26(10):1636-43.
9. Sinha P, Matthay MA, Calfee CS. Is a "Cytokine Storm" Relevant to COVID-19? JAMA Intern Med. 2020;180(9):1152-4.
10. Laing AG, Lorenc A, Del Molino Del Barrio I, Das A, Fish M, Monin L, et al. A dynamic COVID-19 immune signature includes associations with poor prognosis. Nat Med. 2020;26(10):1623-35.
11. Chevrier S, Zurbuchen Y, Cervia C, Adamo S, Raeber ME, de Souza N, et al. A distinct innate immune signature marks progression from mild to severe COVID-19. bioRxiv. 2020:2020.08.04.236315.
12. Mann ER, Menon M, Knight SB, Konkel JE, Jagger C, Shaw TN, et al. Longitudinal immune profiling reveals key myeloid signatures associated with COVID-19. Sci Immunol. 2020;5(51).
13. Abers MS, Delmonte OM, Ricotta EE, Fintzi J, Fink DL, de Jesus AAA, et al. An immune-based biomarker signature is associated with mortality in COVID-19 patients. JCI Insight. 2021;6(1).
14. Zamorano Cuervo N, Grandvaux N. ACE2: Evidence of role as entry receptor for SARS-CoV-2 and implications in comorbidities. Elife. 2020;9.
15. Liao M, Liu Y, Yuan J, Wen Y, Xu G, Zhao J, et al. Single-cell landscape of bronchoalveolar immune cells in patients with COVID-19. Nat Med. 2020;26(6):842-4.

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16. Pandolfi L, Fossali T, Frangipane V, Bozzini S, Morosini M, D'Amato M, et al. Broncho-alveolar inflammation in COVID-19 patients: a correlation with clinical outcome. BMC Pulm Med. 2020;20(1):301.
17. Gallo O, Locatello LG, Mazzoni A, Novelli L, Annunziato F. The central role of the nasal microenvironment in the transmission, modulation, and clinical progression of SARS-CoV-2 infection. Mucosal Immunology. 2020.
18. Habibi MS, Thwaites RS, Chang M, Jozwik A, Paras A, Kirsebom F, et al. Neutrophilic inflammation in the respiratory mucosa predisposes to RSV infection. Science. 2020;370(6513).
19. Jochems SP, Marcon F, Carniel BF, Holloway M, Mitsi E, Smith E, et al. Inflammation induced by influenza virus impairs human innate immune control of pneumococcus. Nat Immunol. 2018;19(12):1299-308.
20. Hou YJ, Okuda K, Edwards CE, Martinez DR, Asakura T, Dinnon KH, 3rd, et al. SARS-CoV-2 Reverse Genetics Reveals a Variable Infection Gradient in the Respiratory Tract. Cell. 2020;182(2):429-46 e14.
21. Amin-Chowdhury Z, Aiano F, Mensah A, Sheppard C, Litt D, Fry NK, et al. Impact of the COVID-19 Pandemic on Invasive Pneumococcal Disease and Risk of Pneumococcal Coinfection with SARS-CoV-2: prospective national cohort study, England. Clin Infect Dis. 2020.
22. Garcia-Vidal C, Sanjuan G, Moreno-Garcia E, Puerta-Alcalde P, Garcia-Pouton N, Chumbita M, et al. Incidence of co-infections and superinfections in hospitalized patients with COVID-19: a retrospective cohort study. Clin Microbiol Infect. 2021;27(1):83-8.
23. Cornick J, Musicha P, Peno C, Saeger E, Toh P-yI, Bennett A, et al. Genomic investigation of a suspected multi-drug resistant Klebsiella pneumoniae outbreak in a neonatal care unit in sub-Saharan Africa. bioRxiv. 2020:2020.08.06.236117.
24. Horby P, Lim WS, Emberson JR, Mafham M, Bell JL, Linsell L, et al. Dexamethasone in Hospitalized Patients with Covid-19 - Preliminary Report. The New England journal of medicine. 2020.
25. Shetty VU, Brotherton BJ, Achilleos A, Akrami KM, Barros LM, Checkley W, et al. Pragmatic Recommendations for Therapeutics of Hospitalized COVID-19 Patients in Low- and Middle-Income Countries. The American journal of tropical medicine and hygiene. 2020.
26. WHO. Clinical management of COVID-19 interim guidance 2020 [Available from: https://www.who.int/publications/i/item/clinical-management-of-covid-19.
27. Moore JB, June CH. Cytokine release syndrome in severe COVID-19. Science. 2020;368(6490):473-4.
28. Chua RL, Lukassen S, Trump S, Hennig BP, Wendisch D, Pott F, et al. COVID-19 severity correlates with airway epithelium-immune cell interactions identified by single-cell analysis. Nat Biotechnol. 2020;38(8):970-9.
29. Trump S, Lukassen S, Anker MS, Chua RL, Liebig J, Thurmann L, et al. Hypertension delays viral clearance and exacerbates airway hyperinflammation in patients with COVID-19. Nat Biotechnol. 2020.
30. Zhou Z, Ren L, Zhang L, Zhong J, Xiao Y, Jia Z, et al. Heightened Innate Immune Responses in the Respiratory Tract of COVID-19 Patients. Cell Host Microbe. 2020;27(6):883-90 e2.
31. Veras FP, Pontelli MC, Silva CM, Toller-Kawahisa JE, de Lima M, Nascimento DC, et al. SARS-CoV-2-triggered neutrophil extracellular traps mediate COVID-19 pathology. J Exp Med. 2020;217(12).

