Clinical studies of epidemic influenza and pandemic COVID-19 to improve the chain of patient care: from bedside diagnostics to long-term complications

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Thesis for the degree of Philosophiae Doctor (PhD)
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"*Obstacles don't have to stop you.*
If you run into a wall, don't turn around and give up.
Figure out how to climb it, go through it, or work around it."
-Michael Jordan

Preface

Influenza is a common respiratory viral pathogen that most people have been exposed to in early childhood. With age and underlying diseases, the risk of severe influenza infection increases. Irregularly, new immune-evading influenza virus strains appear, causing global pandemics. As a young doctor working in the emergency medicine ward from 2016 to 2018, I was introduced to the field of clinical infectious diseases. I became interested in the surge of patients with fever, cough and respiratory compromise that presented during winter, with the arrival of the annual influenza epidemic. Early clinical diagnosis in influenza patients was often flawed due to the symptoms resembling bacterial pneumonia and other viral influenza-like-illnesses (ILI). Both delay of correct treatment or initial excessive treatment came with the risks of adverse events and patient complications. A senior doctor and later my main supervisor, who had extensive patient experience from the 2009 influenza H1N1 pandemic, said that (paraphrased) "*every influenza season is a rehearsal for the next severe influenza pandemic, and we will not perform better in the face of a pandemic, than what we do right here and now"*. With this, I became curious about influenza management in hospital, and in studying how we could change current practice to improve patient outcomes in the epidemic and pandemic setting.

During the work of this thesis, the world abruptly faced a novel pandemic threat, named severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)[1]. The sudden knowledge gap that emerged caused a rapid shift of global attention towards the new virus, affecting both global and local research priorities. Our research group decided to find answers to new and relevant questions concerning SARS-CoV-2. My work became investigative, and with a patient-focused approach. Consequently, this current doctoral work comprises a pre-pandemic observational study of rapid point-of-care influenza diagnostics as well as intra-pandemic comparison of the clinical management of hospitalized influenza and SARS-CoV-2 patients, and long-term follow-up of convalescent SARS-CoV-2 outpatients.

Scientific environment

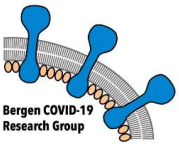
This work was conducted at the Influenza Centre, the Department of Clinical Sciences, University of Bergen (UiB), Bergen, Norway as part of the PhD program at the faculty of medicine, UiB. The PhD was funded intramurally by the Influenza Centre, Department of Microbiology, Haukeland University Hospital, Bergen.

Patient studies were conducted in collaboration with Haukeland University Hospital, Bergen, Norway and Haraldsplass Deaconess Hospital, Bergen, Norway. In addition, after the onset of the Coronavirus 2019 pandemic, the newly formed Bergen COVID-19 Research group started a collaboration with the National Intensive Care and Pandemic Registry (NIPaR). The Influenza Centre, UiB, conducted all laboratory work, except TCR sequencing, which was conducted by Adaptive Biotechnologies.

The mandatory courses for this PhD were conducted at UiB, the International Society for Influenza, and other Respiratory Virus Diseases (ISIRV) Beirut, Lebanon, and the Norwegian Medical Association (NMA).

Associate Professor Kristin G-I Mohn, Professor Rebecca Jane Cox and Doctor Richard Allan Davies provided supervision and guidance.

Additional funding was received by Gades Legat, and the National Graduate School in Infection Biology and Antimicrobials (IBA).



Acknowledgements

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I am also deeply indebted to my two co-supervisors. *Professor Rebecca Jane Cox***,** from our first conversation, I knew that I wanted to join your team at the Influenza Centre. Thank you for allowing me the opportunity. You are a compassionate scientist, and you always impress me with your broad spanning knowledge, and how easily you communicate complex science to a broader audience. I am grateful for your generosity, commitment, and support throughout the work with this thesis. It has been reassuring to know that when needed, you always make time to talk, even when you are on a tight schedule. Thank you so much, *Doctor Richard Allan Davies,* your thorough guidance and help with laboratory assays and academic writing has been very educational. I appreciate all our interesting conversations in the lab, and how you always bring new perspectives on life and science.

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I want to thank *Associate professor Bjørn Blomberg***,** who has provided key assistance with statistics and preparation of manuscripts in this thesis. It has been great to work with such a knowledgeable and positive person. I always leave your office encouraged and in a good mood.

