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委託研究2019冠狀病毒病

- Preparedness and response to pandemic and epidemic
  大流行病和流行病的準備及應變
- Candidate vaccines research and development
  候選疫苗研發
- Candidate therapeutics research and development
  候選療法研發
- Natural history, infectivity and transmissibility of the virus
  病毒的自然史、傳染與傳播
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Health and Medical Research Fund

Commissioned Research on the Novel Coronavirus Disease

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Editorial

In the fight against the COVID-19 pandemic, since April 2020, the Health Bureau and the Health and Medical Research Fund have approved a total of HK$556 million to commission 70 COVID-19-related research studies covering 105 projects. Substantial new knowledge and findings have been generated and have contributed significantly to the fight against the pandemic. In this edition, we present 12 dissemination reports of these commissioned projects. In particular, three projects related to healthcare system response, surveillance, and psychological impact of COVID-19 are highlighted.

In an outbreak of new or emerging infectious disease, for example COVID-19, a system of early detection, assessment, and response (S-EDAR) is essential for control. Yeoh et al[1] conducted an in-depth mixed-methods investigation on how Hong Kong's S-EDAR could be enhanced for control of public health threats across a range of different transmission scenarios to inform future preparedness and response plans. The investigation reviewed COVID-19 pandemic responses among six middle to high income jurisdictions in Asia, conducted interviews and focus groups among key local informants, and gained insights from local and overseas experts. A comprehensive and structured S-EDAR framework was developed, in which 14 domains (including preparedness, readiness, response, and recovery) and 37 major recommendations were presented, enabling preparedness, operational readiness, and timely response for strengthening health system and community resilience for future pandemics.

Surveillance of sewage for evidence of virus genetic material could provide a scalable, cost-effective strategy for measuring population-level infections. Zhang et al[2] tested for the presence of SARS-CoV-2 RNA in community sewage from various sites across Hong Kong to provide early warning signals for re-emergence of COVID-19 in local communities and to supplement clinical tests. The sewage test results obtained provided a basis for identifying buildings and places for statutory public health actions to uncover infected patients, assisting the government in implementing timely actions to achieve early identification, isolation, and treatment of infected patients in local communities. The study demonstrated the feasibility and utility of sewage surveillance and lays the foundation for its wider implementation to supplement clinical surveillance.

The psychological impacts of the COVID-19 pandemic and the factors associated with higher risk of developing mental health issues have not been studied extensively. Shum et al[3] adopted a mixed-methods approach to examine Hong Kong residents' level of psychological trauma and their behaviours regarding COVID-19 infection prevention. They aimed to determine the sociodemographic correlates of high psychological trauma and poor health behaviours, as well as the underlying reasons for people's choice to adopt or not adopt appropriate preventative measures. Among over 3000 survey respondents, the prevalence of potential post-traumatic stress disorder 1 year after the start of the COVID-19 pandemic was 12.4%. Participants who had lower education, were unemployed, had lower personal income, and spent more time watching news reports of COVID-19 had greater psychological trauma. Personal experiences and social networks influenced vaccination barriers and incentives. Trust and confidence in the vaccine were key determinants of motivation for vaccination. Stigma towards healthcare workers, trust in the government, and cultural perceptions of vaccines affected vaccination decisions.

Supplement editors

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Hong Kong's early detection, assessment, and response system (S-EDAR) for infectious disease outbreak: abridged secondary publication

EK Yeoh *, CT Hung, ELY Wong, MKC Chong, D Dong, EYM Leung, SYS Wong, VCH Chung, JZY Yang, JZX Wang

KEY MESSAGES

1. The enhanced system for early detection, assessment and response (S-EDAR), incorporating readiness and recovery, will guide the development of future preparedness, readiness, response, and recovery plans, which have the capacity to strengthen control of public health threats across pandemic trajectories with different transmission scenarios.
2. This multi-level mixed-methods study synthesised the evidence from different sources and types of knowledge derived from the literature and policy document reviews, expert workshops, comparative case studies, modelling studies for control measures, key informant interviews, and focus-group discussions, followed by a group Delphi study to affirm the feasibility and relevance of component statements.
3. We developed an expanded and enhanced S-EDAR, which is a robust, evolutionary, whole-of-government, and whole-of-society system to enable the four phases of preparedness, operational readiness, rapid response, and recovery to strengthen health system and community resilience in future pandemics.

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HMRF project number: COVID190105

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Introduction

The changing epidemiology in the protracted COVID-19 pandemic has created challenges in research methodology for developing an enhanced system of early detection, assessment and response (S-EDAR), which needs to be adaptable to the characteristics of different agents and dynamic contextual factors. In early phases of the pandemic, evidence to inform decisions was often not available and, if available, subject to uncertainty, leaving decision makers to rely on non-codified and contextual knowledge.[1] This study aimed to develop the S-EDAR components from diverse knowlegde sources, using a multi-stage mixed-methods approach. Objectives for the early phase of the study were: (1) to review the early detection, assessment, and response measures for COVID-19, (2) to document and analyse the different components of Hong Kong's S-EDAR to the COVID-19 under different transmission scenarios, (3) to identify barriers and facilitators in the implementation of key response measures, (4) to evaluate the effectiveness of surveillance and detection, and the impact of response measures, and (5) to determine enhancements required for pandemic control. In addition, objectives for the extended phase were: (1) to study the responses to the evolving pandemic, particularly to the surveillance mechanisms, and how these informed decisions for public health and social measures (PHSMs) and response readiness, (2) to review the literature and overseas experiences in the trajectory of protracted pandemic for calibrating PHSMs and health system and community resilience, (3) to assess the impact of the epidemic on the health system and community resilience, (4) to develop a system for calibrating PHSMs, and (5) to update and expand the S-EDAR to include readiness and health system and community resilience, based on the lessons learnt.

Methods

This multi-level mixed-methods study synthesised evidence from the literature and policy document reviews, including (1) a review of Hong Kong's preparedness and response plan, (2) a review of the transmissibility, transmission routes, and clinical manifestations of COVID-19, (3) a review of the effectiveness of non-pharmacological measures and associated implementation barriers and facilitators, (4) a scoping review of the effectiveness, limitations, and barriers of different social-distancing measures, (5) a scoping review of the readiness of preparedness-response plans, and the linkage between readiness

and health system and community resilience, and (6) a literature review on calibration and adjustment of PHSMs.

Comparative case studies were conducted on the containment, control, and mitigation policies and measures in Hong Kong SAR, Japan, Malaysia, South Korea, Singapore, and Shanghai during the early phases of the pandemic. Comparative case studies were also conducted in the later phase of the pandemic on the experiences of Hong Kong SAR, Singapore, and Victoria and New South Wales of Australia regarding how their health systems adapted during outbreaks of the Delta and Omicron variants that overwhelmed their health systems. Modelling studies were conducted to evaluate the transmissibility of the infection and effectiveness of control measures. Key informant interviews were conducted with policymakers, administrators, and managers in healthcare settings, business sector, elderly homes and disability institutions, social service operators from non-governmental organisations, healthcare professionals, and patient groups during the early and recovery phases of the pandemic. Focus-group discussions were conducted with the healthcare sector (including frontline doctors, nurses, and pharmacists) and the community sector (including subdivided unit residents, ethnic minorities, older adults with no family support, and immunocompromised individuals) to document their experiences as well as barriers and facilitators for compliance with PHSMs. In addition, expert workshops were conducted to develop the components and implementation strategies for S-EDAR, which were later reviewed by international experts. Finally, a group Delphi study was conducted to affirm the feasibility and relevance of the components for the enhanced S-EDAR framework for preparedness, readiness, response and recovery.

Results

Conceptualisation of the initial S-EDAR framework and its domains was based on a review of the literature and policy documents. The S-EDAR was further expanded and enhanced based on updated knowledge from the literature, a scoping review on 'readiness', updated findings from the second round of key informant interviews, focus-group discussions, and comparative case studies conducted during the later stage of the pandemic.

Comparative case studies showed that there were limitations in timely response and resource mobilisation despite having preparedness plans. This underscored the importance of 'readiness' in linking effective preparedness to a rapid and effective response. Key enablers for building a resilient health system included operational readiness, a clear and consistent roadmap, effective use of science and technology, private sector engagement, transparent communication, and support for vulnerable populations.

Case finding and contact tracing was a key component of response in the enhanced S-EDAR framework. We conducted modelling studies of the transmissibility of the infection and the effectiveness of control measures at different stages of the pandemic. Early-phase modelling helped characterise the nature of unlinked cases and assess the superspreading risks, which guided targeted interventions. In later phases, modelling studies evaluated the feasibility of contact tracing measures under different scenarios of disease transmissibility, adherence, tracing, and vaccination coverage. At the level of vaccine effectiveness to prevent Omicron infection, the effectiveness of contact tracing in lowering outbreak potential was primarily determined by variant transmissibility. These findings supported better calibration of PHSMs and the design of evidence-informed strategies for the S-EDAR.

The enhanced whole-of-society S-EDAR framework for emergency preparedness, readiness, response, and recovery (Fig 1) is critical for a rapid and effective response in the prevention, control, and

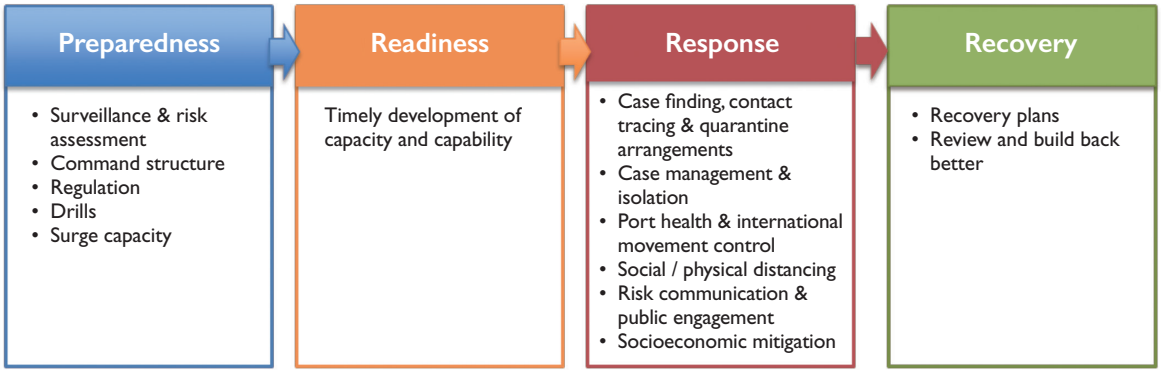

FIG 1. Conceptual framework of the enhanced system for early detection, assessment and response (S-EDAR) for emergency preparedness, readiness, response, and recovery.

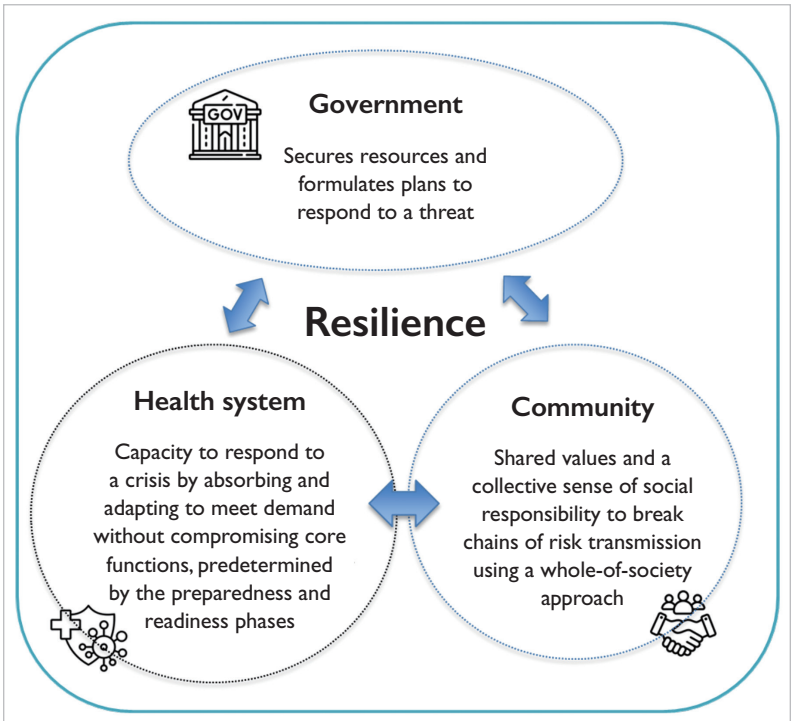

FIG 2. Building resilience through a triad of government, health system, and community.

eradication of the new public health threat. It should be integral to preparedness plans for infectious diseases and other hazards, with engagement from both the private sector and the community. The framework is designed to be comprehensive—covering emerging and re-emerging infectious diseases—and versatile enough to accommodate novel pathogens when little is known of their nature, transmission dynamics, clinical manifestations, and outcomes. Drawing on lessons learnt from the trajectory of different waves during the protracted pandemic, the framework highlighted the importance of building up resilience through a triad of government, health system, and community (Fig 2). It should be dynamic and adaptable to new scientific findings and knowledge, experiences, issues, and challenges, and be continuously reviewed for different transmission scenarios.

The extended study further highlighted the importance of the readiness stage, a concept emphasised by the World Health Organization,[2] to ensure timely development of the capacity and capability to tackle the imminent threat identified, linking effective preparedness to a rapid and efficient response. We then developed an operational readiness framework with domains for readiness assessment. Readiness plans comprise strategies and interventions to enable prompt adaptations of comprehensive 'all-threats' preparedness plans for the specific imminent threat identified for activation, mobilisation of surge capacity, and scaling up of the response according to the levels of public health emergency.

