# Clinical, virological, microbiological, immunological, and laboratory monitoring of patients hospitalised with COVID-19: abridged secondary publication

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KEY MESSAGES

1. Deep throat saliva has suboptimal diagnostic sensitivity and hence a possibility of missed cases, particularly when screening in-bound travellers.
2. Mouth gargle and nasal strip are alternative self-collected specimens with good diagnostic performance and thus may be utilised in community surveillance.
3. Interleukin-38 appears to have a regulatory and protective role in COVID-19. Cytokine and chemokine profiling may have prognostic value.
4. Subgenomic RNA detection may serve as a sensitive marker of infectivity to guide decisions on patient discharge from isolation.

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## Introduction

Most individuals infected with SARS-CoV-2 remain asymptomatic or develop only mild respiratory symptoms; however, approximately 5% develop critical illness.[1] Cytokine release syndrome is proposed as a key driver of inflammation and may contribute to the pathogenesis of severe COVID-19.[2] Infectivity is often monitored using repeated polymerase chain reaction (PCR) testing, although prolonged PCR positivity is frequently observed. Subgenomic RNA (sgRNA) profiling has emerged as a potential alternative.[3] This study aimed to: (1) characterise virological and immunological profiles in relation to clinical outcomes, (2) assess the diagnostic value of various specimen types, and (3) evaluate the performance of molecular diagnostic methods targeting different gene regions.

## Methods

Serial conventional respiratory specimens were collected, including sputum and pooled nasopharyngeal and throat swabs (NPSTS), deep throat saliva (DTS),[4] mouth gargle, and nasal epithelial lining fluid. Cytokine and chemokine responses were evaluated in 85 patients with COVID-19, 50 patients with influenza, and 59 healthy controls. Interleukin (IL)-38 concentrations were measured using the enzyme-linked immunosorbent assay. C-X-C motif chemokine ligand (CXCL) 9, CXCL10, C-C motif chemokine ligand (CCL) 2, CCL5, IL-1β, IL-6, and tumour necrosis factor (TNF)-α were quantified using Cytometric Bead Array Flex Sets.

Early (within 7 days of symptom onset) and late (8 to 12 days from symptom onset) plasma samples were analysed for cytokine profiles in relation to clinical severity (mild, moderate, severe, and critical), as previously described.[5] Forty cytokines were measured using the Milliplex human cytokine multiplex assay, including soluble cluster of differentiation 40 ligand, epidermal growth factor, eotaxin/CCL11, fibroblast growth factor-2, FMS-like tyrosine kinase-3 ligand, fractalkine, granulocyte colony-stimulating factor, granulocyte-macrophage colony-stimulating factor, growth-regulated oncogene, interferon-α, interferon-γ, IL-1α, IL-1β, IL-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, inducible protein (IP)-10, monocyte chemoattractant protein (MCP)-1, MCP-3, macrophage-derived chemokine CCL22, macrophage inflammatory protein (MIP)-1α, MIP-1β, transforming growth factor-α, TNF-α, TNF-β, vascular endothelial growth factor, IL-18, and monokine induced by gamma interferon (MIG)/CXCL9.

A set of subgenomic-specific quantitative reverse transcription–PCR assays was developed to quantify sgRNA fragments corresponding to the E, M, N, open reading frame (ORF) 3a, ORF 6, ORF 7a, ORF 7b, ORF 8, and S regions.

## Results

Among 563 specimens (150 DTS, 309 NPSTS, and 104 sputum) collected during the virus shedding period from 27 female and 23 male patients aged 16 to 72 years, DTS had the lowest overall reverse transcription–PCR-positive rate, compared with sputum and NPSTS (68.7% vs 89.4% vs 80.9%, respectively), and the lowest viral RNA concentration (mean log copy/mL: 3.54 vs 5.03 vs 4.63, respectively). The false-negative rate of DTS was 31.3%, increasing by 2.7-fold among patients without sputum.

Among 49 patients aged 12 to 81 years with 109 pairs of mouth gargle and DTS samples collected between 1 and 19 (mean, 7±4) days from symptom onset, the overall positive rate ranged from 89.9% to 96.3%. No significant differences were detected between the two sample types across all four assays. Diagnostic yield comparison showed strong positive correlations between mouth gargle and DTS ($r$=0.662-0.727).