Clinical studies involve many people, and this thesis would not have been possible without the vigorous efforts of a fantastic team. I am so thankful for all my current and former colleagues at *the Influenza Centre,* and *the Bergen COVID-19 Research Group* for contributing to the conduction of the clinical studies involved in this thesis, all the way from patient recruitment to laboratory analyses.

To all my colleagues on the 5th floor, thank you for the great company. I am grateful to have been surrounded by so many friendly, skilled, and knowledgeable people. *Lena* and *Nina,* I feel so lucky that we started our PhDs together. We have been in the same boat for good and bad, and our friendship has pulled me through this journey. Spending time together we have had so much fun along the way. Thank you for always helping me and supporting me. To *Anders, Abira, and Sarah,* I have really enjoyed your company and it has been great working and traveling to international destinations with you. I would like to thank *Sonja***,** who welcomed me and introduced me to the lab work when I started this Ph.D., and *Chi***,** for support in the lab and for clever advice along the way. Many thanks to my colleagues *Amit*, *Håkon* and *Stefan*, for always being so kind and helpful. To *Therese, Fan, Geir,* and *Juha*, I appreciate the time we have spent together at work and on outings. You are true lab-masters, and you always take your time to answer thoroughly any question regarding your work. I am truly indebted to your dedicated work in various projects. I would like to thank *Karl,* for teaching me about immunology, and for always providing technical support. Thank you, *Helene*, for being a wonderful partner in patient follow-up. To *Linn***,** thank you for your fun company, and for all your help during the Influenza study. Thank you, *Kanika***,** for your significant contributions to the recruitment of the COVID-19 cohort, and for good road trip conversations**.** To my office-partner *Kjersti*, I really enjoyed our motivational afternoon talks over the kitchen dishwasher. Thank you *Türküler*, for your patience and efforts in explaining complex statistics and data handling, I will apply what I have learned from you.

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List of Publications

I. **Fjelltveit E.B.,** Cox R.J., Østensjø J., Blomberg B., Ebbesen M.H., Langeland N., Mohn K. G-I. "Point-of-Care Influenza Testing Impacts Clinical Decision, Patient Flow, and Length of Stay in Hospitalized Adults." *The Journal of Infectious Diseases* 2020

II. **Fjelltveit EB**, Cox R.J., Kittang B.R., Blomberg B., Buanes E.A., Bergen Covid-19 Research Group, Langeland N., Mohn K. G-I. "Lower antibiotic prescription rates in hospitalized COVID-19 patients than influenza patients, a prospective study". *Infectious Diseases* 2022 Feb;54(2):79-89

III. **Fjelltveit E.B.**, Blomberg B., Kuwelker K., Zhou F., Bredholt Onyango T., Brokstad K.A., Elyanow R., Kaplan I.M., Tøndel C., Mohn K.G-I., Özgümüş T., Cox R.J., Langeland N and Bergen COVID-19 Research Group. "Symptom burden and immune dynamics 6 to 18 months following mild SARS-CoV-2 infection: a case-control study". *Clinical Infectious Diseases, 2022 Aug 12th*

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I. Ertesvåg N.U., Xiao J., Zhou F., Ljostveit S., Sandnes H., Lartey S., Sævik M., Hansen L**.**, Madsen A., Mohn K.G.I., **Fjelltveit E.B.**, Olofsson J.S., Tan T.K., Rijal P., Schimanski L., Øyen S., Brokstad K.A., Dunachie S., Jämsén A., James W.S., Harding A.C., Harvala H., Nguyen D., Roberts D., PHE Virology group, Zambon M., Oxford collaborative group, Townsend A., Bergen COVID-19 Research group, Langeland N., Cox R.J.: A rapid antibody screening haemagglutination test for predicting immunity to SARS-CoV-2 variants of concern. *Communications Medicine* 2, 36, (2022).

II. Mohn K. G. I., Bredholt G., Zhou F., Madsen A., Onyango T. B., **Fjelltveit E. B.**, Jalloh S.L., Brokstad K.A., Cantoni D., Mayora-Neto M., Temperton N., Langeland N., Cox R.J., Bergen COVID-19 research group: Durable T-cellular and humoral responses in SARS-CoV-2 hospitalized and community patients. *Plos one*, 17 (2), (2022).