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32. Masso-Silva JA, Moshensky A, Lam MTY, Odish M, Patel A, Xu L, et al. Increased peripheral blood neutrophil activation phenotypes and NETosis in critically ill COVID-19 patients. medRxiv. 2021:2021.01.14.21249831.
33. Radermecker C, Detrembleur N, Guiot J, Cavalier E, Henket M, d'Emal C, et al. Neutrophil extracellular traps infiltrate the lung airway, interstitial, and vascular compartments in severe COVID-19. J Exp Med. 2020;217(12).
34. Thickett DR, Armstrong L, Millar AB. A role for vascular endothelial growth factor in acute and resolving lung injury. Am J Respir Crit Care Med. 2002;166(10):1332-7.
35. Chen G, Wu D, Guo W, Cao Y, Huang D, Wang H, et al. Clinical and immunological features of severe and moderate coronavirus disease 2019. J Clin Invest. 2020;130(5):2620-9.
36. Guan WJ, Ni ZY, Hu Y, Liang WH, Ou CQ, He JX, et al. Clinical Characteristics of Coronavirus Disease 2019 in China. N Engl J Med. 2020;382(18):1708-20.
37. Qun S, Wang Y, Chen J, Huang X, Guo H, Lu Z, et al. Neutrophil-to-Lymphocyte Ratios Are Closely Associated With the Severity and Course of Non-mild COVID-19. Front Immunol. 2020;11:2160.
38. Broughton SE, Dhagat U, Hercus TR, Nero TL, Grimbaldeston MA, Bonder CS, et al. The GM-CSF/IL-3/IL-5 cytokine receptor family: from ligand recognition to initiation of signaling. Immunol Rev. 2012;250(1):277-302.
39. Weber GF, Chousterman BG, He S, Fenn AM, Nairz M, Anzai A, et al. Interleukin-3 amplifies acute inflammation and is a potential therapeutic target in sepsis. Science. 2015;347(6227):1260-5.
40. Patil NK, Bohannon JK, Luan L, Guo Y, Sherwood ER. Preclinical Evaluation of Flt3 Ligand to Improve T Cell Adaptive Immune Response During Sepsis. The FASEB Journal. 2016;30(S1):1202.3-.3.
41. Sitnicka E, Bryder D, Theilgaard-Monch K, Buza-Vidas N, Adolfsson J, Jacobsen SE. Key role of flt3 ligand in regulation of the common lymphoid progenitor but not in maintenance of the hematopoietic stem cell pool. Immunity. 2002;17(4):463-72.
42. Geretti AM, Stockdale AJ, Kelly SH, Cevik M, Collins S, Waters L, et al. Outcomes of COVID-19 related hospitalization among people with HIV in the ISARIC WHO Clinical Characterization Protocol (UK): a prospective observational study. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America. 2020.
43. Bhaskaran K, Rentsch CT, MacKenna B, Schultze A, Mehrkar A, Bates CJ, et al. HIV infection and COVID-19 death: a population-based cohort analysis of UK primary care data and linked national death registrations within the OpenSAFELY platform. Lancet HIV. 2021;8(1):e24-e32.
44. Boulle A, Davies MA, Hussey H, Ismail M, Morden E, Vundle Z, et al. Risk factors for COVID-19 death in a population cohort study from the Western Cape Province, South Africa. Clin Infect Dis. 2020.
45. Cooper TJ, Woodward BL, Alom S, Harky A. Coronavirus disease 2019 (COVID-19) outcomes in HIV/AIDS patients: a systematic review. HIV Med. 2020;21(9):567-77.
46. Sigel K, Swartz T, Golden E, Paranjpe I, Somani S, Richter F, et al. Coronavirus 2019 and People Living With Human Immunodeficiency Virus: Outcomes for Hospitalized Patients in New York City. Clin Infect Dis. 2020;71(11):2933-8.
47. Karim F, Gazy I, Cele S, Zungu Y, Krause R, Bernstein M, et al. HIV infection alters SARS-CoV-2 responsive immune parameters but not clinical outcomes in COVID-19 disease. medRxiv. 2020:2020.11.23.20236828.

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48. ISARIC. Clinical Characterisation Protocol 2020 [Available from: https://isaric.tghn.org/.
49. Banda N, Hara W, Cocker D, Musasa S, Burke RM, Brown C, et al. First case report of a successfully managed severe COVID-19 infection in Malawi. Malawi Med J. 2020.
50. Morton B, Burr S, Jambo K, Rylance J, Henrion M, Banda N, et al. A pneumococcal controlled human infection model in Malawi: Transfer of an established pneumococcal carriage model from Liverpool, UK to Blantyre, Malawi ? A feasibility study [version 1; peer review: awaiting peer review]. Wellcome open research. 2020;5(25).

| | Units | PCR+ (n=41) | PCR-/IgG+ (n=25) | PCR-/IgG- (n=21) | P-value | Healthy Control (n=24) |
|---|---|---|---|---|---|---|
| Male | n (%) | 30 (73) | 18 (72) | 12 (57) | 0.403$^{\alpha}$ | 20 (83) |
| Age | median (IQR) | 50 (42 – 65) | 34 (25 – 51) | 41 (37 – 60) | 0.027$^{\beta}$ | 23 (22 – 25) |
| Days from symptom onset to hospital admission | median (IQR) | 4 (2 – 7) | 6 (2 -13) | 4 (3 – 14) | 0.814$^{\beta}$ | NA |
| HIV seropositive | n (%) | 9 (31*) | 9 (45*) | 9 (64*) | 0.198$^{\alpha}$ | 0 (0) |
| TB positive | n (%) | 1 (2) | 0 (0) | 0 (0) | 0.567$^{\alpha}$ | 0 (0) |
| Malaria positive | n (%) | 2 (5*) | 1 (5*) | 0 (0*) | 0.599$^{\alpha}$ | 0 (0) |
| Cardiac disease | n (%) | 13 (36*) | 5 (25*) | 3 (14) | 0.269$^{\alpha}$ | 0 (0) |
| Pulmonary disease | n (%) | 3 (9*) | 0 (0*) | 0 (0*) | 0.175$^{\alpha}$ | 0 (0) |
| Oxygen required at enrolment | n (%) | 21 (54*) | 12 (48) | 10 (50*) | 0.951$^{\alpha}$ | 0 (0) |
| ISARIC 4C clinical severity score | median (IQR) | 5 (4 – 8) | 4 (3 – 7) | 6 (5 – 8) | 0.075$^{\beta}$ | 0 (0 – 0) |
| UVA score | median (IQR) | 2 (0 – 4) | 2 (1 – 3) | 4 (2 – 5) | 0.025$^{\beta}$ | 0 (0 – 0) |
| Beta-lactam antibiotic administered | n (%) | 32 (78) | 13 (52) | 10 (48) | 0.024$^{\alpha}$ | NA |
| Steroids administered | n (%) | 26 (63) | 1 (4) | 0 (0) | <0.001$^{\alpha}$ | NA |
| Died in hospital | n (%) | 3 (7*) | 5 (20*) | 9 (43*) | 0.004$^{\alpha}$ | NA |
| Hospital length of stay for survivors | median (IQR) | 8 (6 – 17) | 6 (4 – 9) | 6 (3 – 8) | 0.028$^{\beta}$ | NA |

**Table 1: Clinical Characteristics.** PCR+ is RT-qPCR confirmed SARS-CoV-2 severe acute respiratory infection (SARI); PCR-/IgG- is patients with SARI who were RT-qPCR negative but IgG positive for SARS-CoV-2; and PCR-/IgG- is patients with SARI who were both RT-qPCR negative and IgG negative for SARS-CoV-2. Healthy controls were ambulant patients with no intercurrent illness who attended an outpatient clinic appointment. *Proportion (%) positivity calculated using the denominator for individual variables (unknown status classified as missing data). ISARIC 4C score calculated using six clinical variables available within dataset as urea and C-reactive protein were not available. UVA; universal vital assessment score (low income country validated clinical severity score). α: Fisher's exact test; β: Kruskall-Wallis test.