Initial domains and statements of the enhanced S-EDAR were based on findings from comparative case studies, expert workshops, key informant interviews, and inputs from international experts. These were further revised based on ratings and suggestions in the group Delphi study to reach a consensus on acceptability and feasibility, incorporating the components of readiness and health system and community resilience. We developed 37 recommendations across the four phases of preparedness, readiness, response, and recovery.

Discussion

The COVID-19 pandemic exposed the vulnerability of health systems, delivery of social care, and socio-economic systems. Each society has a unique risk profile with deficiencies within and outside the health system, and hence differences in the impact during the pandemic. This speaks for tailored preparedness, readiness, and response plans informed by contextualised public health intelligence to rapidly scale up and stand down response controls in anticipation of dynamic changes in the pandemic trajectory.

The enhanced S-EDAR offers a comprehensive and structured framework and can be applied for effective emergency management of novel infections. It is not a linear framework; when there is unanticipated emergency, a response plan needs to commence even the preparedness and readiness plan is incomplete. To prevent and better manage future pandemics, it is crucial to review and update the emergency-preparedness-readiness-response-recovery plans (EPRRP), derived from the S-EDAR. The operational readiness framework enables deliberations of the immediate actions required to adapt the preparedness plans for a prompt and effective response and enhances health system resilience. Effective implementation of EPRRP in a whole-of-government and whole-of-society response enables effective mitigation of threats. A One Health approach at national and international levels is vital to detect, prevent, and respond to zoonotic diseases with pandemic potential.

Conclusions

The enhanced S-EDAR highlights the criticality of readiness and health system and community resilience for an effective response, which was from lessons learnt in the trajectory of the different waves during the protracted COVID-19 pandemic. Building health system resilience requires strengthening of the capacity to forecast, prevent, detect, absorb, adapt, and respond to a wide range of shocks, while

maintaining essential health services and learning from the lessons for a transformation to a resilient health system. Additionally, engagement with local communities is essential for managing public health emergencies. Community resilience fosters social capital and incorporates equity and social justice principles into various public health initiatives related to preparedness and response. Communities enhance the implementation and coordination of government strategies and interventions, facilitating public health measures and education. Local communities and business enterprises complement the distribution of daily necessities, delivery of health and social care, and mitigation of the socioeconomic impact of the pandemic. Community resilience relies on shared values and a collective sense of social responsibility to break chains of transmission using a whole-of-society approach. The enhanced S-EDAR provides an important reference in building operational effectiveness for health system and community resilience.

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Disclosure

The results of this research have been previously published in:

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Flu-based and PD1-based vaccines for SARS-CoV-2: abridged secondary publication

KY Yuen *, IFN Hung, H Chen, Z Chen

KEY MESSAGES

1. In study 1, the intranasally delivered DelNS1-nCoV-receptor-binding domain (RBD) live attenuated influenza virus (LAIV) vaccine for COVID-19 demonstrated safety and immunogenicity among healthy adults who had not previously received a COVID-19 vaccine.
2. In study 2, the PD1-RBD-DNA vaccine demonstrated safety and immunogenicity among healthy adults who had not previously received a COVID-19 vaccine.
3. In study 3, the intranasally delivered DelNS1-2019-nCoV-RBD-OPT1 vaccine booster demonstrated safety among healthy individuals who had received two doses of the BNT162b2 vaccine, enhancing both pre-existing cellular and mucosal immune responses.

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Introduction

As of 2022, 9.7 billion doses of COVID-19 vaccines have been administered,[1] and 10 vaccines have been approved for emergency use by the World Health Organization.[2] These vaccines demonstrated satisfactory efficacy in preventing severe disease and death. Emerging variants have increased viral transmission and reduced vaccine effectiveness.[3-7] During the Omicron outbreak, neutralising antibody levels decreased 40-fold in individuals who had received two doses of BNT162b2, highlighting the need for a third dose.[8-10] A nebulised viral vector vaccine has shown satisfactory immunogenicity.[11]

Intranasal vaccines can stimulate mucosal immunoglobulin (Ig). Nebulised vaccines pose risks of aerosol generation and unintended spread of vectors or antigens. We previously reported that the intranasally delivered DelNS1-live attenuated influenza virus (LAIV) vaccine conferred complete protection against homologous and heterologous influenza virus challenges in mice.[12] We developed an intranasally delivered DelNS1-nCoV-receptor-binding domain (RBD) LAIV vaccine[13] and evaluated its safety and immunogenicity in COVID-19 vaccine-naïve healthy adults (study 1). Additionally, we evaluated the safety and immunogenicity of PD1-RBD-DNA vaccine via intramuscular electroporation in COVID-19 vaccine-naïve healthy adults (study 2), and the immunogenicity of the DelNS1-2019-nCoV-RBD-OPT1 booster among individuals previously vaccinated with two doses of BNT162b2 (study 3).

Methods

Study 1

A phase 1, randomised, double-blinded, placebo-controlled, dose-escalation study was conducted to evaluate the safety and immunogenicity of the DelNS1-nCoV-RBD LAIV vaccine among healthy adults aged 18 to 55 years who had not received a COVID-19 vaccine. Participants were randomly assigned (in a 4:1 ratio) to receive two doses (4 weeks apart) of low- or high-dose DelNS1-nCoV-RBD LAIV vaccine or placebo.

Reactogenicity was assessed by recording solicited local and systemic events within 14 days of each dose, unsolicited adverse events (AEs) within 28 days, AEs of special interest, and serious AEs. Reactogenicity events were graded using the toxicity scale for healthy adult and adolescent participants in vaccine trials.[14] Unsolicited AEs were graded according to the Common Terminology Criteria for Adverse Events, version 5.0. Immunogenicity—including humoral, cellular, and mucosal responses—was evaluated. Serum samples were collected at multiple time points post-vaccination. RBD-specific IgG was measured using a chemiluminescent microparticle immunoassay. Neutralising antibody titres were assessed using a microneutralisation assay. T-cell responses to an RBD peptide pool were measured using an interferon-gamma ELISpot assay.[15] Influenza A antibody responses were evaluated using a haemagglutination inhibition assay. Saliva samples were used to assess mucosal

immunity; total Ig against SARS-CoV-2 RBD was measured using an in-house assay.[16]

Study 2

A phase 1, randomised, double-blinded, placebo-controlled, dose-escalation study was conducted to evaluate the safety and immunogenicity of the PD1-RBD-DNA vaccine among healthy adults aged 18 to 55 years without prior COVID-19 vaccination. Participants were randomly assigned to receive two doses (3 weeks apart) of high-dose (2 mg) or low-dose (1 mg) PD1-RBD-DNA vaccine or placebo via intramuscular electroporation. Safety (reactogenicity, unsolicited AEs, AEs of special interest, and serious AEs) and immunogenicity (cellular and humoral responses) of the vaccine were assessed.

Study 3

A phase 2, randomised, double-blinded, placebo-controlled study was conducted to assess the immunogenicity of the intranasally delivered DelNS1-2019-nCoV-RBD-OPT1 vaccine as a booster among healthy adults aged 18 to 75 years who were SARS-CoV-2-naïve and had received two doses of BNT162b2. Participants were randomly assigned (in a 1:1 ratio) to receive two doses (3 weeks apart) of the vaccine or placebo. Safety and immunogenicity of the vaccine were evaluated.

Results

Study 1

In total, 29 participants (median age, 26 years; median body mass index, 22.1 kg/m^2) received either low-dose (n=11) or high-dose (n=12) DelNS1-nCoV-RBD LAIV vaccine or placebo (n=6). No participant discontinued vaccination due to AEs. No serious AEs or AEs of special interest occurred within 56 days. The three groups had similar rates of reactogenicity (p=0.595) and unsolicited AEs (p=0.620). One high-dose participant reported self-limiting grade 3 abdominal pain and diarrhoea within 14 days of the first dose. Serum anti-RBD IgG and neutralising antibody titres were undetectable. Median serum T-cell responses were slightly higher in the high-dose group than in the placebo group on day 14 (15 vs 0 spot-forming units [SFU]/10^6 peripheral blood mononuclear cells [PBMCs], p=0.17) and day 42 (14 days after the second vaccination) [12.5 vs 5 SFU/10^6 PBMCs, p=0.18]. They were also higher in the high-dose group than in the low-dose group on day 14 (15 vs 0 SFU/10^6 PBMCs, p=0.09) and day 42 (12.5 vs 0 SFU/10^6 PBMCs, p=0.09). Saliva total Ig against SARS-CoV-2 RBD was higher in the high-dose group than in the placebo group on day 31 (3 days after the second vaccination) [0.24 vs 0.21, p=0.046] and in both vaccine groups than in the placebo group on day 56 (28 days after the second vaccination) [0.31 vs 0.31 vs 0.15, p=0.45]. The haemagglutination inhibition titre on day 28 was also higher in the high-dose group (640 vs 320 vs 240, p=0.29 and p=0.21, respectively).

Study 2

In total, 11 participants (five men) received either high-dose (n=1; median age, 43 years) or low-dose (n=8; median age, 43 years) PD1-RBD-DNA vaccine or placebo (n=2; median age, 46 years). Three low-dose participants discontinued, including two with confirmed COVID-19. No serious AEs or AEs of special interest occurred within 50 days. Pain was reported only in the high-dose group. Most reactogenicities were grade 1 or 2; one low-dose participant experienced grade 3 malaise. Neutralising antibodies were undetectable in all groups. Anti-RBD antibody was detected in both vaccine groups after two doses. Compared with the low-dose group, the high-dose group had higher geometric mean titres (GMTs) of anti-RBD antibody (246.0 vs 38.8) and higher seroconversion rates (100% vs 25%) on day 36 and day 50 (146.5 vs 70.3 and 100% vs 50%, respectively). In the low-dose group, the GMT of anti-RBD antibody significantly increased from day 1 to day 50 (70.3 vs 25.0, p=0.0057), whereas no such antibody was detected in the placebo group. On day 22, median T-cell responses increased from 0.0 to 28.0 SFU/10^6 PBMCs in the high-dose group and from 12.5 to 48.8 SFU/10^6 PBMCs in the low-dose group; differences between groups were not significant. No increase was observed in the placebo group. By day 50, the median T-cell response significantly increased in the low-dose group from 12.5 (day 1) to 77.5 SFU/10^6 PBMCs (p=0.0018), relative to 580.0 SFU/10^6 PBMCs in the high-dose group and 182.8 SFU/10^6 PBMCs in the placebo group.

Study 3

In total, 106 participants received either the DelNS1-2019-nCoV-RBD-OPT1 vaccine (n=53) or placebo (n=53). No serious AEs or AEs of special interest were reported within 14 days of each dose. In the vaccine group, one participant reported grade 3 nasal irritation and congestion, and another reported grade 3 malaise. One participant experienced grade 3 reactogenicities. By day 50, saliva total Ig levels in the vaccine group had increased against Delta (1.2-fold), Omicron BA.1 (1.4-fold), and BA.2 (1.4-fold) variants; no change occurred in the placebo group. On day 50, neutralising antibody titres (viral microneutralisation GMTs) in the vaccine group remained stable against the ancestral strain (1.1-fold), Delta variant (0.9-fold), and Omicron BA.1 variant (1.0-fold). Median anti-RBD IgG levels slightly declined in both groups, but the decline was

significant in the placebo group (p=0.012). T-cell responses against the spike protein on day 50 were 1.2-fold in the vaccine group and 0.8-fold in the placebo group. T-cell activity increased from 46.1 to 54.7 SFU/2×10^5 PBMCs in the vaccine group but decreased from 43.2 to 31.8 SFU/2×10^5 PBMCs in the placebo group. Four vaccine recipients and three placebo recipients developed SARS-CoV-2 infection.

Discussion

Study 1

The intranasally delivered DelNS1-nCoV-RBD LAIV vaccine showed safety and immunogenicity among healthy adults. Common reactogenicities—malaise, myalgia, and sneezing—were mild and self-limiting, consistent with other LAIV vaccines.[17] Although no humoral immune response was detected, the vaccine elicited sustained cellular and mucosal responses, as evidenced by persistent increases in T-cell activity and saliva total Ig against SARS-CoV-2 in the high-dose group. Mucosal immunity is critical for preventing SARS-CoV-2 infection and transmission, particularly regarding the Omicron variant, which predominantly affects the upper respiratory tract. Local mucosal Ig protects the nasal and upper airway mucosa by blocking viral attachment, thus reducing replication and spread. Current injectable COVID-19 vaccines do not induce mucosal immunity and fail to prevent nasal infection or asymptomatic transmission.[18,19] However, intranasal administration of single-dose chimpanzee adenovirus-vectored vaccine,[20] helper-dependent adenoviral vector vaccine,[21] and RBD nanoparticles in animal models has elicited strong mucosal and systemic immunity against SARS-CoV-2. These approaches have demonstrated virus-specific $CD8^+$ T-cell responses and increased numbers of interferon-gamma-producing cells and IgA-secreting B cells in the nasal mucosa, trachea, lungs, and spleen, thus protecting both upper and lower respiratory tracts. Previous mouse model studies demonstrated that intranasal influenza-based vaccines (LAIV-CA4-RBD and LAIV-HK68-RBD) and the intramuscular PD1-RBD-DNA vaccine induced robust mucosal and systemic immunity. Bronchoalveolar lavage IgA/IgG levels and polyfunctional memory CD8+ T cells in the lungs provided effective protection against SARS-CoV-2 infection in upper and lower respiratory tracts; they also cross-neutralised variants of concern.[13] Accordingly, the intranasal vaccine may serve as a booster for individuals previously vaccinated with injectable COVID-19 vaccines or those who have recovered from infection.