III. Blomberg B.., Mohn K. G-I., Brokstad K.A., Zhou F., Linchausen D.W., Hansen B-A., Lartey S.J., Onyango T.B, Kuwelker K., Sævik M., Bartsch H, Tøndel C., Reiakvam B.K., (**Fjellveit E.B.,** as a member of) Bergen COVID-19 Research Group, Cox R.J, Langeland N. "Long COVID in a prospective cohort of home-isolated patients" *Nature Medicine* (2021)

Summary

Current knowledge indicates that early diagnosis along with timely and targeted management have the potential to improve the outcomes after Influenza and SARS-CoV-2. During the influenza season 2018/2019, we investigated the effect of implementing an ultra-rapid molecular influenza point-of-care test (POCT) in the emergency department (ED) (intervention hospital), compared to the use of rapid laboratory-based diagnostics (control hospital). We showed that influenza POCT was more rapid, reducing the time from triage to testing, allowing correct isolation of patients, and reduced the length of stay. Both hospitals similarly prescribed antivirals to >80% of influenza patients. The influenza POCT was not associated with reduced rate (>70% overall) or duration of antibiotic treatment, suggesting that antibiotic stewardship measures beyond the ED are important to improve targeted antibiotic use. The concern of overuse of antibiotics in respiratory viral infections increased in the SARS-CoV-2 pandemic. Hence, we investigated antibiotic treatment in patients hospitalized during the first COVID-19-wave in Bergen, and compared to antibiotic treatment of our influenza patients and all nationally registered COVID-19 hospitalised patients. COVID-19 patients were prescribed fewer antibiotics than influenza patients, although more resistance-driving antibiotics were used. There was a positive development from the first to second COVID-19 pandemic wave, with reduced antibiotic use. We then investigated the long-term complications of non-severe COVID-19 in home isolated patients up to 18 months after acute infection, named long COVID. We found that up to 18 months, almost half of the patients had one or more residual symptoms, with fatigue, memory problems, concentration problems and dyspnea being most common. The symptom burden at 12 months was significantly higher after infection compared to age- and time-period matched seronegative controls, and we found humoral and cellular SARS-CoV-2 specific immune correlates of symptom sequelae. Overall, our studies demonstrated an excess risk of multiple symptoms, associated with COVID-19, and that recovery from symptoms is slow in most individuals. In conclusion, this work has shown the importance of timely diagnostics for reducing patient length of stay and timely antiviral treatment and defined the long-term complications after mild COVID-19.

Oppsummering

Tidlig diagnostikk og rask og målrettet behandling kan forbedre utfallet av influensa og Covid-19 sykdom. Gjennom prospektive, kontrollerte observasjonsstudier har vi vist betydningen av rask diagnostikk for å redusere liggetid og starte tidlig antiviral behandling mot influensa, samt definert langtidsplager etter mild Covid-19 infeksjon. Under influensasesongen i 2018/2019 undersøkte vi effekten av å bruke en pasientnær nukleinsyrebasert influensa hurtigtest i akuttmottaket på Haukeland Sykehus sammenlignet med rask, standard laboratoriediagnostikk på Haraldsplass Diakonale Sykehus. Vi fant at den pasientnære hurtigtesten var raskere, og reduserte tidsbruk fra triage til influensatesting. Bruk av hurtigtest var forbundet med mer målrettet isolasjonsbruk og kortere sykehusopphold. Begge sykehus initierte antiviral behandling i >80% av bekreftede influensatilfeller. Pasientnær hurtigtest var ikke forbundet med lavere forbruk av antibiotika (gitt til >70%) eller kortere antibiotikakurer, som antyder at anibiotikastyrings-verktøy i forløpet etter akuttmottak kan være vel så viktig for å forbedre forskrivningspraksis. Bekymring om overforbruk av antibiotika økte under Covid-19 pandemien. Derfor undersøkte vi antibiotikabruk blant innlagte koronapasienter i Bergen under den første bølgen av pandemien. Vi sammenlignet med 2018/2019 influensapasienter fra samme sykehus, samt nasjonale tall over alle Covid-19 relaterte sykehus-innleggelser i Norge i 2020. Vi så at Covid-19 pasienter fikk færrest antibiotikakurer, men det var et høyere forbruk av resistensdrivende antibiotika. Videre så vi en positiv utvikling med redusert antibiotikabruk hos Covid-19 pasienter som ble innlagt i andre bølge av pandemien, sammenlignet med den første. Til slutt undersøkte vi forekomst av restplager, også kalt «long Covid» blant hjemmeisolerte Covid-19 pasienter opp til 18 måneder etter akutt Covid-19 sykdom. Nesten halvparten av pasientene hadde restplager. Vanligst var utmattelse, hukommelse- og konsentrasjonsvansker og tungpust, og ved 12 måneder var forekomsten av disse plagene mye høyere enn blant en aldersjustert seronegativ kontrollgruppe. SARS-CoV-2 spesifikke immunsvar korrelerte også med restplager. Totalt sett så vi at Covid-19 pasienter hadde økt risiko for en rekke symptomer, og at for de fleste tar det lang tid å bli kvitt plagene.