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**Figure Legends**

**Figure 1. Study recruitment timeline and SARS-CoV-2 diagnosis.** a) Suspected and SARS-CoV-2 RT-qPCR confirmed COVID-19 patients recruited at Queen Elizabeth Central Hospital, Blantyre (positive (red) and negative (blue)) reported as cases/week compared to the national data (light gray). National data includes SARS-CoV-2 RT-qPCR confirmed symptomatic and non-symptomatic COVID-19. b) SARS-CoV-2 Spike protein 2 (S2) and nucleoprotein (NP) IgG antibodies in PCR-negative and positive individuals. Pre-pandemic historical samples (2016-2019) were tested for SARS-CoV-2 S2 and NP IgG antibodies. The data are reported as the ratio of OD in the test samples to the assay threshold control. The horizontal bars represent the median and interquartile range (IQR). Data were analysed using Kruskal-Wallis test (Historical samples, n=132; PCR-positive, n=38; PCR-negative, n=45). Severe acute respiratory syndrome coronavirus 2; COVID-19, coronavirus disease of 2019; PCR, polymerase chain reaction; IgG, immunoglobulin G; SARI, severe acute respiratory infection.

**Figure 2. Cytokine concentrations in nasal lining fluid and serum.** a) Volcano plots showing differential cytokine concentrations in nasal lining fluid and serum of PCR-confirmed COVID-19, PCR-/IgG+ SARI and PCR-/IgG- SARI patients compared to health controls. The horizontal dotted line represents a cut-off for statistical significance, while the vertical dotted line represents a cut-off point for determining whether the levels of the cytokines were higher (right, red) or lower (left, blue) compared to healthy controls. b) Venn diagrams showing similarities in cytokine concentrations among the PCR-confirmed COVID-19 (green), PCR-/IgG+ SARI (turquoise) and PCR-/IgG- SARI (purple) patients in nasal lining fluid and serum relative to healthy controls. Numbers in Venn diagrams represent the number of cytokines that were either higher (red) or lower (blue) than healthy controls common among the study groups. Data were analysed using empirical Bayes moderated t-tests (Healthy controls, n=25; PCR-confirmed COVID-19; n=25; PCR-/IgG+ SARI, n=16; PCR-/IgG- SARI, n=11). SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; COVID-19, coronavirus disease of 2019; PCR, polymerase chain reaction; IgG, immunoglobulin G; SARI, severe acute respiratory infection

**Figure 3. Nasal and serum cytokine profiles.** Principal component analysis (PCA) of the 38 analytes (37 cytokines and sCD40L) in a) nasal lining fluid and b) serum of healthy controls, PCR-confirmed COVID-19, PCR-/IgG+ SARI and PCR-/IgG- SARI patients; showing similarity of nasal cytokine responses between PCR-confirmed COVID-19 and PCR-/IgG+ SARI patients. Correlogram of cytokine interactions in c) nasal lining fluid and d) serum amongst the different study groups, showing

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induction of similar immune process in PCR-confirmed COVID-19 and PCR-/IgG+ SARI patients. Healthy controls, n=25; PCR confirmed COVID-19; n=25; PCR-/IgG+ SARI, n=16; PCR-/IgG- SARI, n=11. SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; COVID-19, coronavirus disease of 2019; PCR, polymerase chain reaction; IgG, immunoglobulin G; SARI, severe acute respiratory infection; PC1, principal component 1; PC2, principal component 2.

**Figure 4. Nasal cell composition in healthy controls, confirmed and suspected COVID-19 patients.** a) Representative flow cytometry plots for cellular composition in the nasal mucosa of healthy controls, PCR-confirmed COVID-19 and PCR-/IgG+ SARI patients. b) Proportions of T cells, B cells, neutrophils and monocytes in nasal mucosa of healthy controls and COVID-19 patients. The horizontal bars represent the median and interquartile range (IQR). Data were analysed using Kruskal-Wallis test (Healthy controls, n=20; PCR confirmed COVID-19; n=20; PCR-/IgG+ SARI, n=11). SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; COVID-19, coronavirus disease of 2019; PCR, polymerase chain reaction; IgG, immunoglobulin G; SARI, severe acute respiratory infection

**Figure 5. Co-colonisation/infection status of suspected and confirmed COVID-19 patients.** a) Profile of co-colonisation/infection pathogens in nasopharyngeal/throat swabs. Prevalence of b) *Staphylococcus aureus* and c) *Streptococcus pneumoniae* co-colonisation in suspected and confirmed COVID-19. The horizontal bars represent the median and interquartile range (IQR). Data were analysed using Kruskal-Wallis test (PCR-confirmed COVID-19; n=39; PCR-/IgG+ SARI, n=23; PCR-/IgG- SARI, n=22). COVID-19, coronavirus disease of 2019.

**Figure 6. Cytokine concentrations in nasal lining fluid and serum based on HIV-coinfection status.** PCR confirmed COVID-19 and PCR-/IgG+ SARI patients were combined (n=41) into one COVID-19 patient group. Only cytokines that showed differential responses between patients with healthy controls were included in the analysis. a) Cytokine concentrations between HIV-infected and uninfected COVID-19 patients in nasal lining fluid. b) Cytokine concentrations between HIV-infected and uninfected COVID-19 patients in serum. The horizontal bars represent the median and interquartile range (IQR). Data were analysed using Kruskal-Wallis test (HIV-, n=20; HIV+, n=10). COVID-19, coronavirus disease of 2019; PCR, polymerase chain reaction; SARI, severe acute respiratory infection

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**Supplementary material**

**Figure S1. Sample collection and assays.** Nasal and peripheral blood samples were collected at a single time point.

**Figure S2. Levels of analytes in nasal lining fluid.** The horizontal bars represent the median and interquartile range (IQR). Data were analysed using Kruskal-Wallis test (Healthy controls, n=25; PCR confirmed COVID-19; n=25; PCR-/IgG+ SARI, n=16; PCR-/IgG- SARI, n=11). SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; COVID-19, coronavirus disease of 2019; PCR, polymerase chain reaction; IgG, immunoglobulin G; SARI, severe acute respiratory infection.

**Figure S3. Levels of analytes in serum.** The horizontal bars represent the median and interquartile range (IQR). Data were analysed using Kruskal-Wallis test (Healthy controls, n=25; PCR confirmed COVID-19; n=25; PCR-/IgG+ SARI, n=16; PCR-/IgG- SARI, n=11). SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; COVID-19, coronavirus disease of 2019; PCR, polymerase chain reaction; IgG, immunoglobulin G; SARI, severe acute respiratory infection.

**Figure S4. Prevalence of co-colonisation/infection in suspected and confirmed COVID-19 based on HIV status.** a) Prevalence rate of pathogens per study group. b) Prevalence of *Klebsiella pneumoniae* co-colonisation in suspected and confirmed COVID-19 in those with and without HIV infection. Data were analysed using Kruskal-Wallis test (PCR-confirmed COVID-19, n=38; PCR-/IgG+ SARI, n=24; PCR-/IgG- SARI, n=18). SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; COVID-19, coronavirus disease of 2019; PCR, polymerase chain reaction; IgG, immunoglobulin G; SARI, severe acute respiratory infection.

Figure 1.