Nasal strip results were correlated with NPSTS (P=0.0003) and DTS (P=0.01), with concordance rates of 94% (17/18) and 100% (3/3) for NPSTS-positive and NPSTS-negative samples, respectively; and 93% (14/15) and 14% (1/7) for DTS-positive and DTS-negative samples, respectively. Viral RNA remained detectable after 24 and 72 hours of storage at room temperature.

Serum IL-38 was significantly elevated in patients with COVID-19 and negatively correlated with serum C-reactive protein, lactate dehydrogenase, and duration of hospitalisation. These findings suggest that IL-38 affects disease severity and hence a potential therapeutic target for COVID-19.

Among the 40 cytokines analysed, 23 showed progressive changes in concentration corresponding to disease severity. From mild to severe/critical illness in both early and late phases, levels of growth-regulated oncogene-α, IL-1RA, IL-6, IL-8, IL-10, IP-10, and MIG increased, whereas levels of fibroblast growth factor-2, IL-5, MDC, and MIP-1α decreased. Intensive care unit length of stay was positively correlated with levels of eotaxin (rho=0.592, P=0.012) and MCP-1 (rho=0.587, P=0.013). Duration of mechanical ventilation was correlated with levels of IL-9 (rho= -0.482, P=0.05) and MCP-1 (rho=0.609, P=0.009). Norepinephrine dose was correlated with levels of MCP-1 (rho=0.586, P=0.014) and TNF-α (rho= -0.135, P=0.605).

All culture-positive cases, and culture-negative cases with a genomic RNA PCR Ct value ≤27, exhibited a full spectrum of sgRNA. Respiratory and stool specimens often remained genomic PCR-positive for 3 to 4 weeks after symptom onset; however, a full spectrum of sgRNA was rarely detectable beyond day 10. Most stool samples were sgRNA-negative, suggesting the presence of non-viable virus.

## Discussion

Self-collected specimens offer logistical advantages. DTS—widely used in Hong Kong—showed suboptimal diagnostic performance. In contrast to saliva, mouth gargle is non-viscous and technically more manageable, making it suitable for mass screening of asymptomatic individuals. Nasal strip is preferable to NPSTS for specimen collection because it causes less irritation and hence more appropriate for use in children.

IL-38 expression was correlated with SARS-CoV-2 infection. Similar to SARS-CoV-1 in 2003, severe and critical COVID-19 cases were associated with elevated levels of Th1 cytokines such as IL-18, IP-10, and MIG. Increase in IL-18 levels during the late phase coincided with intensive care unit admission. Viral loads did not differ between patients with mild/moderate disease and those with severe/critical disease. These findings suggest that deterioration, typically observed during the middle phase (days 8 to 12 from symptom onset), is not driven by uncontrolled viral replication. Several cytokines (IL-6, MCP-1, IL-1RA, and IL-8) previously associated with non-COVID-19-related acute respiratory distress syndrome were significantly elevated among patients with severe COVID-19. IL-6 levels elevated shortly after symptom onset in patients who later developed severe/critical disease. Early measurement of IL-6 can help to identify individuals for IL-6 inhibition.

Our sgRNA profiling study suggested that prolonged positivity in genomic RNA PCR does not reflect infectivity. Unlike virus isolation, sgRNA PCR does not require high-level biosafety containment facilities, making it a more feasible and reliable method for assessing patient infectivity. The use of sgRNA PCR as a criterion for discharge from isolation should be considered.

## Conclusions

DTS is suboptimal compared with conventional respiratory specimens. Mouth gargle is suitable for large-scale screening of asymptomatic infections in the community. Nasal strip offers good diagnostic yield and is particularly appropriate for use in children. Th1 helper responses and acute respiratory distress syndrome–associated cytokines are correlated with disease severity. MCP-1 is predictive of the duration of mechanical ventilation, vasopressor requirements, and intensive care unit length of stay. PCR targeting viral sgRNA does not require a high biosafety facility and may serve as a more practical and reliable tool for monitoring infectivity.

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## Disclosure

The results of this research have been previously published in:

1. Lai CKC, Chen Z, Lui G, et al. Prospective study comparing deep throat saliva with other respiratory tract specimens in the diagnosis of novel coronavirus disease 2019. J Infect Dis 2020;222:1612-9.
2. Chan PKS, Lui G, Hachim A, et al. Serologic responses in healthy adult with SARS-CoV-2 reinfection, Hong Kong, August 2020. Emerg Infect Dis 2020;26:3076-8.
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