Omicron variant infections are characterised by reduced replication efficiency and fusion activity, milder symptoms, and higher transmissibility. Neutralising antibodies generated after vaccination wane within 6 months. Winter surges of SARS-CoV-2 in the northern hemisphere may requires annual vaccination. The DelNS1-nCoV-RBD LAIV could serve as a combined COVID-19 and influenza vaccine, potentially improving uptake, particularly among children. Additionally, it can be administered at home and provided in a prefilled syringe. In contrast, aerosolised vaccines require 30 to 60 seconds of administration and a specialised nebuliser.[11]

Study 2

The PD1-RBD-DNA vaccine demonstrated safety; no participant withdrew due to AEs. Although neutralising antibodies were not detected after full vaccination, anti-RBD antibody levels had significantly increased by day 50 in the low-dose group. The vaccine also enhanced T-cell responses in both vaccine groups by day 50.

Dendritic cells activate both adaptive and innate immunity.[12] The PD1-RBD-DNA vaccine leverages PD1-ligand interactions to improve antigen uptake by dendritic cells, leading to antigen-specific immune activation.[8] In mice, two doses of the vaccine induced anti-RBD and neutralising antibodies, as well as cellular and mucosal immune responses by day 30.[9] Similar humoral and cellular responses were observed in vaccinated adults. This antigen-PD1 fusion strategy has also been applied to an HIV vaccine (ICVAX), which combines soluble PD1 with Gap-P41.[8]

The RBD of the spike protein is a key SARS-CoV-2 antigen that induces neutralising antibodies. After PD1-RBD-DNA vaccination, only anti-RBD antibodies were detected, whereas in an animal study, neutralising activity was observed after two doses. This discrepancy may be due to differences in assay methods: the animal study used a pseudovirus-based neutralisation assay, whereas we used a live virus microneutralisation assay. Furthermore, the animal study used a much higher relative dose (2.5 vs 0.02 mg/kg).

Study 3

The intranasally delivered DelNS1-2019-nCoV-RBD-OPT1 vaccine demonstrated safety as a booster in healthy adults previously vaccinated with two doses of BNT162b2; no serious AEs were reported. In the vaccine group, saliva total Ig levels against Delta, BA.1, and BA.2 variants increased by day 50. The vaccine group also maintained pre-existing neutralising antibodies—induced by BNT162b2—against the ancestral strain, Delta, and BA.1 variants. The T-cell response on day 50 was 1.2-fold in the vaccine group, compared with 0.8-fold in the placebo group.

In an animal study, mice receiving one BNT162b2 dose followed by one DelNS1-2019-nCoV-RBD-OPT1 dose exhibited higher levels of bronchoalveolar lavage fluid IgA, serum neutralising antibodies, and T-cell responses than those given two BNT162b2 doses.[13] These results suggest that the DelNS1-2019-nCoV-RBD-OPT1 vaccine enhances immune responses primed by the BNT162b2 vaccine. Vaccine-induced immunity against SARS-CoV-2, particularly against the Omicron variant, begins to wane within 6 months.[22-25] As SARS-CoV-2 continues to evolve and co-circulate with influenza, annual COVID-19 and influenza vaccination may be necessary.

Repeated homologous mRNA vaccination may diminish immunogenicity.[26-28] mRNA vaccines rely on cellular uptake and intracellular antigen expression. Modified mRNA can be recognised by immune cells, potentially triggering an anti-mRNA immune response.[26,27] In animal models, such immunity substantially reduced antigen expression after booster doses.[28] Heterologous vaccination may help overcome this limitation. The intranasally delivered DelNS1-2019-nCoV-RBD-OPT1 vaccine could serve as a booster for those who have received three or more doses of mRNA vaccines.

Intranasal vaccines can induce mucosal immune responses, which are essential for preventing viral infection and transmission. Animal studies have demonstrated that intranasal vaccines—including RBD nanoparticles, LAIV-CA4-RBD, adenoviral vectors, and spike-based vaccines—elicit strong mucosal immunity against SARS-CoV-2.[13,21,29,30] However, the DelNS1-2019-nCoV-RBD-OPT1 vaccine showed only moderate mucosal responses to Delta and Omicron variants. This difference may be due to the use of saliva samples in our study versus the use of bronchoalveolar lavage fluid in the animal study.[13] Among individuals given one intramuscular mRNA vaccine dose followed by an intranasal adenovirus-vectored vaccine dose, mucosal antibodies were detectable in saliva.[31]

Conclusions

Both the intranasally delivered DelNS1-nCoV-RBD LAIV vaccine and the PD1-RBD-DNA vaccine showed safety and immunogenicity among healthy adults. The intranasally delivered DelNS1-2019-nCoV-RBD-OPT1 vaccine showed safety as a booster dose and enhanced pre-existing mucosal and cellular immune responses among healthy adults who had received two doses of an mRNA vaccine.

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Disclosure

The results of this research have been previously published in:

1. Zhang R, Chan KH, Wang P, et al. A phase 1, randomized, double-blinded, placebo-controlled and dose-escalation study to evaluate the safety and immunogenicity of the intranasal DelNS1-nCoV-RBD LAIV for COVID-19 in healthy adults. Vaccines (Basel) 2023;11:723.

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Single and combined anti-COVID-19 drugs among hospitalised patients: abridged secondary publication

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KEY MESSAGES

1. Early initiation (within 2 days of hospital admission) of a 5-day remdesivir regimen was associated with improved clinical outcomes and a reduced risk of in-hospital death among patients with moderate COVID-19 who did not require oxygen therapy upon admission.
2. Initiation of remdesivir prior to or concurrently with dexamethasone was associated with a significantly shorter time to clinical improvement and seroconversion as well as a lower risk of in-hospital death among patients hospitalised with moderate COVID-19.
3. Early administration of interferon-β-1b, either alone or in combination with oral ribavirin, was associated with improved survival and reduced need for mechanical ventilation and intensive care among patients with mild to moderate COVID-19.
4. Cardiovascular disorders were the most common complications among post-discharge patients, followed by nephrological and hepatic, haematological, and respiratory disorders.

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Introduction

In response to the COVID-19 pandemic, various pharmaceutical agents were repurposed for the management of hospitalised patients. These included antivirals such as lopinavir-ritonavir, ribavirin, and remdesivir, as well as immunomodulators such as corticosteroids and interferons. The safety and efficacy of these drugs, which act via different mechanisms, may depend on initiation timing and disease severity, given the progression of infection from viral replication to a host hyperinflammatory response. This study aimed to evaluate the effectiveness and adverse effects of antiviral and immunomodulatory drugs in hospitalised patients with COVID-19 in Hong Kong.

Methods

Data from consecutive patients with confirmed COVID-19 admitted to public hospitals in Hong Kong between 6 December 2020 and 31 January 2021 were retrospectively retrieved and analysed. Between 6 December 2020 and 21 January 2021, all patients with a positive polymerase chain reaction result were admitted to public hospitals regardless of disease severity. Patients were categorised based on treatments received during hospitalisation and the time of drug initiation since symptom onset. Patients were followed up until in-hospital death, discharge, treatment crossover, or censoring, whichever occurred first. Outcomes included a composite of death, invasive mechanical ventilation (IMV), or admission to the intensive care unit (ICU) or high dependency unit, as well as time from hospital admission to discharge. Discharge was based on two consecutive negative tests 24 hours apart and clinical assessment by the attending physician.

To determine remdesivir effectiveness, patients who received early intravenous remdesivir (within the first 2 days of admission) were compared with those who received remdesivir after 2 days of admission or who did not receive remdesivir. To determine the effectiveness of combined remdesivir and dexamethasone, patients who received remdesivir before or on the same day as dexamethasone initiation were compared with those who received remdesivir after dexamethasone initiation or those who did not receive remdesivir. To determine adverse events and complications of COVID-19, patients who received lopinavir-ritonavir, ribavirin, remdesivir, interferon-β-1b, dexamethasone, and/or corticosteroids were compared with those who did

not receive any of these medications.

Disease severity was assessed using the World Health Organization (WHO) Clinical Progression Scale (CPS), ranging from 0 (uninfected) to 10 (death).[1] Study outcomes included time to clinical improvement (defined as an improvement of ≥1 point on the WHO CPS), hospital discharge (WHO CPS score ≤3), recovery without oxygen therapy (WHO CPS score ≤4), viral clearance (first negative polymerase chain reaction result), low viral load (cycle threshold value ≥35), first positive immunoglobulin G (IgG) antibody, composite outcome of in-hospital death or IMV (WHO CPS score ≥7), composite outcome of in-hospital death, IMV, or ICU admission, in-hospital death, acute respiratory distress syndrome, hospital length of stay (LOS), and mean change in WHO CPS score from baseline to day 90. Adverse events and complications included newly diagnosed clinical conditions within 18 months after hospital discharge, all-cause mortality, and any disorders. Patients with a prior diagnosis of a specific outcome were excluded from analyses of that outcome. Incidence (events per 10 000 person-years) was estimated for each outcome among cases, controls, and both.

Results

Among patients with mild to moderate COVID-19, use of interferon-β-1b was associated with an improved composite outcome (odds ratio [OR]=0.55, 95% confidence interval [CI]=0.38-0.80) and a shorter LOS (-8.8 days), compared with non-use. Use of oral ribavirin within 7 days of symptom onset was associated with a lower risk of the composite outcome (OR=0.51, 95% CI=0.29-0.90). Use of lopinavir-ritonavir, corticosteroids, and antibiotics did not demonstrate consistent clinical benefit. Co-administration of interferon-β-1b and ribavirin was associated with an improved composite outcome (OR=0.50, 95% CI=0.32-0.78) and a shorter LOS (-2.35 days), compared with interferon-β-1b monotherapy. The combination of interferon-β-1b with lopinavir-ritonavir, with or without ribavirin, yielded results comparable to interferon-β-1b monotherapy in terms of the composite outcome (Table 1).

Among patients with moderate COVID-19, early remdesivir treatment was associated with significantly lower WHO CPS scores from day 30 onwards, compared with matched controls. Early remdesivir use was associated with a significantly shorter time to clinical improvement (median, 13 vs 14 days; hazard ratio [HR]=1.14, 95% CI=1.01-1.29) and a shorter LOS (-2.56 days), as well as a marginally lower risk of in-hospital death (HR=0.58, 95% CI=0.34-0.99). Early remdesivir treatment was also associated with a significantly greater increase in cycle threshold value on day 7. Remdesivir use was significantly associated with a shorter time to achieving a low viral load (median, 9 vs 10 days; HR=1.51, 95% CI=1.24-1.83) and a positive IgG antibody result (median, 6 vs 7 days; HR=1.50, 95% CI=1.31-1.70), compared with matched controls (Table 2).

Compared with dexamethasone alone, combined use of remdesivir and dexamethasone was associated with a significantly shorter time to clinical improvement (median, 12 vs 13 days; HR=1.23, 95% CI=1.02-1.49), a significantly shorter time to a positive IgG antibody result (median, 5 vs 6 days; HR=1.22, 95% CI=1.02-1.46), lower WHO CPS scores from day 5 onwards, a shorter LOS among survivors by 2.65 days, and lower risks of composite outcomes and in-hospital death (HR=0.59, 95% CI=0.36-0.98) [Table 2]. In addition, more rapid recovery was observed in the subgroup of patients who received remdesivir prior to dexamethasone, compared with those who received remdesivir later or not at all. Faster seroconversion and significantly lower risks of composite outcomes and acute respiratory distress syndrome were also observed among those who received remdesivir prior to or concurrently with dexamethasone, compared with those who received remdesivir after dexamethasone.

During the 18-month follow-up period after hospital discharge, patients who had received any of the medications generally showed higher crude incidences of all-cause mortality and various disorders, compared with those who had not received such treatments. Cardiovascular disorders were most common, followed by nephrological and hepatic, haematological, and respiratory disorders (Table 3).

Discussion

In patients with mild to moderate COVID-19, use of interferon-β-1b was associated with improved survival, reduced need for IMV and ICU admission, and a shorter LOS. Compared with the use of lopinavir-ritonavir alone, the combination of lopinavir-ritonavir, ribavirin, and interferon-β-1b was associated with faster symptom resolution, more rapid viral clearance, and reduced LOS in patients with mild to moderate COVID-19.[2] However, the triple-drug regimen did not demonstrate superiority over interferon-β-1b monotherapy. The absence of clinical benefit from lopinavir-ritonavir was consistent with findings from other major trials, possibly due to the ineffectiveness of an acceptable dosage for treating COVID-19.

In a randomised trial involving over 80% of patients hospitalised with moderate COVID-19 who did not require supplemental oxygen, remdesivir was initiated at a median of 2 days after admission. A 5-day course of remdesivir was associated with improved clinical status on days 11 and 14, compared

TABLE 1. Composite outcome of death, invasive mechanical ventilation, or intensive care unit admission among symptomatic COVID-19 patients.