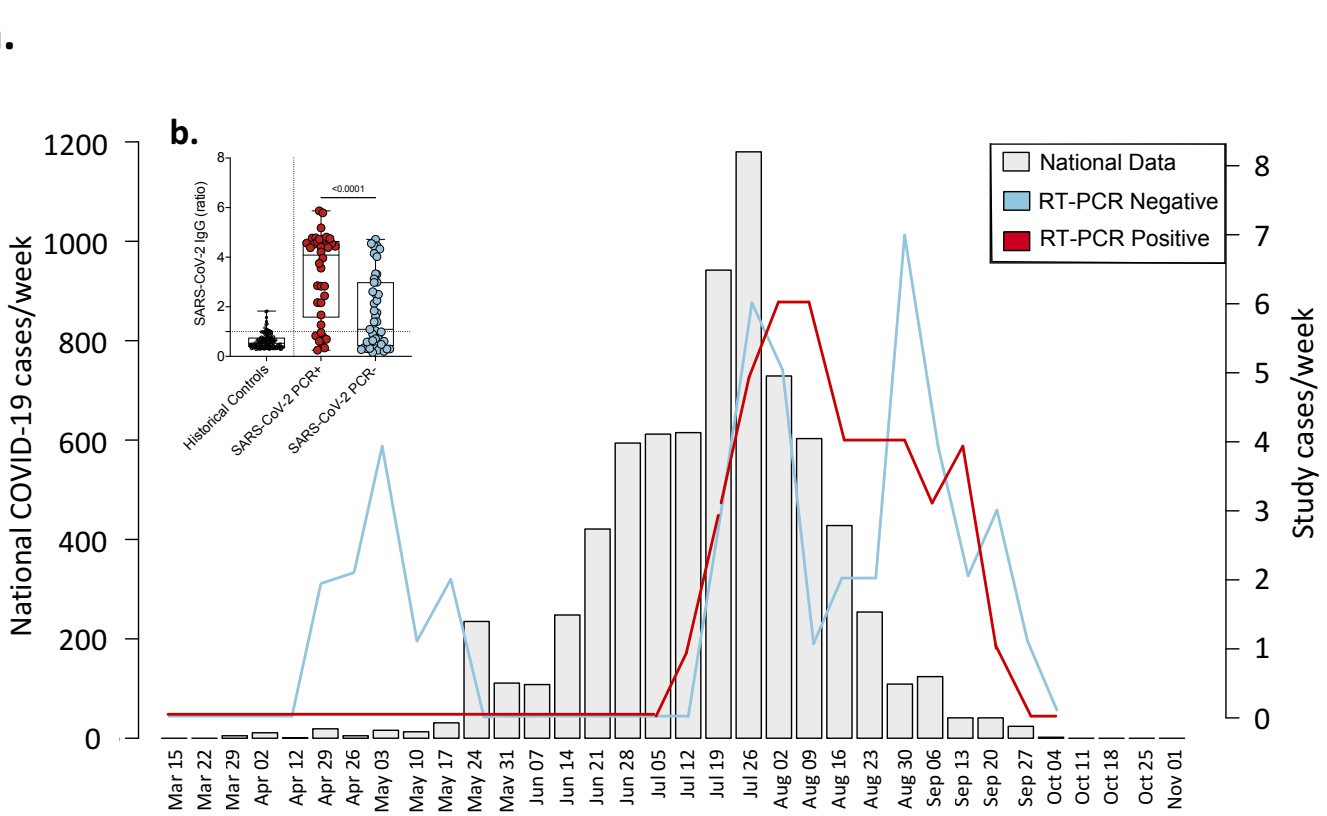
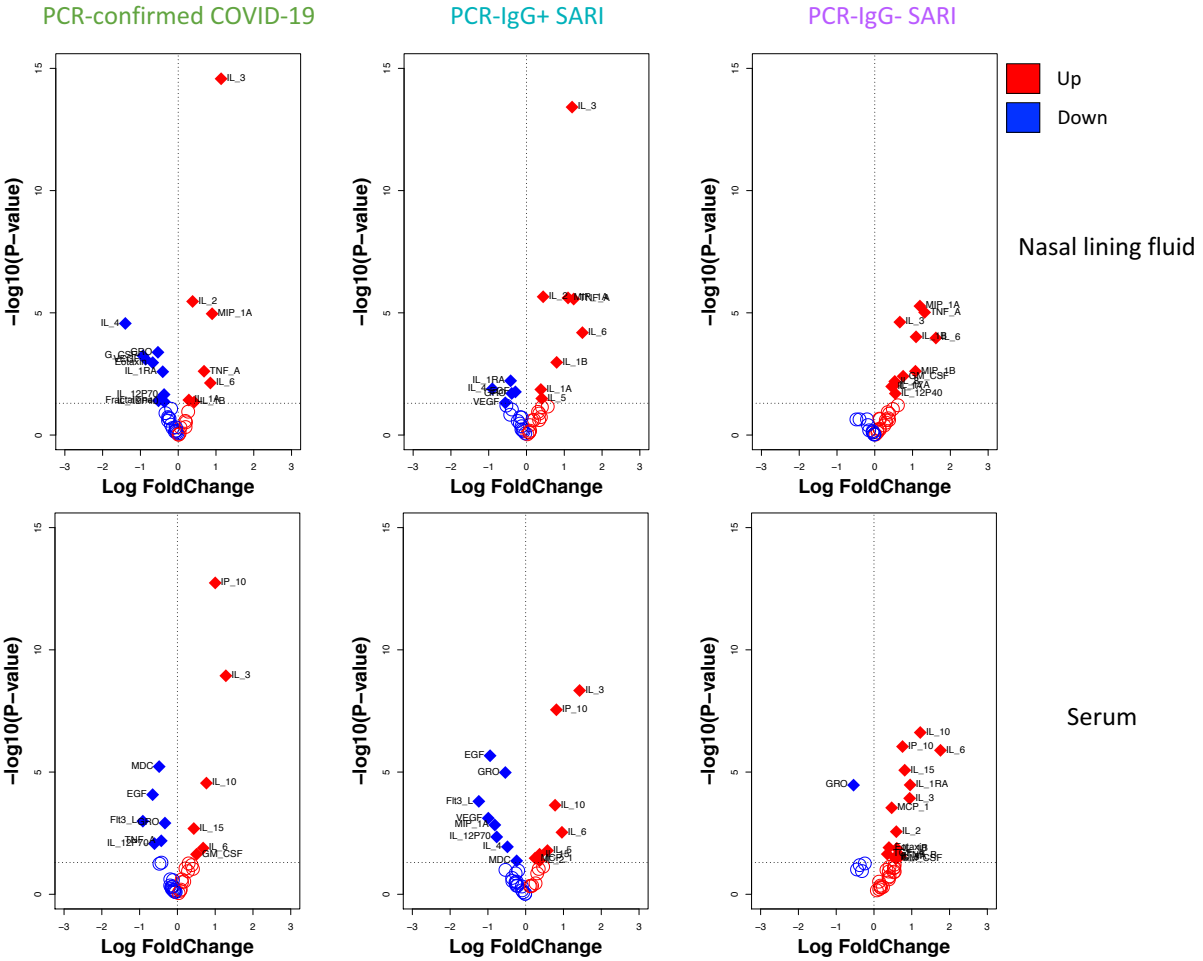

a.
b.
National COVID-19 cases/week
Study cases/week
National Data
RT-PCR Negative
RT-PCR Positive
SARS-CoV-2 IgG (ratio)
<0.0001
Historical Controls
SARS-CoV-2 PCR+
SARS-CoV-2 PCR-
Mar 15
Mar 22
Mar 29
Apr 02
Apr 12
Apr 29
Apr 26
May 03
May 10
May 17
May 24
May 31
Jun 07
Jun 14
Jun 21
Jun 28
Jul 05
Jul 12
Jul 19
Jul 26
Aug 02
Aug 09
Aug 16
Aug 23
Aug 30
Sep 06
Sep 13
Sep 20
Sep 27
Oct 04
Oct 11
Oct 18
Oct 25
Nov 01

Figure 2.