Abbreviations

Ab	Antibody
A/H1N1pdm09	The 2009 H1N1 pandemic virus strain
ACE2	Angiotensin Converting Enzyme 2
AIDS	Acquired immunodeficiency syndrome
AMR	Antimicrobial resistance
APC	Antigen presenting cell
ARDS	Acute respiratory distress syndrome
ARTI	Acute respiratory tract infection
CAP	Community Acquired Pneumonia
CD	Cluster of Differentiation
CDC	Centers for Disease Control and Prevention (USA)
CO_2	Carbon dioxide
COBRA	Computationally Optimized Broadly Reactive Antigen
COVID-19	Coronavirus disease 2019
CRP	C-reactive protein
DNA	Deoxyribonucleic acid
ds	Double stranded
E	Envelope protein
EBM	Evidence-based medicine
ED	Emergency Department
EDTA	Ethylenediaminetetraacetic acid
ELISA	Enzyme-linked immunosorbent assay
ER	Endoplasmic Reticulum
EVD	Ebola virus disease
eGFR	Estimated glomerular filtration rate
GMT	Geometric mean titer
HA	Hemagglutinin
HAI	Hemagglutination Inhibition
HAP	Hospital Acquired Pneumonia

HCl	Hydrochloric acid
HCoVs	Human coronaviruses
HCW	Health care worker
HI	Hemagglutinin inhibition assay
HIV	Human immunodeficiency virus
HRP	Horseradish peroxidase
ICU	Intensive care unit
IFN	Interferon
Ig	Immunoglobulin
IIV	Inactivated influenza vaccine
i.v.	Intravenous
IL	Interleukin
ILI	Influenza-like-illness
LAIV	Live attenuated influenza vaccine
M1/M2	Matrix protein 1 or 2
MERS-CoV	Middle East Respiratory syndrome coronavirus
MHC	Major Histocompatibility Complex
MN	Microneutralization
N	Nucleocapsid protein
NA	Neuraminidase
NAAT	Nucleic Acid Amplification Test
NI	Neuraminidase Inhibition
NAI	Neuraminidase Inhibitor
NIPH	Norwegian Institute of Public Health
NK	Natural killer cell
NP	Nucleoprotein
O_2	Oxygen
OD	Optical density
OPD	O-phenylenediamine dihydrochloride
ORF	Open reading frame
PB	Polymerase protein basic

POC	Point-of-care
POCT	Point-of-care test
PPE	Personal Protective Equipment
PRR	Pathogen recognition receptor
RBD	Receptor Binding Domain
RIDT	Rapid Influenza diagnostic test
RTI	Respiratory tract infection
RT-PCR	Reverse-transcriptase Polymerase Chain Reaction
RNA	Ribonucleic Acid
S	Spike protein of SARS-CoV-2
SA	Sialic acid
SARS	Severe acute respiratory syndrome
SARS-CoV	Severe Acute Respiratory Syndrome Coronavirus
SARS-CoV-2	Severe Acute Respiratory Syndrome Coronavirus 2
SATS	South African Triage Scale
ss	Single stranded
TCR	T cell receptor
Tfh	T follicular helper cell
Th	T helper cell
TLR	Toll-like receptor
TMB	3,3',5,5'-tetramethylbenzidine
TNF	Tumor necrosis factor
VOC	Variant of concern
WHO	World Health Organization
WWI	World war I

Contents

Introduction

Epidemics and pandemics.

The development and spread of contagious diseases.