Outcome	No treatment		Treatment		Adjusted odds ratio (95% confidence interval)	Adjusted P value
	Total	No. (%) of events	Total	No. (%) of events		
Interventions initiated regardless of timing						
Antivirals						
Lopinavir-ritonavir	3087	32 (1.0)	1436	51 (3.6)	1.27 (0.81-1.98)	1.000
Ribavirin	3285	52 (1.6)	1238	31 (2.5)	0.58 (0.36-0.92)	0.009
Immunomodulators						
Corticosteroids	3865	7 (0.2)	658	76 (11.6)	1.74 (1.17-2.58)	<0.001
Dexamethasone	3865	7 (0.2)	573	71 (12.4)	3.49 (2.34-5.20)	<0.001
Hydrocortisone	3865	7 (0.2)	96	15 (15.6)	0.27 (0.11-0.64)	<0.001
Methylprednisolone	3865	7 (0.2)	6	2 (33.3)	3.79 (0.31-46.13)	1.000
Prednisolone	3865	7 (0.2)	37	3 (8.1)	0.88 (0.15-5.27)	1.000
Interferon-β-1b	2568	10 (0.4)	1955	73 (3.7)	0.55 (0.38-0.80)	<0.001
Antibiotics	2946	5 (0.2)	1577	78 (4.9)	2.74 (1.56-4.80)	<0.001
Interventions initiated within 7 days of symptom onset						
Antivirals						
Lopinavir-ritonavir	3087	32 (1.0)	1109	40 (3.6)	1.40 (0.88-2.25)	0.370
Ribavirin	3285	52 (1.6)	884	19 (2.1)	0.51 (0.29-0.90)	0.010
Immunomodulators						
Corticosteroids	3865	7 (0.2)	276	42 (15.2)	1.57 (0.97-2.55)	0.084
Dexamethasone	3865	7 (0.2)	225	37 (16.4)	3.46 (2.10-5.72)	<0.001
Hydrocortisone	3865	7 (0.2)	42	6 (14.3)	0.31 (0.09-0.99)	0.046
Methylprednisolone	3865	7 (0.2)	2	0	-	-
Prednisolone	3865	7 (0.2)	14	1 (7.1)	-	-
Interferon-β-1b	2568	10 (0.4)	1581	60 (3.8)	0.60 (0.41-0.88)	0.002
Antibiotics	2946	5 (0.2)	1128	63 (5.6)	3.10 (1.76-5.43)	<0.001
Interventions initiated >7 days after symptom onset						
Antivirals						
Lopinavir-ritonavir	3087	32 (1.0)	327	11 (3.4)	1.01 (0.52-1.94)	1.000
Ribavirin	3285	52 (1.6)	354	12 (3.4)	0.66 (0.36-1.22)	0.556
Immunomodulators						
Corticosteroids	3865	7 (0.2)	382	34 (8.9)	1.85 (1.20-2.87)	<0.001
Dexamethasone	3865	7 (0.2)	348	34 (9.8)	3.50 (2.26-5.43)	<0.001
Hydrocortisone	3865	7 (0.2)	54	9 (16.7)	0.24 (0.07-0.79)	0.008
Methylprednisolone	3865	7 (0.2)	4	2 (50.0)	5.51 (0.44-69.38)	0.556
Prednisolone	3865	7 (0.2)	23	2 (8.7)	0.91 (0.08-10.47)	1.000
Interferon-β-1b	2568	10 (0.4)	374	13 (3.5)	0.39 (0.16-0.91)	0.018
Antibiotics	2946	5 (0.2)	449	15 (3.3)	1.86 (0.82-4.24)	0.322
Composite outcome						
Interferon-β-1b monotherapy	-	-	161	9 (5.6)	Reference	-
Interferon-β-1b + ribavirin			634	16 (2.5)	0.50 (0.32-0.78)	<0.001
Initiated within 3 days of symptom onset	-	-	127	4 (3.1)	1.36 (0.67-2.76)	0.667
Initiated 3 to 7 days after symptom onset	-	-	362	8 (2.2)	Reference	-
Initiated >7 days after symptom onset	-	-	145	4 (2.8)	0.63 (0.26-1.53)	0.489
Interferon-β-1b + lopinavir-ritonavir			752	35 (4.7)	0.88 (0.61-1.28)	1.000
Initiated within 3 days of symptom onset	-	-	194	11 (5.7)	1.14 (0.67-1.96)	1.000
Initiated 3 to 7 days after symptom onset	-	-	424	18 (4.2)	Reference	-
Initiated >7 days after symptom onset	-	-	134	6 (4.5)	0.73 (0.40-1.33)	0.467
Interferon-β-1b + lopinavir-ritonavir + ribavirin			408	13 (3.2)	1.11 (0.77-1.59)	1.000
Initiated within 3 days of symptom onset	-	-	123	8 (6.5)	4.47 (1.46-13.68)	0.005
Initiated 3 to 7 days after symptom onset	-	-	227	3 (1.3)	Reference	-
Initiated >7 days after symptom onset	-	-	58	2 (3.4)	0.70 (0.15-3.25)	1.000

TABLE 2. Comparison of outcomes between COVID-19 patients who received early remdesivir treatment and those who did not, and between those who received remdesivir and dexamethasone and those who received dexamethasone alone.

Outcome	% of patients	% of patients	Hazard ratio (95% confidence interval)	P value
	Early remdesivir	**Control**		
Clinical improvement (≥1 score on WHO CPS)	96.3	84.0	1.14 (1.01-1.29)	0.038
Hospital discharge (score ≤3)	94.0	81.3	1.06 (0.93-1.20)	0.372
Recovery (score ≤4)	83.6	59.6	1.16 (0.87-1.57)	0.314
Viral clearance (first negative PCR result)	36.1	30.4	1.06 (0.87-1.30)	0.552
Low viral load (Ct value ≥35)	40.6	28.1	1.51 (1.24-1.83)	<0.001
Immunoglobulin G antibody	94.0	80.4	1.50 (1.31-1.70)	<0.001
In-hospital death or invasive mechanical ventilation (score ≥7)	10.7	11.3	0.95 (0.67-1.37)	0.796
In-hospital death or invasive mechanical ventilation (score ≥7) or intensive care unit admission	5.9	6.8	0.92 (0.55-1.53)	0.747
In-hospital death, invasive mechanical ventilation, vasopressors, dialysis, or ECMO (score ≥9)	6.0	6.6	0.87 (0.55-1.38)	0.556
In-hospital death (score=10)	4.3	6.7	0.58 (0.34-0.99)	0.045
	Remdesivir-dexamethasone	**Dexamethasone**		
Clinical improvement (≥1 score on WHO CPS)	92.1	88.1	1.23 (1.02-1.49)	0.032
Hospital discharge (score ≤3)	90.6	87.1	1.18 (0.97-1.43)	0.090
Recovery (score ≤4)	79.2	74.5	0.94 (0.72-1.23)	0.663
Viral clearance (first negative PCR result)	32.7	31.6	1.29 (0.93-1.79)	0.126
Low viral load (Ct value ≥35)	31.3	31.2	1.25 (0.91-1.72)	0.177
Immunoglobulin G antibody	97.1	91.7	1.22 (1.02-1.46)	0.029
In-hospital death or invasive mechanical ventilation (score ≥7)	14.5	18.8	0.67 (0.46-0.96)	0.031
In-hospital death or invasive mechanical ventilation (score ≥7) or intensive care unit admission	11.3	17.4	0.64 (0.43-0.97)	0.034
In-hospital death (score=10)	7.7	11.6	0.59 (0.36-0.98)	0.042
Acute respiratory distress syndrome	10.7	8.2	0.99 (0.59-1.66)	0.965

Abbreviations: Ct=cycle threshold, ECMO=extracorporeal membrane oxygenation, PCR=polymerase chain reaction, WHO CPS=World Health Organization Clinical Progression Scale

with standard care, although the time to clinical improvement did not significantly differ.[3] Our findings suggested that early remdesivir treatment resulted in more rapid clinical improvement (reflected by shorter hospital LOS) and lower mortality risk. Such findings may have important implications in healthcare settings with limited resources.

Similarly, combined use of remdesivir and dexamethasone was found to reduce mortality and need for mechanical ventilation, compared with standard care.[4] Our subgroup analyses indicated that remdesivir administration prior to dexamethasone was associated with better clinical outcomes, compared with delayed or no antiviral use. The effect may be more pronounced when remdesivir is introduced prior to or concurrently with dexamethasone, rather than at a later stage. These findings are consistent with the progression of viral infections, where early antiviral administration may help to inhibit viral replication and potentially prevent a cytokine storm. Anti-inflammatory agents subsequently mitigate the hyperinflammatory response, if it arises.

Higher incidences of all-cause mortality and various disorders in the drug exposure group, compared with the non-exposure group, may be explained by the fact that patients who received antivirals and/or immunomodulators had more severe disease at admission and/or more disease progression during hospitalisation, thus requiring initiation of such treatments. Common complications of COVID-19 include thrombosis, cardiovascular events, and acute kidney or liver injury.[5]

Conclusions

Early administration of interferon-β-1b, either alone or in combination with oral ribavirin, was associated with a reduced risk of death or serious complications

TABLE 3. Crude incidences of all-cause mortality and other disorders among COVID-19 patients after discharge.

Outcome	Estimated crude incidence (95% confidence interval)	Person-years
Overall (n=9511)		
All-cause mortality	32.23 (21.74-46.00)	9309
Neurological disorder	40.64 (28.62-56.02)	9104
Psychiatric disorder	157.62 (132.24-186.45)	8628
Respiratory disorder	320.49 (282.57-362.08)	8050
Cardiovascular disorder	3831.70 (3599.12-4075.37)	2639
Haematological disorder	455.76 (408.73-506.71)	7504
Endocrine disorder	165.95 (139.33-196.18)	8255
Nephrological and hepatic disorder	724.96 (664.93-788.95)	7407
Gastrointestinal disorder	19.44 (11.52-30.72)	9260
Dermatological disorder	56.08 (41.75-73.73)	9095
Lopinavir-ritonavir (n=1795)		
All-cause mortality	40.67 (18.60-77.20)	2213
Neurological disorder	46.38 (22.24-85.29)	2156
Psychiatric disorder	246.88 (182.64-326.38)	1985
Respiratory disorder	368.39 (284.91-468.68)	1792
Cardiovascular disorder	6460.01 (5727.97-7259.69)	437
Haematological disorder	1007.45 (857.39-1176.21)	1588
Endocrine disorder	192.51 (134.83-266.52)	1870
Nephrological and hepatic disorder	1263.28 (1098.57-1445.73)	1670
Gastrointestinal disorder	18.19 (4.96-46.56)	2199
Dermatological disorder	88.60 (53.34-138.36)	2145
Ribavirin (n=2117)		
All-cause mortality	49.08 (24.50-87.81)	2241
Neurological disorder	32.23 (12.96-66.41)	2172
Psychiatric disorder	193.22 (137.40-264.14)	2018
Respiratory disorder	366.63 (283.55-466.44)	1800
Cardiovascular disorder	5013.46 (4374.22-5719.83)	441
Haematological disorder	736.69 (607.13-885.70)	1534
Endocrine disorder	216.28 (155.21-293.41)	1896
Nephrological and hepatic disorder	1199.52 (1034.59-1383.25)	1576
Gastrointestinal disorder	27.00 (9.91-58.76)	2222
Dermatological disorder	54.91 (28.37-95.91)	2186
Remdesivir (n=787)		
All-cause mortality	68.48 (18.66-175.34)	584
Neurological disorder	143.21 (61.83-282.18)	559
Psychiatric disorder	224.29 (111.97-401.32)	490
Respiratory disorder	955.89 (661.98-1335.77)	356
Cardiovascular disorder	1331.49 (535.33-2743.38)	53
Haematological disorder	455.47 (183.12-938.44)	154
Endocrine disorder	389.23 (222.48-632.09)	411
Nephrological and hepatic disorder	1035.94 (703.87-1470.44)	299
Gastrointestinal disorder	17.35 (0.44-96.68)	576
Dermatological disorder	53.14 (10.96-155.31)	565

TABLE 3. (cont'd)

Outcome	Estimated crude incidence (95% confidence interval)	Person-years
Interferon-β-1b (n=3229)		
All-cause mortality	68.97 (43.72-103.49)	3335
Neurological disorder	55.89 (33.12-88.33)	3221
Psychiatric disorder	215.68 (166.10-275.42)	2967
Respiratory disorder	481.89 (400.81-574.55)	2573
Cardiovascular disorder	6203.79 (5581.94-6875.98)	585
Haematological disorder	1111.06 (972.97-1263.26)	2097
Endocrine disorder	269.27 (211.06-338.56)	2711
Nephrological and hepatic disorder	1264.09 (1123.24-1417.71)	2310
Gastrointestinal disorder	33.28 (16.61-59.54)	3306
Dermatological disorder	92.98 (62.73-132.73)	3227
Dexamethasone (n=1358)		
All-cause mortality	93.47 (46.66-167.24)	1177
Neurological disorder	98.20 (49.02-175.70)	1120
Psychiatric disorder	285.47 (189.69-412.58)	981
Respiratory disorder	955.39 (743.35-1209.11)	722
Cardiovascular disorder	5672.22 (4453.92-7120.96)	130
Haematological disorder	261 578.29 (224 050.90-303 593.27)	7
Endocrine disorder	397.71 (273.77-558.53)	830
Nephrological and hepatic disorder	2175.11 (1809.15-2593.37)	570
Gastrointestinal disorder	17.24 (2.09-62.29)	1160
Dermatological disorder	132.84 (74.35-219.11)	1129
Corticosteroids (n=1517)		
All-cause mortality	88.14 (45.54-153.96)	1361
Neurological disorder	84.98 (42.42-152.05)	1294
Psychiatric disorder	273.52 (185.84-388.23)	1133
Respiratory disorder	899.88 (710.17-1124.70)	856
Cardiovascular disorder	6034.19 (4839.60-7434.29)	146
Haematological disorder	19 167.97 (16 552.45-22 079.41)	100
Endocrine disorder	378.42 (266.44-521.60)	978
Nephrological and hepatic disorder	2113.33 (1781.16-2489.47)	677
Gastrointestinal disorder	22.38 (4.62-65.40)	1341
Dermatological disorder	130.11 (75.80-208.32)	1307
Without any antivirals (n=5469)		
All-cause mortality	11.70 (4.29-25.46)	5130
Neurological disorder	29.61 (16.57-48.84)	5066
Psychiatric disorder	108.24 (81.08-141.59)	4896
Respiratory disorder	219.59 (179.60-265.83)	4782
Cardiovascular disorder	3094.61 (2848.68-3356.09)	1887
Haematological disorder	169.95 (135.37-210.68)	4884
Endocrine disorder	107.42 (80.23-140.87)	4841
Nephrological and hepatic disorder	421.97 (364.10-486.42)	4503
Gastrointestinal disorder	9.77 (3.17-22.81)	5116
Dermatological disorder	33.71 (19.64-53.97)	5043

in patients with mild to moderate COVID-19; no such association was observed after combined use of lopinavir and ritonavir. Among patients with moderate COVID-19, initiation of a 5-day course of remdesivir within 2 days of admission was associated with substantial clinical and virological benefits. Among dexamethasone users, early or concurrent initiation of remdesivir was superior to delayed or no remdesivir use. When drug supply and healthcare resources permit, early remdesivir treatment should be offered to hospitalised patients with COVID-19. The combination of remdesivir and dexamethasone as well as the initiation of remdesivir prior to dexamethasone are supported.