**a.**


PCR-confirmed COVID-19
PCR-IgG+ SARI
PCR-IgG- SARI
Up
Down
Nasal lining fluid
Serum
−log10(P−value)
Log FoldChange

**b.**

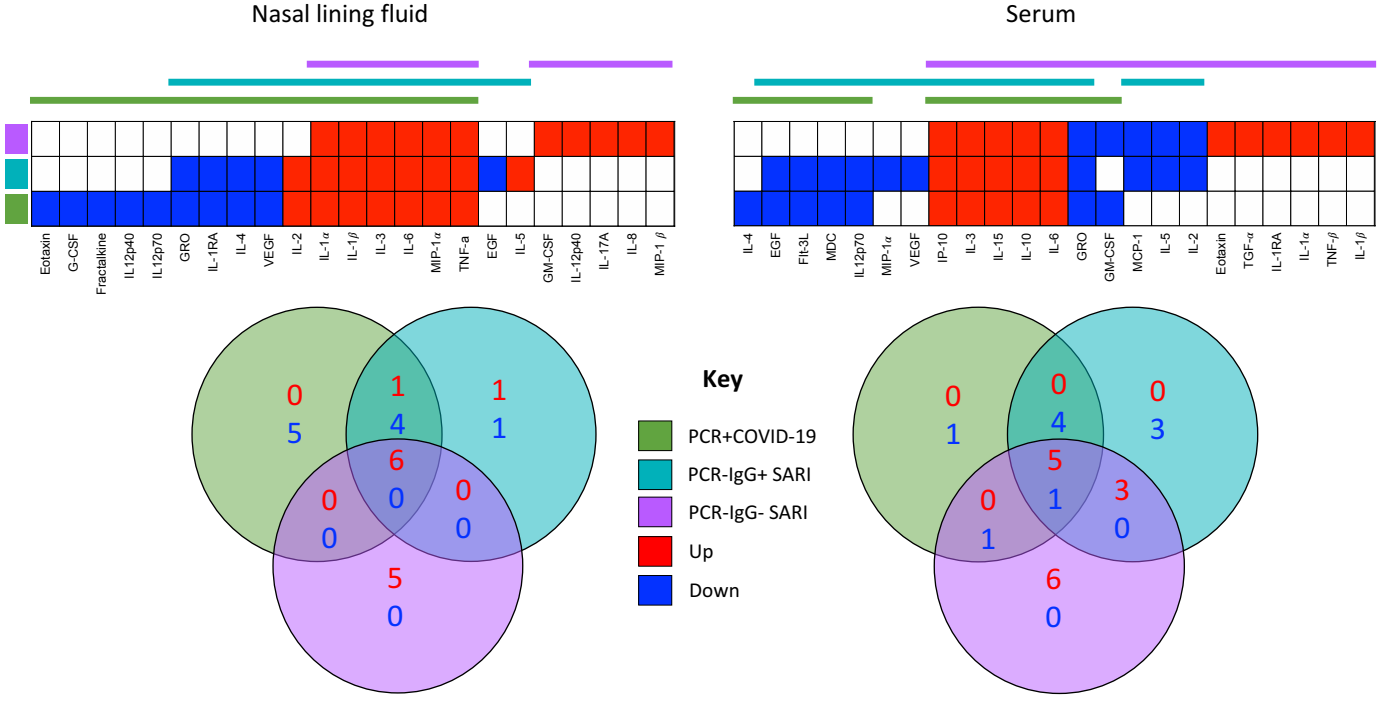

Nasal lining fluid
Serum
Eotaxin
G-CSF
Fractalkine
IL12p40
IL12p70
GRO
IL-1RA
IL-4
VEGF
IL-2
IL-1α
IL-1β
IL-3
IL-6
MIP-1α
TNF-a
EGF
IL-5
GM-CSF
IL-12p40
IL-17A
IL-8
MIP-1 β
IL-4
EGF
Flt-3L
MDC
IL12p70
MIP-1α
VEGF
IP-10
IL-3
IL-15
IL-10
IL-6
GRO
GM-CSF
MCP-1
IL-5
IL-2
Eotaxin
TGF-α
IL-1RA
IL-1α
TNF-β
IL-1β
0 5
1 4 6 0
1 1
0 0
0 0
5 0
Key
PCR+COVID-19
PCR-IgG+ SARI
PCR-IgG- SARI
Up
Down
0 1
0 4 5 1
0 3
0 1
3 0
6 0

Figure 3.