This thesis was conducted under to different disease outbreak scenarios; a seasonal influenza epidemic and a global SARS-CoV-2 pandemic. The word "epidemic" originates from the Greek *epi* (upon) and *dêmos* (people). It is defined as the occurrence of a communicable disease within a limited area and period that exceed the normal disease frequency of the population[2]. A "pandemic" is defined as a wide-spreading epidemic, that crosses international boundaries, and normally affects a considerable number of people[2]. Paleo archaeological findings, meaning archaeology of deep time, document the presence of infectious diseases back in the era of hunters and gatherers. Contagious diseases on the other hand, first efficiently started spreading with the establishment of larger agricultural communities 10000-12000 years ago[3]. This societal shift involved closer contact between people and animals through both crowded living conditions and domestication, which helped the spread of zoonoses, infectious diseases that transmits between animals and humans. As communities expanded, and trade and travel increased contacts between communities, this promoted the spread of communicable diseases to immunologically naïve individuals. Devastating disease outbreaks have been reported since ancient Greece, including the Antonine plague (2nd century A.D) and the Justinian plague (6th century A.D)[4]. During the industrial revolution (1760-1840), urbanization accelerated the spread of infectious diseases due to crowded and bad housing conditions, poor sanitation, and polluted working environments. Frequent community outbreaks of both airborne (e.g., tuberculosis) and waterborne communicable diseases (e.g., cholera, typhoid) caused poor health and short life expectancies[5].

The Miasma theory, explaining that diseases were caused by "bad air" originated in the Ancient Greece, and still dominated in the 19th century[6]. Although the theory

contributed to sanitation reforms, the theory lacked an explanation of the true causes of infectious diseases, limiting advances in infection control interventions. By the end of the 19th century, medical science took a huge leap forward by establishing the new germ theory of disease claiming the role of specific agents causing infectious diseases. Scientific pioneers Louis Pasteur (creator of the germ theory, 1861)[7], Robert Koch (isolation and pure culture of *Vibrio cholerae* and *Mycobacterium tuberculosis* 1882-1883)[8], Joseph Lister (antiseptic surgery 1865-1869)[9] and John Snow (tracking a cholera outbreak to contaminated water, first epidemiologist) amongst others, contributed with important discoveries in this paradigm shift. Viruses were discovered in 1892 by Dmitri Ivanovsky's[10], but their role in human infectious diseases, like influenza, was first established years after the Spanish flu (1918-1919), the most lethal pandemic of all times. In the 1930s, influenza viruses of the H1N1 subtype were isolated from pigs and humans and soon linked to the 1918 pandemic through studies of neutralizing antibodies in sera[11-13]. Following the discoveries of bacteria and viruses, medical science rapidly advanced. Today we have improved hygiene, intervention of vaccines, targeted antimicrobial and antiviral therapy, infection control strategies, and surveillance that makes humankind better equipped than ever to combat communicable infectious diseases. Still, the emergence of previously undetected or unknown infectious diseases, termed emerging infectious diseases, including viral diseases acquired immunodeficiency syndrome (AIDS), severe acute respiratory syndrome (SARS), Middle East respiratory syndrome (MERS), and Ebola virus disease (EVD) shift both research and political attention. Due to its recurrent nature, the pandemic threat of influenza A has claimed much attention over the years and remains a puzzle. No other pathogen has caused more frequent pandemics than influenza, or comparable fatalities within a brief period. There are still many unknowns about the pathogenicity and origin of previous pandemic pathogens, and epidemic diseases continue to re-emerge. Increased human interference with wild animal habitats, climate change and drug resistance are key factors of concern as they make us further vulnerable to new pandemic threats. At this moment, since the emergence of the coronavirus SARS-CoV-2 in 2019, we are once again facing the consequences of this human, animal interference.