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Disclosure

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Grid monitoring of SARS-CoV-2 in sewage for early warning of community outbreaks: abridged secondary publication

T Zhang*, Y Deng, GM Leung, LLM Poon, HM Tun, XQ Xu, XW Zheng

KEY MESSAGE

Sewage surveillance for SARS-CoV-2 may offer a cost-effective approach to measuring infections at the population level. It can uncover hidden transmission chains and serve as a supplementary tool to clinical testing to support public health actions.

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Introduction

Sewage surveillance for SARS-CoV-2 may serve as an early-warning, cost-effective, community-level measure to guide public health interventions for the control of COVID-19.[1] Infected individuals shed the virus in faeces, with reported positivity rates ranging from 15.3% to 100%, regardless of symptom presentation.[2] The presence of SARS-CoV-2 RNA in sewage was first reported in the sewersheds of Amersfoort, Netherlands, in April 2020.[3] Subsequent studies revealed similar findings.[4-6] Viral signals in sewage are strongly correlated with the number of clinical cases and may provide early warning of SARS-CoV-2 circulation at the population level, with an estimated lead time of 6 to 8 days or 4 to 10 days ahead of clinical testing results.[7,8] Sewage surveillance for SARS-CoV-2 has been adopted in over 50 countries.[9]

Methods

In Hong Kong, sewage surveillance was first implemented at residential care homes during the fourth wave in October 2020. It was subsequently extended to ad hoc sites at residential buildings and city blocks with infection clusters in December 2020, and later to fixed monitoring sites for early warning after the fourth wave subsided in March 2021. The number of sampling points exceeded 1500.

Virus concentration and extraction and quantification of viral RNA were performed using reverse transcription quantitative polymerase chain reaction; samples were then classified as negative or positive based on the results for two primers. A primer was classified as 'having a signal' if a cycle threshold value ≤40 was observed in at least one reaction, as negative if no signal was detected for both primers or only one showed a signal, or as positive if both primers showed a signal.

Results

Sewage testing results were largely consistent with clinical testing results. To monitor the re-emergence of SARS-CoV-2 circulation within communities, sewage samples were collected from 26 sites across Hong Kong. Sewage surveillance successfully detected the upward trend in clinical cases during the fourth wave, beginning in mid-November 2020. From December 2020 to February 2021, routine sewage testing at the 26 sites was temporarily redirected to estates with identified infection clusters. The results served as the basis for public health actions including compulsory testing operations at selected buildings and locations, leading to the identification of over 50 confirmed cases and the likely interruption of hidden transmission chains. As the fourth wave subsided in February 2021, routine monitoring at the 26 sites resumed and revealed a downward trend.

Discussion

The identification of 10 COVID-19 cases at Choi Wan (II) Estate between December 2020 and January 2021 was the first instance in Hong Kong, in which community cases were uncovered through compulsory testing operations prompted by sewage test results. Between December 2020 and March 2021, the government conducted compulsory testing at over 110 buildings with positive sewage results, uncovering >50 hidden cases. These findings provided a basis for statutory public health actions to identify infected individuals and enable timely

interventions such as isolation and treatment at the community level.

Given the limitations of sewage surveillance (the random nature of sampling, temporal variation in viral excretion, and the variable faecal positivity rates among patients), it is difficult to determine the precise number of infected individuals in a given sewershed. Nonetheless, sewage surveillance demonstrated predictive value and practical utility in guiding public health actions for early identification and isolation of infected individuals and their close contacts. The findings of this study led to the adoption of sewage surveillance strategies in other countries under various epidemiological conditions.

Conclusions

In Hong Kong, sewage surveillance for SARS-CoV-2 has provided early warning of COVID-19 outbreaks, tracked community transmission trends, and supported monitoring efforts at estates with infection clusters.

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Disclosure

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Whole genome stability of SARS-CoV-2: abridged secondary publication

MH Wang *, MKC Chong, ELY Wong, PKS Chan, Z Chen, CKC Lai, RCW Chan

KEY MESSAGES

1. Understanding virus evolution is important for pandemic preparedness and response. Characterisation of genome stability of SARS-CoV-2 enables evaluation of the virus's evolutionary rate and the potential risk of COVID-19 vaccine breakthrough.
2. The receptor-binding domain region of the spike protein was associated with a significant risk of COVID-19 vaccine breakthrough.

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Introduction

The continuous evolution of SARS-CoV-2 has generated successive epidemic waves, posing a major threat to COVID-19 prevention and control.[1] Evaluations of viral evolutionary traits help predict future trajectories and support assessment of the health burden and disease risks posed by emerging variants.[2] The substitution rate, or evolutionary rate, is a key metric for understanding the evolutionary characteristics of a species.[3] At the genomic level, the substitution rate of SARS-CoV-2 aggregates information across its constituent proteins.[4] However, various genes and genomic regions are subject to different degrees of immune pressure. For example, surface proteins or epitope sites of the influenza virus tend to have more mutations that lead to antigenic escape or positive selection.[5] Therefore, analyses of segment-specific substitution rates may better reveal the evolutionary characteristics of SARS-CoV-2. Such information is valuable for monitoring and assessing the risks posed by circulating variants. This study aimed to evaluate substitution rates across specific genome segments of SARS-CoV-2.

Methods

The dataset used included 73 062 SARS-CoV-2 sequences retrieved from the Global Initiative on Sharing All Influenza Data[6] and 110 viral genomes sequenced at the Prince of Wales Hospital, Hong Kong SAR between March 2020 and March 2022. Substitution rates were estimated using BEAST v1.10 for Bayesian phylogenetic analysis.[7]

We developed a method to evaluate the effect of mutations in major immunodominant regions on the reduction of vaccine effectiveness at the population level.[8] Cumulative genetic distance was calculated between the antigen sequences of the COVID-19 of circulating viruses. Real-world vaccine effectiveness data from observational studies were collected. A model was then constructed to capture the relationship between genetic mutations and vaccine protection.[8]

Results

During the early phase of the pandemic, cumulative mutation activity across the SARS-CoV-2 genome was profiled over time. Increasing trends in mutation activity were observed, particularly in the S protein, ORF1b, and the N protein.[9] The basic framework for profiling mutation activity was based on average genetic distance from the ancestral Wuhan strain.

The estimated genomic-level substitution rate was 0.86 (95% confidence interval [CI]=0.76-0.96) $\times 10^{-3}$ substitutions per nucleotide site per year (s/n/y), consistent with values reported in the literature.[4,10] The highest substitution rates were observed in the N and ORF8 proteins at 2.27 and 2.19 $\times 10^{-3}$ s/n/y, respectively—approximately 2.5-fold higher than the genomic average, followed by the S protein at 1.64 $\times 10^{-3}$ s/n/y—approximately 1.9-fold higher than the genomic average, and then the E and ORF10 proteins at 0.17 and 0.35 $\times 10^{-3}$ s/n/y, respectively.

Based on the vaccine effectiveness–genetic distance model, as of March 2022, a single mutation in the receptor-binding domain region corresponded to an absolute reduction in vaccine effectiveness of 5.2% (95% CI=2.4-8.0) for mRNA vaccines and 15.8% (95% CI=12.4-19.3) for inactivated vaccines.

Discussion

Genome stability of SARS-CoV-2 was characterised on a gene segment basis. The N, ORF8, and S proteins were identified to be the fastest-evolving among all evaluated genomic segments. The receptor-binding domain region of the spike protein was associated with a significant risk of COVID-19 vaccine breakthrough. All results presented should be interpreted with regard to the time of data collection.

Conclusions

Substitution rates in the SARS-CoV-2 genome vary. Conserved genomic segments containing epitopes may serve as potential vaccine antigen candidates. Regular reporting on viral genome stability may assist health authorities in understanding local viral genetic diversity and transmission patterns. Regular estimation of vaccine protection levels for both COVID-19 and influenza may support timely issuance of health alerts and allocation of hospital resources.

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Disclosure

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SARS-CoV-2 antibodies for specificity and function in clinical infection and asymptomatic cases: abridged secondary publication

S Valkenburg *, JT Wu, K Leung, M Peiris

KEY MESSAGES

1. Antibody responses after SARS-CoV-2 infection are strongest against nucleocapsid, followed by ORF8, ORF3d, and ORF7a, surpassing the spike protein as a diagnostic target.
2. ORF8 is a sensitive and specific diagnostic target; use of combined antigens can further enhance specificity.
3. Children exhibit lower-magnitude antibody responses but demonstrate higher avidity and greater Fc receptor function per antibody. Their responses appear more specific and directly mature against SARS-CoV-2.
4. The Luciferase Immunoprecipitation System is a useful experimental approach for antigen discovery against novel outbreak viruses, particularly when reagents are limited and only viral RNA is available. This platform may be adapted for other viruses of interest.
5. Fc receptor functions of spike antibodies should be assessed after vaccination.

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Introduction

SARS-CoV-2 virions comprise structural proteins. During infection, up to 30 additional proteins may be expressed based on putative open reading frames (ORFs) in the viral genome. Some of these proteins modulate cellular processes through direct interactions. Their truncations may influence disease pathogenesis and serve as antigenic targets for more specific serological assays. In addition to structural proteins, the ORF1a/b polyprotein and accessory proteins can elicit antibody responses during infection. Antibodies targeting non-structural proteins may affect viral infection via Fc-mediated effector functions and via interactions during virus entry, fusion, replication, and egress within infected cells. Because antibodies against non-surface proteins cannot directly mediate neutralisation, secondary Fc functions become critical. The balance of Fc binding is associated with COVID-19 outcomes. Characterisation of serological responses to these additional proteins provides a snapshot of the 'antibody landscape', including antibody magnitude, antigenic specificity, and biological relevance of SARS-CoV-2 proteins.

This study aimed to (1) develop a novel Luciferase Immunoprecipitation System (LIPS) assay as a diagnostic tool for clinical SARS-CoV-2 infection; (2) determine the immunodominance hierarchy of antibodies in asymptomatic SARS-CoV-2 infection; and (3) evaluate antibody effector functions targeting non-neutralising SARS-CoV-2 proteins.

Methods

Based on previous studies describing the structure of the SARS-CoV-2 genome,[1,2] a panel of 14 proteins (S1, S2, S2', E, M, N, NSP1, ORF3a, 3b, 6, 7a, 7b, 8, and 10) was selected for antibody testing using the LIPS assay. Primers and cloning protocols for the amplification of SARS-CoV-2 proteins followed previously published methods.[3] Constructs containing the SARS-CoV-2 antigen of interest were cloned into the pREN2–Renilla luciferase (Ruc) plasmid, transfected into Cos1 cells, and prepared as previously described.[3]

The LIPS assays were performed with modifications[4] to established protocols.[3] Briefly, Ruc-antigens (at an equal concentration of 10^7 per well) and heat-inactivated plasma (diluted 1:100) were incubated for 2 hours with shaking at 800 rpm. UltraLink protein A/G beads were added to the Ruc-antigen and plasma mixture and incubated for a further 2 hours with shaking at 800 rpm. The entire volume was then transferred to high-throughput screening plates and washed as previously described. Plates were then read and analysed. Experimental controls included blank wells without plasma but containing Ruc-antigens, as well as negative control

plasma from healthy donors collected prior to the COVID-19 pandemic. Background signal was defined as the luminescence detected from each Ruc-fusion antigen incubated with protein A/G and substrate in the absence of plasma.