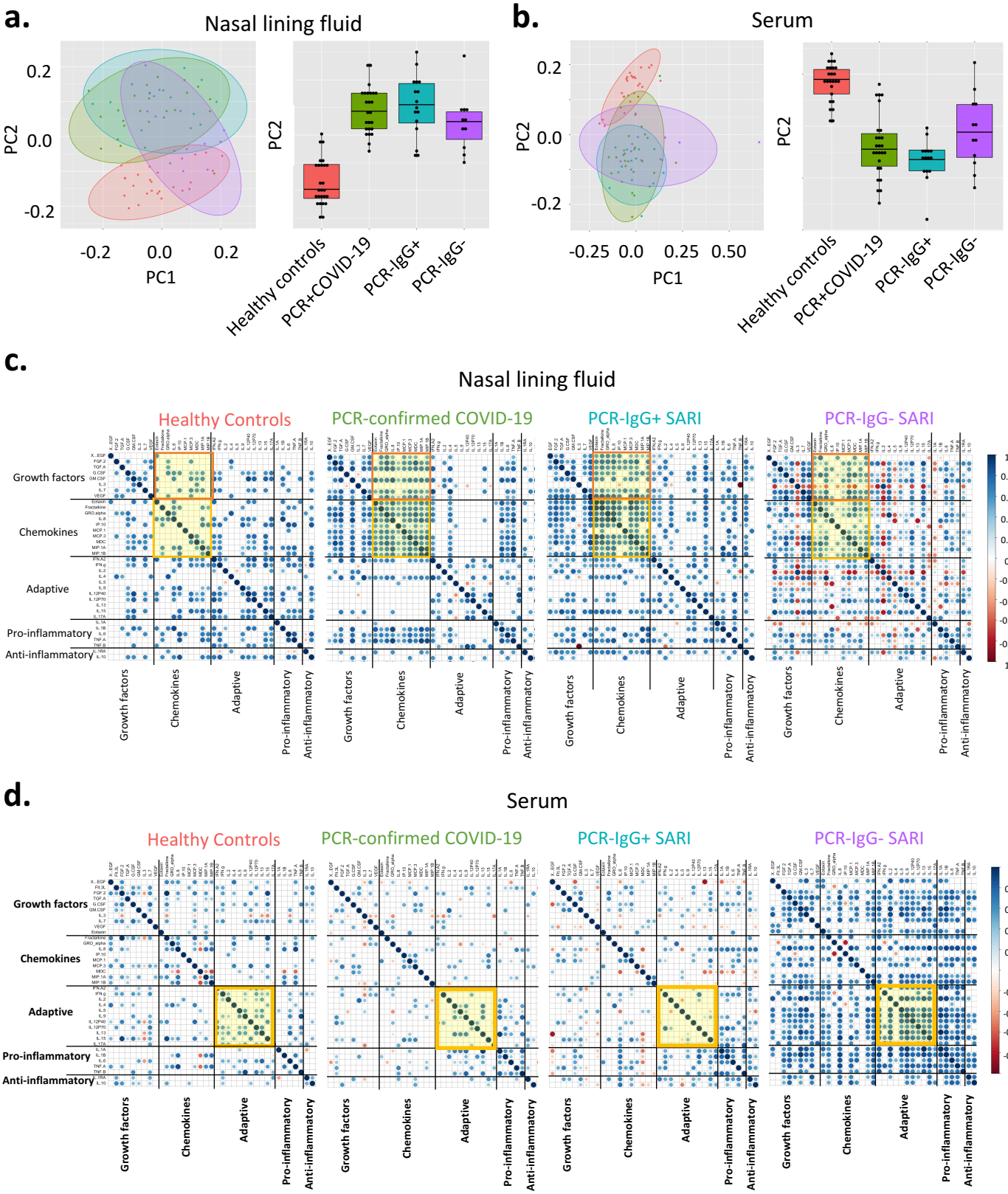
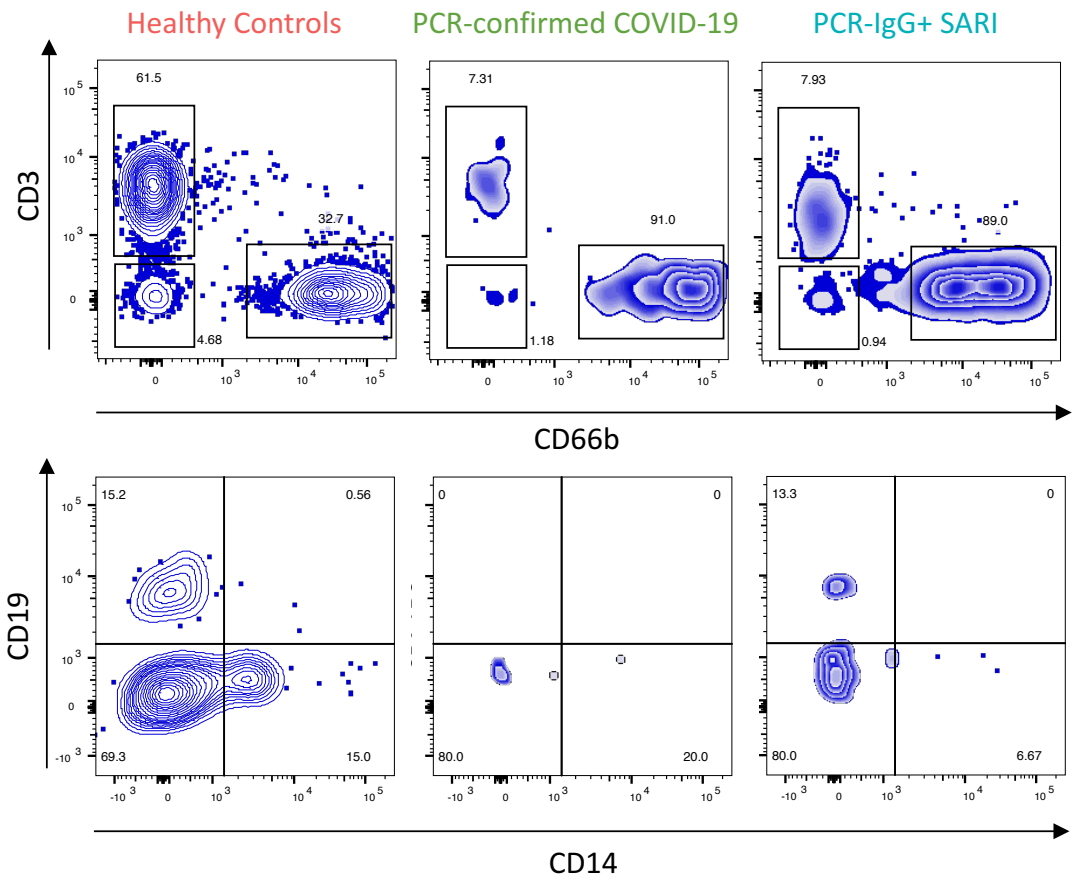

a.
Nasal lining fluid
PC2
PC1
Healthy controls
PCR+COVID-19
PCR-IgG+
PCR-IgG-
b.
Serum
c.
Nasal lining fluid
Healthy Controls
PCR-confirmed COVID-19
PCR-IgG+ SARI
PCR-IgG- SARI
Growth factors
Chemokines
Adaptive
Pro-inflammatory
Anti-inflammatory
d.
Serum

Figure 4.

**a.**


Healthy Controls
PCR-confirmed COVID-19
PCR-IgG+ SARI
CD3
CD66b
61.5
32.7
4.68
7.31
91.0
1.18
7.93
89.0
0.94
CD19
CD14
15.2
0.56
69.3
15.0
0
0
80.0
20.0
13.3
0
80.0
6.67