Influenza –from mild epidemics to lethal pandemics

Influenza is a respiratory viral zoonosis[14-16] that has been capable of adapting and surviving all current medical advances and is present in all parts of the world as an epidemic disease. Human influenza infection, or "the flu", is characterized by a sudden onset of fever, headache, myalgia, cough, and other respiratory symptoms[17]. Reports of symptoms that may be attributable to influenza date back to the Ancient Greece, where Hippocrates described the "cough of Perinthos" in the 5th century BC[18,19]. Since the Middle Ages, many epidemics and at least 13 pandemic outbreaks have been attributed to influenza. Influenza A and B are the main disease-causing agents in humans, but only influenza A has a pandemic potential and is found in many different animal species, including birds, swine, and horses. Wild aquatic birds are the natural reservoir for most subtypes of influenza A[20]. Whereas influenza A was discovered in 1933, influenza B was not discovered until 1940[21]. Influenza A viruses can infect different mammals, adapt genetically, and further cause transmission between individuals in the new hosts[22,23]. Different variants of structural surface glycoproteins Hemagglutinin (HA) and Neuraminidase (NA) coat the influenza A and B viruses. The subtypes of the influenza A viruses are named by the combination of these proteins[24,25], whereas influenza B are divided into two main lineages, Victoria and Yamagata[26]. Influenza A has the highest mutational rate of the four influenza types and human strains evolve in two ways: through coincidental genetic point mutations of the surface proteins (HA and NA), termed "antigenic drift," and by reassortment of genetic segments from different influenza viruses when they haphazardly infect the same host, termed "antigenic shift". Mixing of genetic material may result in novel progeny viruses that are antigenically distinct from other influenza viruses found in humans, and able to escape prior immunity. Such a virus may show increased transmissibility, virulence, or both. If the virus also develops capacity for sustained human-to-human transmission, and appears at the right time and place, it can be the origin of a new pandemic. The 1918 "Spanish flu" was the first pandemic identifying an influenza virus, a human adaption of a H1N1 virus of avian origin, as the causative agent of influenza disease[27,28]. Studying this pandemic has brought great insight into the

dissemination of pandemic infections. Reports of early outbreaks have been derived from US military camps, but during the spring of 1918, the world rapidly experienced parallel disease outbreaks over distant geographic areas, and the origin of the pandemic is still unresolved[29,30]. World War I (WWI) played a key role in the spread of the devastating influenza pandemic as it swept through war-disrupted cities and battlefield trenches, with military censorship inhibiting the spread of information on the disease outbreak. Being a neutral country, Spain was the first to report on the novel and lethal disease, and later got stuck with the name, the Spanish flu[31]. The pandemic spread in three recurring waves within a year from 1918-1919, the second wave being the most lethal. Globally, over 500 million people were infected and over 50 million people died, ten times more than the fatalities caused by the war itself [32]. Most deaths were caused by acute respiratory complications, some of rapidly progressing character like the acute respiratory distress syndrome (ARDS), but more commonly, people succumbed more slowly to pneumonia with secondary bacterial infection[33,34]. The mortality pattern during the Spanish flu was unique compared to other influenza outbreaks, as the infection was most lethal in the age-group 20-40 years, as well as the expected youngest and oldest individuals, creating a W-shaped mortality curve [35,36]. The rate of secondary infection was also higher than in seasonal influenza. The reason for the unusual distribution of infection and deaths is still not completely resolved, but there are several theories. Host factors were likely to play a significant role, with aberrant immune responses in young adults possibly due to the childhood exposure of the 1875-1900 birth cohort to an immunologically distant influenza subtype (H3N8) resulting in compromised immunity to the pandemic virus[37]. Immune-mediated tissue damages through a cytokine storm, high susceptibility to new bacterial pathogens in socially displaced military populations, as well as the widespread use of aspirin and smoking habits of young adults have also been suggested as contributing factors[38,39]. After the 1918 pandemic ended, the H1N1 influenza virus continued to circulate causing mild seasonal influenza until it disappeared in 1957 when it was replaced by an emerging avian/human reassortant H2N2 virus, causing the 1957 Asian flu virus pandemic[40]. This virus led to the second largest influenza pandemic in the 20th century (***figure 1***). In 1968, a new reassortment between the H2N2 virus and avian influenza resulted in

the circulation of an immunologically distinct H3N2 virus, and the third influenza pandemic, the Hong Kong flu. Due to pre-existing N2-immunity the mortality rates were lower compared to the previous pandemic[41]. In 1977, the 1918 H1N1 mysteriously reappeared and circumstantial hypothesis of its origin includes either a laboratory accident, an ineffectively attenuated live vaccine, or deliberate release[42,43]. The 1977 outbreak manifested as mild influenza mostly affecting children, not qualifying as a pandemic.