Plates were coated with 80 ng/mL of spike, nucleocapsid, or ORF8 proteins. Plates were rinsed, blocked with 1% fetal bovine serum in phosphate-buffered saline, incubated with 1:100 heat-inactivated plasma diluted in 0.05% Tween-20 / 0.1% fetal bovine serum in phosphate-buffered saline for 2 hours, and rinsed again. To measure antibody FcγRIIIa binding, plates were instead coated with 500 ng/mL of protein, then incubated with 1:50 heat-inactivated plasma. Plates were subsequently incubated with biotinylated dimeric FcγRIIIa-V158 (2) at 50 ng/mL for 1 hour at 37°C, rinsed, and incubated with streptavidin-horseradish peroxidase (1:10 000). Horseradish peroxidase activity was detected using stabilised hydrogen peroxide and tetramethylbenzidine for 20 minutes, stopped with 2 M H_2SO_4, and read at 450 nm using an absorbance microplate reader.

Results

From January 2020, at the onset of the pandemic, we cloned 14 different ORFs for application in the LIPS assay. Plasmids were shared internationally with collaborators in the United States, Germany, Singapore, and Australia, thereby facilitating global research efforts. Due to limited case numbers and sample volumes in Hong Kong prior to July 2020, we used 15 infected samples and pre-pandemic controls to establish the assay. The LIPS platform was successfully adapted for serological investigation of the SARS-CoV-2 immune response.

We conducted LIPS screening on hundreds of plasma samples from adults, children, asymptomatic individuals, and longitudinal collections, targeting 14 distinct SARS-CoV-2 protein regions. Pre-pandemic negative control samples were used to define assay sensitivity and specificity, with cut-off values set at the negative mean plus three standard deviations to ensure high stringency. Based on the mean difference compared with negative controls, the strongest antibody responses were observed against the nucleocapsid protein, followed by ORF8, ORF3b, and ORF7a. No difference in response magnitude was identified between symptomatic and asymptomatic cases. However, age-dependent variation in antibody response magnitude was evident, likely reflecting reduced viral replication in paediatric cases.

The most robust antibody responses were directed against the nucleocapsid and ORF8 proteins, which are abundantly expressed during infection but absent from the virion surface, in contrast to the spike protein. High-quality ORF8 protein was obtained. Protein-binding enzyme-linked immunosorbent assays were subsequently adapted to assess Fc receptor binding for spike-, nucleocapsid-, and ORF8-specific antibody responses, enabling evaluation of antibody effector functions. Fc receptor binding and antibody avidity against spike and nucleocapsid proteins were higher in children than in adults.

Discussion

The relevance of antibodies targeting non-neutralising and internal proteins is increasing. Unbiased and quantitative platforms such as the LIPS assay offer valuable tools for serosurveillance in the post-COVID-19 era, allowing identification of key immunogenic targets in emerging viruses. Memory B cells specific to nucleocapsid and ORF8 proteins exhibit substantial maturation over time. However, monoclonal antibodies against these internal targets do not confer protection in vivo in murine models,[5] suggesting limited therapeutic potential despite having diagnostic value.

Conclusions

SARS-CoV-2 infection induces an antibody response extending beyond the spike protein, which may improve diagnostic accuracy in both adults and children through the use of unique targets such as ORF8. FcγRIIIa effector functions are enhanced against spike in children relative to adults, and against nucleocapsid-specific responses, indicating a targeted immune response consistent with mild pathogenesis.

Funding

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Disclosure

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Stability and transmissibility of SARS-CoV-2: abridged secondary publication

LLM Poon *, HL Yen, WHA Chin, M Huang

KEY MESSAGES

1. The stability of SARS-CoV-2 under various environmental conditions, including temperature and pH, is demonstrated. Droplet transmission of SARS-CoV-2 is efficient.
2. SARS-CoV-2 remains stable on smooth surfaces for days but becomes inactive relatively quickly on porous surfaces.
3. Copper-containing stainless steel, metal oxide-containing, and porous surfaces effectively inactivate SARS-CoV-2.
4. SARS-CoV-2 is sensitive to commonly used disinfectants.
5. Contaminated N95 masks can be disinfected by heating at 70°C for 1 hour in an oven.

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Introduction

SARS-CoV-2 is the causative agent of the COVID-19 pandemic.[1-3] Epidemiological studies indicate that COVID-19 is primarily transmitted through droplets between individuals in close contact. However, indirect contact transmission is also possible (touching the eyes, nose, or mouth after contact with a virus-contaminated surface or object).[4-6] Additionally, facilities occupied by patients are heavily contaminated with SARS-CoV-2.[7,8] We conducted a series of in vitro and in vivo studies to examine the virus' stability and infectivity under various environmental conditions, to identify effective methods for decontamination, and to determine the potential role of droplets or droplet nuclei in COVID-19 transmission.

Methods

SARS-CoV-2 (BetaCoV/Hong Kong/VM20001061/2020) was isolated using Vero E6 cells, and virus stock was prepared and stored. The virus was exposed to various environmental conditions for specific durations. After incubation, infectious viral titre was quantified using the 50% tissue culture infectious dose assay. The efficiency of droplet transmission was determined using an established hamster model.[9] At 2 days post-infection, an infected hamster and contact hamsters were placed in separate containers within the same chamber. The contact hamsters were then housed separately and monitored for signs of infection.

Results

Effect of temperature on SARS-CoV-2 stability

SARS-CoV-2 in infection medium was incubated at various temperatures. At 4°C, the virus remained stable for at least 14 days. At room temperature (22°C), the infectious titre was reduced by at least 3 logs after approximately 7 days. Infectivity decreased by at least 99.9% after 30 minutes of incubation at 56°C, and infectious virus was not detected by the 50% tissue culture infectious dose assay after 5 minutes of incubation at 70°C. These results indicate that SARS-CoV-2 exhibits reduced stability at higher temperatures.

Effect of pH on SARS-CoV-2 stability

To determine the stability of SARS-CoV-2 at various pH levels ranging from 3 to 10, the virus was spiked into viral transport medium (VTM) adjusted to pH levels. After incubation for 1 hour at room temperature, viral titre decreased by <1 log across all tested pH levels. These findings suggest that SARS-CoV-2 remains stable and retains infectivity between pH 3 and pH 10.

SARS-CoV-2 stability in various clinical samples

The stability of the virus in respiratory specimens—including nasal/throat swabs, nasopharyngeal aspirates, and sputum—was comparable to that observed in the VTM control at both room temperature and 37°C. In contrast, viral stability in faecal samples was significantly reduced at both temperatures.

Stability of SARS-CoV-2 viral RNA in various transport media

Comparable quantities of viral RNA were detected when the virus was stored in VTM, phosphate-buffered saline (PBS), or ethanol for up to 7 days at both room temperature and 37°C. These results indicate that PBS or ethanol can be used instead of VTM for the transport of clinical specimens.

SARS-CoV-2 stability on different surfaces

The virus remained relatively stable on smooth surfaces such as stainless steel, glass, and plastic. A reduction of 99.9% infectivity required 1 to 4 days. Surprisingly, infectious virus was still detectable on the surface of a surgical mask—composed of polypropylene fibre—after incubation for 7 days. On porous surfaces such as tissue paper and printing paper, infectivity decreased much more rapidly. Infectious viral titre decreased by >99.9% within 6 hours on paper and within 30 minutes on wooden surfaces. These results indicate that SARS-CoV-2 can remain infectious on smooth surfaces including stainless steel, plastic, and glass, whereas infectivity rapidly decreases on porous surfaces.

Effect of copper-containing stainless steel on SARS-CoV-2 inactivation

Copper has been shown to inactivate SARS-CoV-2.[10] We fabricated copper-containing stainless steel samples with varying copper content using powder metallurgy to investigate their virus-inactivating properties. Samples containing ≥10% copper inactivated SARS-CoV-2 more rapidly than the standard stainless steel control. These materials may be applied to frequently touched surfaces to reduce the risk of fomite-mediated transmission.

Effect of metal oxide-containing surface coatings on SARS-CoV-2 inactivation

Several surface coatings containing copper oxide or zinc oxide were tested. These coatings substantially reduced viral infectivity within 1 to 3 hours. Additional advantages include hydrophilicity, which enables absorption of larger droplets, and the availability of sprayable, transparent formulations suitable for application on touch-screen devices. Overall, we demonstrated that multiple surface coatings could inactivate SARS-CoV-2. Their use may help to reduce the risk of COVID-19 transmission via the fomite route.

Effect of different disinfectants on SARS-CoV-2

The virus was mixed with various commonly used disinfectants at their working concentrations for 5 to 15 minutes. SARS-CoV-2 demonstrated sensitivity to all tested disinfectants, including household bleach, ethanol, povidone-iodine surgical scrub, chloroxylenol, chlorhexidine, benzalkonium chloride, and a 1:49 hand soap solution.

Decontamination of SARS-CoV-2-contaminated N95 masks with heat

We developed an evidence-based method to disinfect used masks. SARS-CoV-2 applied to various 3M N95 masks was fully inactivated after 1 hour of heating in an oven at 70°C. Repeated heat treatment did not compromise mask fit on wearers.

Droplet transmission in hamsters

There was droplet transmission in hamsters under various conditions, including different humidity levels.

Discussion

SARS-CoV-2 showed rapid inactivation at 70°C, and we developed a heat-based disinfection protocol using an oven for contaminated or used N95 masks. This protocol may help to alleviate supply shortage, particularly during the early stages of an outbreak. Additionally, SARS-CoV-2 remained stable at 4°C, retaining infectivity for up to 2 weeks. This result implies that transmission via cold-chain transport, such as the packaging of refrigerated food, is possible. Indeed, reports from China have documented cases of fomite transmission via cold-chain transport.[11,12] Our findings may support implementation of precautionary measures when handling items transported under cold-chain conditions. The virus remined stable across a range of pH levels and was susceptible to commonly used disinfectants. Given that rapid and accurate diagnosis is critical for controlling the spread of COVID-19, our results indicate that PBS or ethanol can be used as alternative

transport media for clinical samples from the point of collection to laboratories for nucleic acid testing. In countries with limited resources where VTM may not be readily available, PBS and ethanol are more readily available and economical reagents, thus strengthening diagnostic capacity for COVID-19 control. The virus can remain infectious on smooth surfaces for 1 to 2 days, highlighting the potential for fomite transmission. Furthermore, SARS-CoV-2 exhibits high stability on surgical masks, underscoring the importance of hand hygiene after touching potentially contaminated surfaces and removal of personal protective equipment. Copper-containing stainless steel and anti-COVID-19 surface coatings may be applied to frequently touched surfaces in daily life to reduce the risk of transmission. Further research is warranted to evaluate the effectiveness of these materials in healthcare settings and community environments.

Conclusions

We demonstrated multiple properties of SARS-CoV-2, including its sensitivity to heat, pH, and a range of disinfectants. These findings support evidence-based recommendations for disinfection in laboratories, healthcare settings, and the community. Several materials and surface coatings developed in this study efficiently inactivated the virus, contributing to the reduction of transmission risk via the fomite route.

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Emotions, knowledge, attitudes, and behaviours during the COVID-19 pandemic: a mixed-methods study (abridged secondary publication)

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KEY MESSAGES

1. The prevalence of potential post-traumatic stress disorder 1 year after the onset of the COVID-19 pandemic was 12.4%.
2. Greater psychological trauma symptoms were associated with a lower education level, an unemployed status, no income, and spending ≥1 hour per day watching pandemic-related news.
3. Participants' perceptions of, and hesitancy toward, COVID-19 vaccination were affected by various factors across individual (trust, confidence, and social support networks), microsocial (stigma toward healthcare workers), intermediate-social (government), and macrosocial (cultural stereotypes, civic and collective responsibility, and economic considerations) levels.

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Introduction

Socially disadvantaged groups, such as older adults, are more vulnerable to the impacts of infectious diseases. This study aimed to examine the psychological trauma and behaviours regarding COVID-19 prevention among Hong Kong residents, and to determine sociodemographic factors associated with psychological trauma and health behaviours. Reasons for adopting appropriate preventive measures were explored.

Methods

For the quantitative study, data were collected between 18 December 2020 and 2 February 2021 through a telephone survey targeting Cantonese-speaking Hong Kong residents aged ≥18 years. Symptoms of post-traumatic stress disorder (PTSD) were measured using the Revised Impact of Event Scale.[1] Compliance with preventive measures, vaccine acceptance, and willingness to participate in voluntary testing were measured using the Questionnaire of Knowledge, Attitudes and Practice Towards COVID-19.[2] This questionnaire includes 14 items focused on preventive behaviour, rated on a four-point Likert scale from 3 (always) to 0 (never). Questions regarding exposure to the COVID-19-related news were also included.

For the qualitative study, 31 older adults (age ≥65 years) who had not received the COVID-19 vaccine at the time of the study and could communicate in Cantonese were recruited using purposive sampling. Additionally, 38 ethnic minority individuals aged ≥18 years who had resided in Hong Kong prior to January 2020 (the start of the pandemic) were recruited using purposive sampling. They were interviewed to explore the motivations and reasons for their health behaviour decisions.

Results

For the quantitative study, 3011 individuals completed the survey; 12.4% of them scored ≥33 on the Revised Impact of Event Scale, suggesting potential PTSD, which was associated with having completed primary education or below, being unemployed, having no personal income, and spending ≥1 hour per day watching pandemic-related news. The mean score on the Questionnaire of Knowledge, Attitudes and Practice Towards COVID-19 was 2.42±0.41, suggesting frequent adherence to government health advice. The vaccine acceptance rate was 45.6%. Female, older, and more educated participants showed better compliance with preventive measures.