**b.**

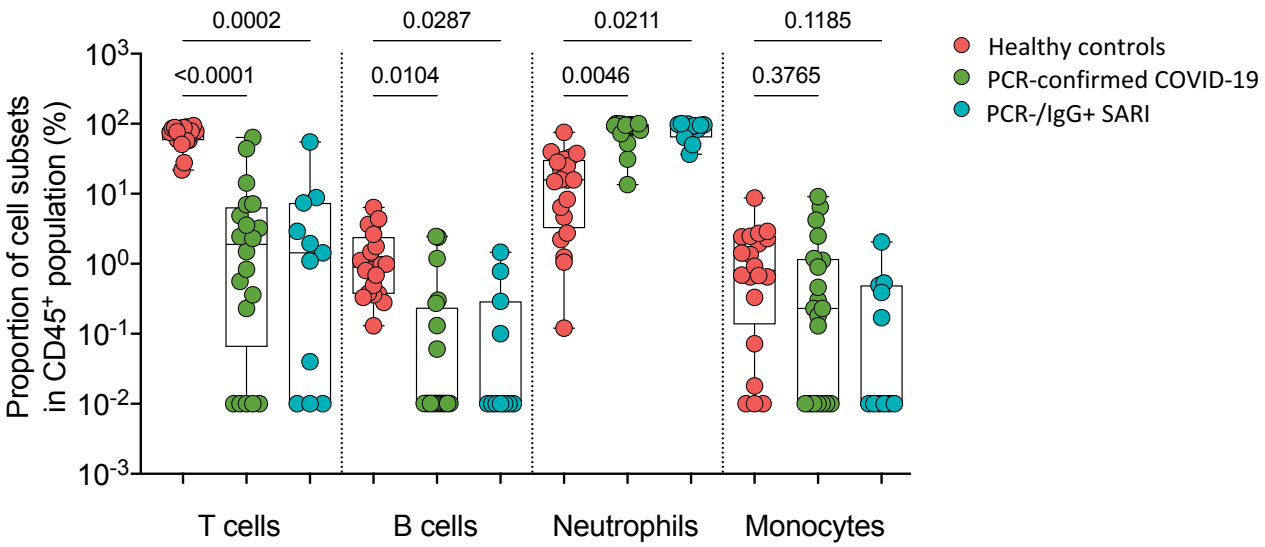
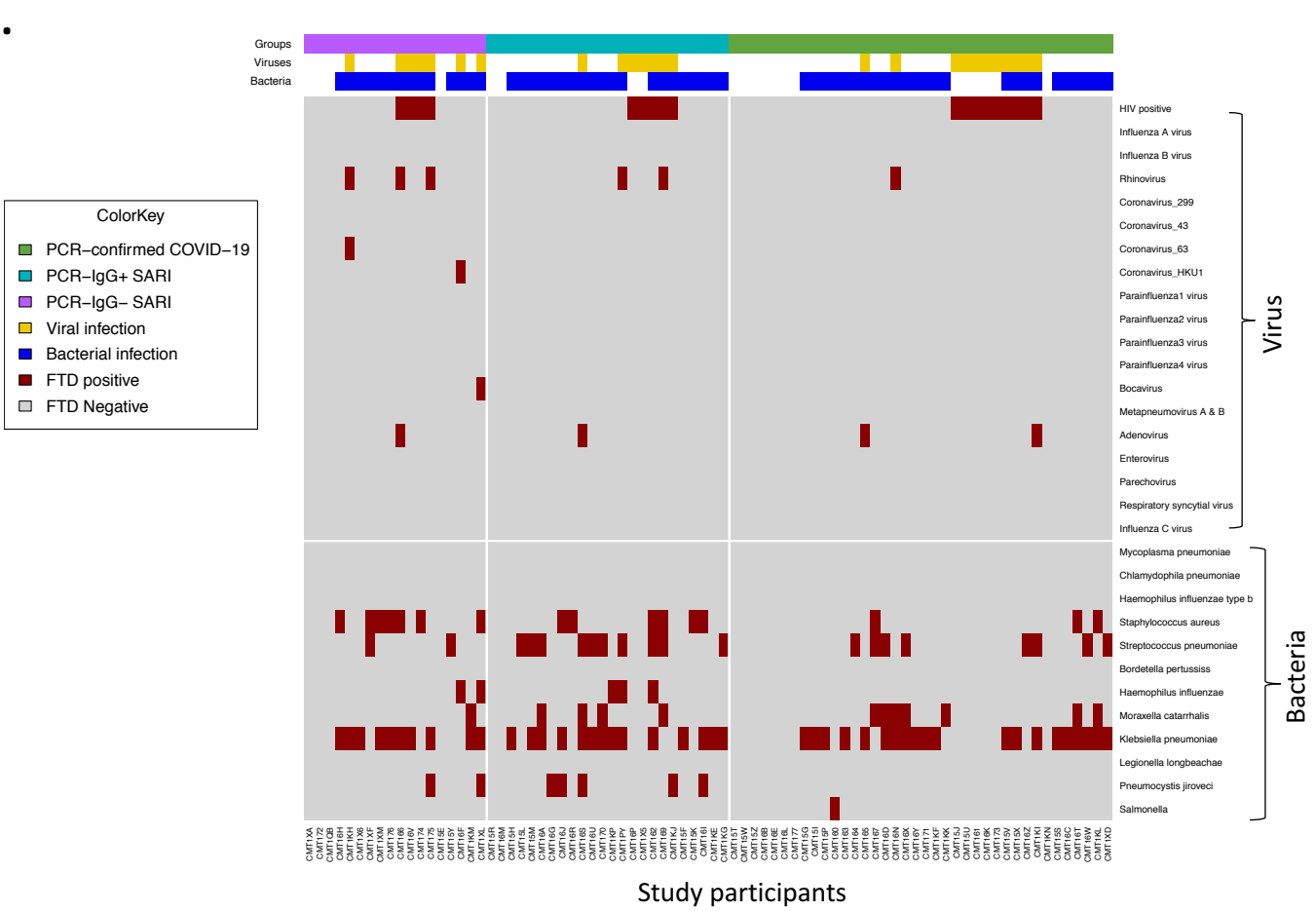
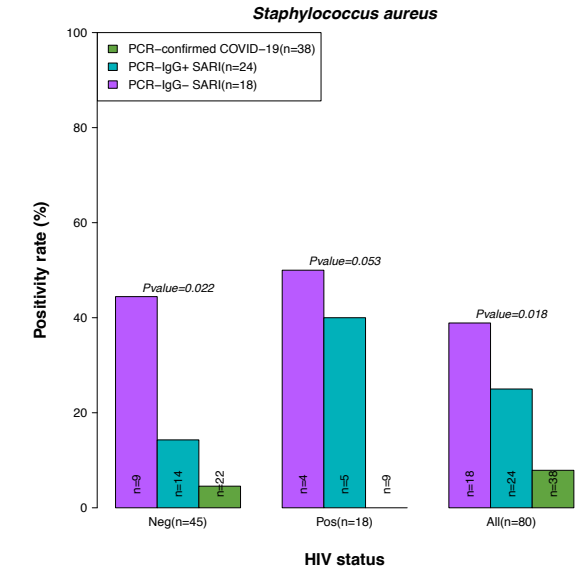

0.0002
<0.0001
0.0287
0.0104
0.0211
0.0046
0.1185
0.3765
Proportion of cell subsets in CD45+ population (%)
T cells
B cells
Neutrophils
Monocytes
Healthy controls
PCR-confirmed COVID-19
PCR-/IgG+ SARI

Figure 5.