For the qualitative study, among older adults, barriers to COVID-19 vaccination included a lack of trust in the vaccine, safety concerns, perceptions of limited long-term efficacy, feelings of personal unsuitability for vaccination, peer pressure, and concerns about insufficient support. Vaccine hesitancy was influenced by stigma towards

healthcare workers during the pandemic, a lack of trust in government accountability for adverse events, cultural beliefs, and perceptions of vaccines as toxic or involving viral injection. Participants who were male, older, married, or had lower education levels showed greater acceptance of vaccination and voluntary testing. Perceptions of, and hesitancy toward, vaccination were affected by various factors across individual (trust, confidence, and social support networks), microsocial (stigma toward healthcare workers), intermediate-social (government), and macrosocial (cultural stereotypes, civic and collective responsibility, and economic considerations) levels.

Ethnic minorities were often viewed as a high-risk group and scapegoated for virus transmission, leading to experiences of segregation and seclusion. South Asian participants, in particular, reported such experiences, which were rooted in pre-existing stereotypes and stigmatisation of ethnic minorities. The Government's focus on ethnic identity when reporting COVID-19 cases contributed to this seclusion. Consequently, ethnic minorities had difficulty accessing infection control information, and many experienced job loss. The pandemic acted as a catalyst, amplifying existing issues of segregation and seclusion among ethnic minorities in Hong Kong.

Discussion

This study found that 12.4% of participants exhibited symptoms indicative of potential PTSD, probably due to uncertainty and repeated outbreaks. Unemployment and lack of personal income were associated with a higher likelihood of PTSD-like symptoms. Spending more time watching pandemic-related news was associated with more severe PTSD symptoms and also with increased compliance with preventive measures. Personal experiences and social networks influenced vaccination barriers and incentives. Trust and confidence in the vaccine were key determinants of motivation for vaccination. Stigma towards healthcare workers during the pandemic and the lack of trust in government impacted vaccination decisions at the microsocial and intermediate-social levels. Cultural perceptions of vaccines contributed to hesitancy at the macrosocial level.

Mental health support for lower-income individuals is required, in addition to financial assistance. Accurate health messages should be disseminated to the public to encourage adherence to preventive measures. The public should be advised to avoid repetitive exposure to identical COVID-19-related news, with the goal of protecting mental health. The public should be reminded to verify information before relying on or sharing it with family and friends. Social support should be prioritised when promoting COVID-19 vaccination among older adults. Strengthening social support networks or offering targeted assistance after vaccination may reduce hesitancy and increase motivation to receive the vaccine among older adults.

Conclusions

Greater psychological trauma symptoms were associated with a lower education level, an unemployed status, no income, and spending ≥1 hour per day watching pandemic-related news. Participants' perceptions of, and hesitancy toward, COVID-19 vaccination were affected by various factors across individual, microsocial, intermediate-social, and macrosocial levels. Socially disadvantaged groups have difficulty adopting preventive health behaviours, due to individual perceptions, economic constraints, and social isolation. Participants reported relying on non-governmental organisations for support during the pandemic. This highlights the importance of collaboration between government agencies and community organisations to promote the well-being of socially disadvantaged groups during future public health crises.

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Disclosure

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Gut microbiota in the pathogenesis of COVID-19: viral replication and transmission (abridged secondary publication)

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KEY MESSAGES

1. Prolonged and active SARS-CoV-2 virus remained in the gut of patients with COVID-19, even after recovery.
2. Both gut bacterial and viral microbiota were disrupted in patients with COVID-19, persisting for up to 6 months after disease resolution.
3. Several gut commensal bacteria with known immunomodulatory potential—*Faecalibacterium prausnitzii*, *Eubacterium rectale*, and *Bifidobacteria*—and two RNA virus species derived from pepper plants were underrepresented in these patients.
4. Depletion of these bacterial and viral taxa was associated with more severe disease and higher levels of inflammatory cytokines and blood markers.

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Introduction

Although most COVID-19 cases are mild, the illness can result in hospitalisation, respiratory failure, or death.[1] The prevalence of patients exhibiting gastrointestinal symptoms, including diarrhoea, was 2% to 10% in early reports from Wuhan and was up to 20% in a meta-analysis.[2-5] SARS-CoV-2 was detected in anal swabs and stool samples of almost 50% of patients, suggesting that the digestive tract can be a site for viral replication and activity.[6,7] Moreover, faecal calprotectin was elevated in COVID-19 patients with diarrhoea,[8] indicating an inflammatory response in the gut. SARS-CoV-2 enters host cells via the angiotensin-converting enzyme 2 (ACE2) receptor, which is highly expressed in both the respiratory and gastrointestinal tracts.[9-11] ACE2 also plays important roles in controlling intestinal inflammation and gut microbial ecology.[12] The gut microbiome affects immune response and metabolism. The commensal microbiota is dynamic and can be modulated by invading viruses to elicit either stimulatory or suppressive responses.[13] Respiratory viral infections may alter the gut microbiome, increasing susceptibility to secondary bacterial infections.[14,15] Meta-transcriptomic analysis of bronchoalveolar lavage fluid from infected patients revealed dominance of pathogens or commensal bacteria in the oral and upper respiratory tracts.[16] Comorbidities in patients with severe COVID-19 have been linked to shifts in bacterial taxa from the phyla *Bacteroidetes* and *Firmicutes*,[17-20] which regulate *ACE2* expression in rodents.[21] Host microbial perturbations may impact response to infection and the efficacy of future immune interventions such as vaccines.[22] We hypothesised that SARS-CoV-2 replicates in the gastrointestinal tract and alters gut microbiota, leading to worsened clinical manifestations. This study aimed to explore the roles of the gastrointestinal tract and gut microbiota in the pathogenesis of COVID-19.

Methods

In total, 50 hospitalised patients with laboratory-confirmed SARS-CoV-2 infection, 30 hospitalised patients with community-acquired pneumonia (pneumonia controls), and 30 healthy controls were recruited. Stool and plasma samples were collected. Clinical data were collected prospectively using a standardised template developed by the International Severe Acute Respiratory and Emerging Infection Consortium.

Laboratory investigations included measurement of viral load in faecal samples, profiling of faecal microbes (including viruses and bacteria), inflammatory cytokine profiling (interleukin [IL]-1β, IL-6, IL-10, IL-12p70, tumour necrosis factor-α, C-X-C motif chemokine ligand [CXCL] 8, CXCL9, CXCL10, C-C motif chemokine ligand [CCL] 2, and CCL5), *ACE2* gene expression analysis, and bacterial co-culture studies of Caco-2 and HT-29 cells.

Results

SARS-CoV-2 nucleic acid was detected in the faeces

of 73.3% of patients during hospitalisation (median viral load, 3.86 $\times 10^3$ copies per mL inoculum), whereas active SARS-CoV-2 infection was confirmed in 46.7% of patients, with substantially higher genomic coverage at the 3′ end relative to the 5′ end of the SARS-CoV-2 genome in faecal viral metagenomes, even after clinical recovery. Patients with COVID-19 showed disrupted bacterial and viral microbiota, which persisted for up to 6 months after recovery. Several gut commensal bacteria with known immunomodulatory properties—*Faecalibacterium prausnitzii*, *Eubacterium rectale*, and *Bifidobacteria*—and two RNA virus species derived from pepper plants were underrepresented in patients. Decreased abundances of these bacterial and viral taxa were associated with more severe disease and elevated concentrations of inflammatory cytokines and blood markers including C-reactive protein, lactate dehydrogenase, aspartate aminotransferase, and gamma-glutamyl transferase. No significant correlations were observed between *ACE2* gene expression and disease severity, blood biomarkers, or gut microbiota composition. *Bacteroidetes* species (*Bacteroides dorei*, *Bacteroides thetaiotaomicron*, and *Bacteroides massiliensis*) did not downregulate *ACE2* expression in epithelial cells.

Discussion

Patients with COVID-19 had substantial active viral infection and replication in the gastrointestinal tract, which in some cases persisted after respiratory clearance of SARS-CoV-2. Clearance of gastrointestinal infection appeared to be delayed. The implications of this delayed clearance for viral transmission remain unclear.

The gut microbiome was disrupted in patients with COVID-19, characterised by enrichment of opportunistic pathogens and depletion of beneficial commensals. Loss of salutary species persisted in most patients for up to 6 months after clearance of SARS-CoV-2. Given that many recovered patients reported symptoms such as fatigue, dyspnoea, and joint pain, we speculate that a dysbiotic gut microbiome contributes to post-COVID-19 complications. Further investigations are needed to determine whether dysbiosis or specific microbial imbalances predispose individuals to future health issues.

SARS-CoV-2 infection may induce dysfunctional immune responses and cytokine storm syndrome in a subset of patients, resulting in more severe disease. The gut microbiota plays a key role in regulating the development and function of both the innate and adaptive immune systems. This study showed that depletion of several gut commensal bacteria—*F prausnitzii*, *E rectale*, and *Bifidobacteria*—and two RNA virus species derived from pepper plants was associated with more severe disease and elevated levels of inflammatory cytokines and blood markers. *F prausnitzii* has been shown to prime human colonic regulatory T cells to secrete the anti-inflammatory cytokine IL-10.[19] Higher relative abundances of *E rectale* in the gut have been linked to reduced inflammation in Alzheimer's disease,[10] and *Bifidobacterium adolescentis* is able to suppress the activation of nuclear factor-κB, a protein that promotes expression of pro-inflammatory cytokines.[21] The pepper-derived RNA virus is the most abundant and prevalent plant RNA virus found in human faeces; it has been proposed as an indicator of faecal contamination in aquatic environments and water treatment systems. SARS-CoV-2 may modulate host immunity and create an unfavourable environment for certain RNA viruses. The lysis or clearance of these viruses in the gut may lead to the release of nucleic acids, proteins, and lipids that act as pathogen-associated molecules that trigger inflammation. These gut microorganisms play a broader role in modulating systemic inflammation; their depletion in COVID-19 may contribute to severe disease and inflammatory symptoms via dysregulation of host immune responses.

Conclusion

Prolonged and active SARS-CoV-2 was detected in the gut of patients with COVID-19, even after recovery, highlighting the importance of long-term surveillance and the potential risk of faecal-oral transmission. Both gut bacterial and viral microbiota were disrupted in patients with COVID-19 and could persist for up to 6 months after recovery; the disruption was associated with disease severity and immune responses. These findings underscore the urgent need to elucidate the specific roles of gut microorganisms in immune regulation and systemic inflammation in the context of COVID-19.

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Disclosure

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1. Zuo T, Zhang F, Lui GC, et al. Alterations in gut microbiota of patients with COVID-19 during time of hospitalization. Gastroenterology 2020;159:944-55.
2. Zuo T, Zhan H, Zhang F, et al. Alterations in fecal fungal microbiome of patients with COVID-19

during time of hospitalization until discharge. Gastroenterology 2020;159:1302-10.
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Artificial intelligence analysis of chest X-ray and computed tomographic images for COVID-19 patient management: abridged secondary publication

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KEY MESSAGES

1. The artificial intelligence (AI)-enhanced chest X-ray (CXR) model increased lung signal and suppressed rib signal, enabling more accurate classification of COVID-19 conditions.
2. The AI-based segmentation model delineated lung contours and infected regions in computed tomographic and CXR images with high Dice scores.
3. The multi-view approach significantly improved the area under the curve compared with the use of a single feature type.
4. The developed computer programme supports COVID-19 diagnosis and patient management.

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Introduction

Chest X-ray (CXR) and computed tomography (CT) are essential for diagnosing, assessing, and monitoring patients with COVID-19.[1] Artificial intelligence (AI) and big-data analytics facilitate early detection and diagnosis of COVID-19.[2] This study aimed to evaluate the application of AI and big-data analytics in the detection, diagnosis, surveillance, and management of COVID-19.

Methods

In total, 1818 CXR or CT images were retrospectively collected from Queen Elizabeth Hospital and Pamela Youde Nethersole Eastern Hospital, as were >3000 images from public datasets. The AI-enhanced CXR model was developed using a multi-strategy framework comprising lung segmentation, bone suppression, and super-resolution. Robust, self-adaptive AI-based segmentation frameworks were developed with an image pre-processing pipeline, residual learning, and data augmentation. A multiple kernel combination method was applied within our existing multi-view framework[3] and incorporated into conventional classifiers, including support vector machines and kernel ridge regression. The computer programme was integrated with the developed models and compiled using 3D Slicer.

Results

The AI-enhanced CXR images achieved a peak signal-to-noise ratio of 43.21±3.14 and a root mean square error of 0.0074±0.0029. Average Dice scores for infected regions based on the AI-based segmentation model were 0.93 for CT and 0.85 for CXR. The proposed multi-view analysis model substantially improved the area under the curve when combining radiomics and clinical features. The area under the curve for assessing COVID-19 pneumonia severity using the multi-view analysis was 0.98, considerably greater than that based on image features alone (0.60). The developed computer programme incorporated CXR image enhancement, CT and CXR image segmentation, and multi-view analysis, enabling automatic processing and analysis of input CT and CXR images for COVID-19.

Discussion

We developed a multi-strategy model comprising AI-enhanced CXR, AI-based segmentation, multi-view analysis, and an integrated image analysis programme to support auxiliary diagnosis for patients with COVID-19. The results of the multi-view analysis confirmed that the integration of feature types improved classification performance. The introduction of radiomics features also enhanced

the interpretability of the classification model; this is essential for clinical adoption of AI technologies. Future studies should incorporate larger and more diverse datasets, evaluate model performance across varied settings and populations, and investigate the added value of other imaging features or patient information.

Conclusions

Our AI techniques offer safer, more accurate, and more efficient imaging solutions for managing COVID-19, providing actionable insights for clinical assessment and personalised treatment. Our models can predict patients at risk of developing severe symptoms, facilitating hospital resource planning. The AI-empowered CXR technique is highly accessible and demonstrates robust diagnostic and monitoring accuracy for COVID-19. It is suitable for implementation across hospitals at all levels.

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Disclosure

The results of this research have been previously published in:

1. Ren G, Xiao H, Lam SK, et al. Deep learning-based bone suppression in chest radiographs using CT-derived features: a feasibility study. Quant Imaging Med Surg 2021;11:4807-19.
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Clinical, virological, microbiological, immunological, and laboratory monitoring of patients hospitalised with COVID-19: abridged secondary publication

PKS Chan *, CKC Lai, GCY Lui, L Ling, A Li, RWY Ng

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

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Nowcasting COVID-19 transmission dynamics, severity, and effectiveness of control measures: abridged secondary publication

BJ Cowling *, P Wu, JT Wu

KEY MESSAGES

1. Our model yielded unbiased estimates of the time-varying reproductive number of COVID-19 cases by accounting for differences in infectiousness between local and imported cases.
2. The case fatality risk of COVID-19 increased with age in 2020 in Hong Kong.
3. Incorporation of changes over time in the serial interval distribution enabled more accurate estimation of the reproductive number.

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Introduction

During the initial period of the COVID-19 pandemic in Hong Kong, containment and suppression measures included intensive surveillance of infections among both incoming travellers and the local community. Once identified, patients were isolated until recovery and cessation of virus shedding. Their close contacts were traced (from 2 days prior to illness onset) and quarantined in designated facilities. Because not every infected person could be identified, containment was only effective when accompanied by social distancing measures and behavioural changes that reduced undetected community transmission.

To assess the effectiveness of suppression measures, we monitored the transmissibility of COVID-19 over time by estimating the effective reproductive number in real time. We also monitored the clinical severity profile by age and estimated the impact of various control measures on COVID-19 transmissibility. We aimed to provide evidence-based guidance on the most appropriate public health strategies for suppressing COVID-19 transmission.[1]

Methods

COVID-19 data regarding infections, illnesses, and hospitalisations were compiled into a single database. Data sources included: (1) daily reports of laboratory-confirmed cases from the Centre for Health Protection; (2) detailed time-to-event interval data from a subset of cases; (3) detailed data on laboratory-confirmed and probable cases from the Hospital Authority, with regular updates; (4) a complete dataset from December 2020 regarding hospital admissions for pneumonia and other respiratory causes, used as a reference to estimate the overall morbidity impact and epidemiology of COVID-19 in Hong Kong; and (5) snapshot data on community illness prevalence, obtained through telephone surveys and crowdsourced reporting platforms.

Advanced analytic techniques were used to estimate daily changes in the effective reproductive number (Rt), adjusting for delays between illness onset and reporting, via time series of confirmed cases, probable cases, pneumonia hospitalisations, and relevant data. Given the delay between onset and case notification, data were augmented for recent days using the estimated onset-to-notification distribution. We developed models to triangulate the various sources of information regarding infection incidence and transmissibility, including models assessing age-specific differences in transmissibility.

Severity was monitored via hospital fatality risk, symptomatic case fatality risk, and infection fatality risk, both overall and by age. Using very early data from cases in Wuhan, we estimated the hospital fatality risk to be 14%.[2] Care was taken to avoid estimating mortality risk among laboratory-confirmed cases in Mainland China because testing practices varied over time due to changes in clinical case definitions, laboratory procedures, and testing methods. Similarly, we avoided estimating fatality risk among laboratory-confirmed cases in Hong Kong. We aimed to characterise the clinical severity profile within a single Bayesian model for comprehensive assessment of age-specific severity.

The impact of school closures and other interventions on Rt was estimated by examining changes in that number over time. Using age-specific data on illnesses and hospitalisations, we constructed a detailed mathematical model to

simulate pandemic trajectories under alternative hypothetical scenarios. Model parameters were derived from available datasets and the literature. Comparisons of pandemic curves with and without various interventions, as well as comparisons across locations, provided estimates of the impact of those measures.

Results

Estimating reproductive number in real time throughout the pandemic

The framework developed by Cori et al[3] was extended to estimate Rt for both imported and local cases by adjusting their differences in infectiousness. Surge in local case numbers corresponded to an estimated Rt of >1 during 14 to 26 March 2020, prior to tightening of public health measures. Rt then steadily declined after the implementation of special work arrangements for civil servants and additional social distancing measures (Fig 1). In contrast, during early March, Rt for imported cases fell <1, despite daily numbers >10, secondary to the enforcement of the 14-day quarantine for inbound travellers.

Estimating severity in real time throughout the pandemic

In Hong Kong, all confirmed COVID-19 cases were hospitalised during the study period. Using severity profiles of 5088 laboratory-confirmed cases, we estimated the confirmed case fatality risk (cCFR). Cases were stratified by date of confirmation: 23 January to 30 September 2020 (waves 1-3) and after 30 September 2020 (after wave 3).

The adjusted cCFR was estimated by dividing the cumulative number of deaths by the sum of cumulative deaths and recoveries, whereas the overall cCFR was estimated by directly standardising the age-specific cCFR using the cumulative number of confirmed cases during waves 1-3. The all-ages adjusted cCFRs were 2.2% (95% confidence interval [CI]=1.8%-2.5%) during waves 1 to 3 and 1.4% (95% CI=1.1%-1.7%) after wave 3. The point estimates of the adjusted cCFR increased with age in both periods. Specifically, the adjusted cCFR rose from 0% in those aged <20 years to 12.2% in those aged ≥65 years during waves 1 to 3, and from 0% in those aged <20 years to 7.32% in those aged ≥65 years after wave 3 (Fig 2).

Temporal change in serial intervals during the pandemic

Contact tracing data were categorised into transmission pairs to explore temporal changes in serial intervals. Serial intervals were defined as the number of days between symptom onset in the infector and symptom onset in the infectee for each transmission pair. Transmission pairs were classified into pre-peak (9-22 January 2020), peak week

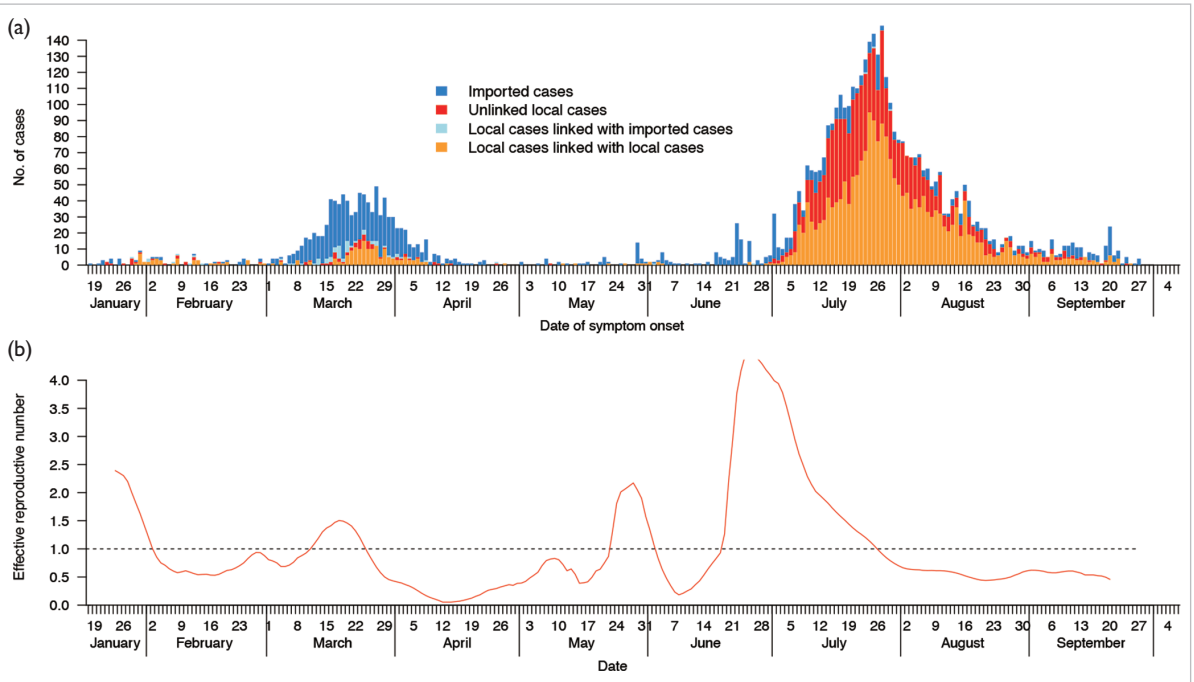

FIG 1. (a) Numbers of local and imported confirmed COVID-19 cases in Hong Kong as of 27 September 2020. (b) Estimates of the daily effective reproductive number (Rt) over time for local cases, with the shaded area indicating the uncertainty range. The dotted line indicates the critical threshold of Rt=1; if Rt >1 for a prolonged period, a pandemic is expected to occur.

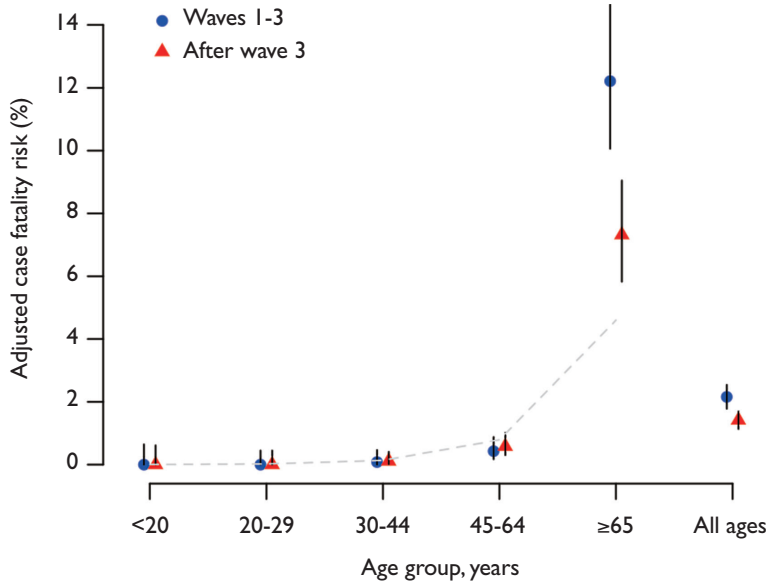

FIG 2. Confirmed case fatality risk of COVID-19 in Hong Kong, stratified by age group and pandemic wave.

(23-29 January 2020), and post-peak (30 January to 13 February 2020) periods, based on the symptom onset dates of infectors. After normal distributions were fitted to the empirical serial interval data, serial interval decreased from an average of 7.8 (95% CI=7.0-8.6) days during the pre-peak period to 2.6 (95% CI=1.9-3.2) days during the post-peak period. Stratification by isolation delay showed a similar trend such that average serial intervals were shortened by at least threefold. Additionally, estimated Rt obtained using a constant serial interval distribution versus a time-varying effective serial interval distribution was compared. The differences in estimated Rt were more pronounced during the pre- and post-peak periods but were relatively minor during the peak week when Rt was approximately 1.

There were positive associations between isolation delay and effective serial intervals. In a linear multivariable regression model, isolation delay alone explained up to 51.5% of variability in daily empirical serial intervals, indicating a primary predictor. Inclusion of either non-pharmaceutical intervention strategies or the accumulation of population immunity explained an additional 15.6% to 20.3% of variability.

Discussion

The extended framework for separately estimating Rt for local and imported cases was compared based on assumptions of (1) equal infectiousness of local and imported cases, and (2) all cases being local, which resulted in underestimation and overestimation of local transmission, respectively. To account for presymptomatic transmission, a deconvolution approach was used instead of serial intervals to reduce bias arising from misspecification of the infectiousness profile. A bootstrap method was used to adjust for uncertainties in the analysis.

The cCFR increased with age across all waves, and the cCFR generally lowered after the third wave, likely due to improved detection of mild cases and better clinical management of patients. These findings were consistent with studies conducted in other countries.[4] Similarly, a systematic review of the 2009 influenza A (H1N1) pandemic showed that mortality risk among symptomatic cases increased monotonically with age.[5]

A notable observation was a minimum threefold reduction in serial intervals over the 36-day period from 9 January to 13 February 2020. Non-pharmaceutical interventions designed to reduce isolation delays was the primary factor in shortening serial intervals over time. Specifically, serial intervals were shortened by >3 days among transmission pairs in which infectors were isolated promptly after symptom onset, compared with those isolated later. Our findings aligned with results of a study that showed a 60% reduction in COVID-19 transmissibility when case isolation and contact quarantine occurred within 1 day of symptom onset.

Conclusions

In Hong Kong, suppression of COVID-19 transmission during three pandemic waves was

achieved through a combination of public health measures, including travel restrictions, early and near-complete case identification, rapid interruption of transmission chains through contact tracing and quarantine, social distancing measures, and widespread use of face masks by the population. Maintenance of these interventions and population behaviours, along with strategies to address public fatigue, may help prevent future pandemic waves.

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Disclosure

The results of this research have been previously published in:

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