a.
Groups
Viruses
Bacteria
ColorKey
PCR−confirmed COVID−19
PCR−IgG+ SARI
PCR−IgG− SARI
Viral infection
Bacterial infection
FTD positive
FTD Negative
HIV positive
Influenza A virus
Influenza B virus
Rhinovirus
Coronavirus_299
Coronavirus_43
Coronavirus_63
Coronavirus_HKU1
Parainfluenza1 virus
Parainfluenza2 virus
Parainfluenza3 virus
Parainfluenza4 virus
Bocavirus
Metapneumovirus A & B
Adenovirus
Enterovirus
Parechovirus
Respiratory syncytial virus
Influenza C virus
Virus
Mycoplasma pneumoniae
Chlamydophila pneumoniae
Haemophilus influenzae type b
Staphylococcus aureus
Streptococcus pneumoniae
Bordetella pertussiss
Haemophilus influenzae
Moraxella catarrhalis
Klebsiella pneumoniae
Legionella longbeachae
Pneumocystis jiroveci
Salmonella
Bacteria
Study participants


b.
Staphylococcus aureus
PCR−confirmed COVID−19(n=38)
PCR−IgG+ SARI(n=24)
PCR−IgG− SARI(n=18)
Positivity rate (%)
Pvalue=0.022
Pvalue=0.053
Pvalue=0.018
n=9
n=14
n=22
n=4
n=5
n=9
n=18
n=24
n=38
Neg(n=45)
Pos(n=18)
All(n=80)
HIV status

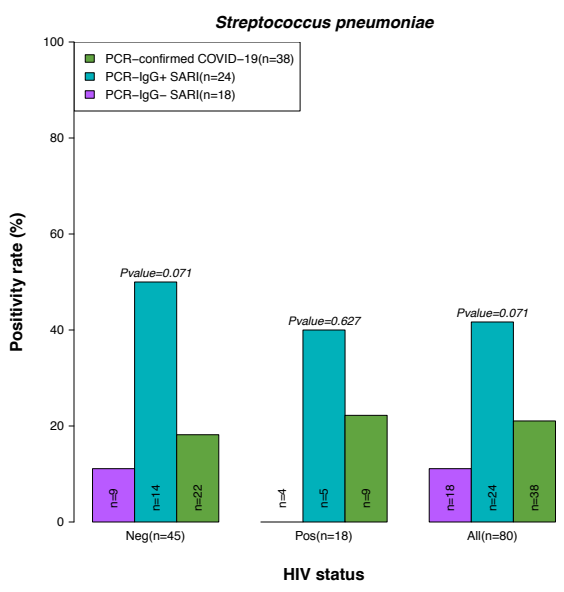
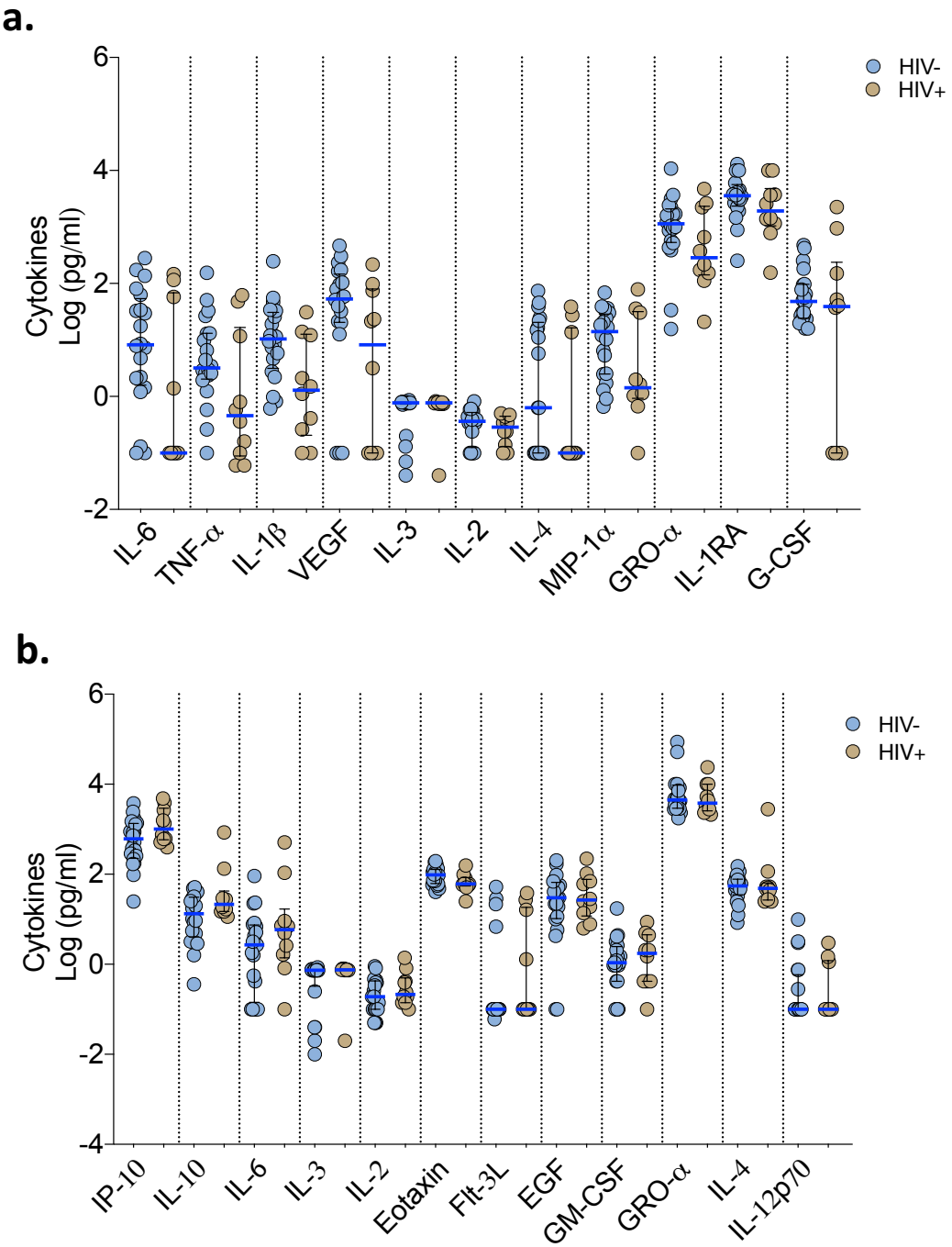

c.
Streptococcus pneumoniae
PCR−confirmed COVID−19(n=38)
PCR−IgG+ SARI(n=24)
PCR−IgG− SARI(n=18)
Positivity rate (%)
Pvalue=0.071
Pvalue=0.627
Pvalue=0.071
n=9
n=14
n=22
n=4
n=5
n=9
n=18
n=24
n=38
Neg(n=45)
Pos(n=18)
All(n=80)
HIV status

Figure 6.


a.
HIV-
HIV+
Cytokines Log (pg/ml)
6
4
2
0
-2
IL-6
TNF-α
IL-1β
VEGF
IL-3
IL-2
IL-4
MIP-1α
GRO-α
IL-1RA
G-CSF
b.
HIV-
HIV+
Cytokines Log (pg/ml)
6
4
2
0
-2
-4
IP-10
IL-10
IL-6
IL-3
IL-2
Eotaxin
Flt-3L
EGF
GM-CSF
GRO-α
IL-4
IL-12p70