By the end of the 1990s Johan Hult recovered the 1918 pandemic virus from lung tissue of victims buried in the Alaskan permafrost. This led to the completion of the genetic sequencing of the 1918 H1N1virus, and the conclusion that viruses responsible for all three of the subsequent pandemics, and seasonal influenza outbreaks until present were descendants of this subtype[44]. In the 21st century, a new descendant of the 1918 H1N1 virus, the 2009 swine (A/H1N1pdm09) pandemic virus was the result of a new reassortment with 1918 swine, seasonal H3N2 and avian influenza subtypes[45]. This time, hospitalized patients were treated with stockpiled influenza antivirals, and the rapid genetic sequencing of the virus led to the production of efficient vaccines within six months of the first case report, providing important tools to manage the outbreak. Although influenza pandemics of the past had markedly increased population mortality compared to seasonal influenza, the 2009 Swine flu pandemic did not show increased mortality rates. However, a signature age shift was observed as many deaths occurred in previously healthy young adults, causing many more life-years lost[46].

In between pandemics, influenza has circulated (**Figure 1**), with seasonal peaks in the Northern and Southern Hemispheres, and year-round activity in the tropic areas[47]. In recent years, two influenza A subtypes have co-circulated with the two influenza B lineages. Antigenic drift affecting virulence factors, present population immunity, vaccine efficacy and vaccine coverage influence the annual disease burden of influenza. Together, influenza infections cause between 290 000 and 650 000 deaths annually by respiratory disease alone[48].

Surveillance of animal reservoirs for highly pathogenic avian influenza A virus subtypes circulating in wild and domestic birds are important risk assessment tools for future influenza pandemics. In addition, the surveillance of genetic changes in human influenza viruses and knowledge of current population immunity is necessary to select suitable vaccine strains for annual influenza vaccination. However, there are still challenges in accurate forecasting of the next circulating influenza subtypes and strains. This poses challenges in both annual vaccination and pandemic preparedness.

SARS-CoV-2: The ongoing pandemic

SARS-CoV-2 is the seventh addition to the human coronaviruses (HCoVs). The HCoVs were first discovered in the 1960s[49,50] and four species are currently circulating endemically, causing 15-29% of common colds[51]. Apart from respiratory symptoms, HCoVs can also cause variable degrees of gastrointestinal disease. Coronaviruses exist in both birds and mammals, and molecular clock analysis suggest that the last common ancestor between them diverged around 10000 years ago[52]. Mathematical modeling estimate that the viral lineage of which SARS-CoV-2 has originated, has been circulating in bats for decades[53]. The emergence of SARS-CoV-2 is the third time in the 21st century that a new, highly pathogenic coronavirus has emerged from animal reservoirs and adapted to transmission between humans. Additionally, researchers have recently hypothesized that the 1889 “Russian flu” may have been a coronavirus pandemic, arguing that molecular dating of HCoV variants, similarities of symptoms and epidemiological characteristics with coronavirus disease-19 (COVID-19)[54-57] provide important clues for this point of view.

In 2003, the severe acute respiratory syndrome coronavirus (SARS-CoV) spread from China to 29 countries, causing over 900 deaths (case fatality rate 11%)[58]. The outbreak was halted through meticulous contact tracing, isolation and quarantine procedures[59], and the virus later disappeared. Ten years later in 2013 a pathogen identified and named Middle East respiratory syndrome coronavirus (MERS-CoV) infected Saudi Arabian patients, causing influenza-like symptoms, severe acute pneumonia, acute respiratory distress syndrome (ARDS), and multi-organ failure[60]. Over 2200 cases have been

identified, and fatal disease is common (>35% case fatality rate)[61]. New cases occur sporadically to this day, possibly due to zoonotic spill-over from dromedary camels[62]. In Wuhan, China, in December 2019, a mysterious accumulation of patients with severe respiratory symptoms and fever, concerned local doctors[63]. China publicly acknowledged a disease outbreak on December 31st, when the Chinese WHO was informed of 27 cases of pneumonia with unknown aetiology, all connected to a Wuhan seafood market[64]. Twelve days later, the novel pathogen was isolated, and the SARS-CoV-2 genome deposited on GISAID platform, an open data platform, sharing genetic sequence data from influenza and coronaviruses with scientists from the entire world. The 22nd of January 2020 human-to-human transmission was confirmed[65]. On the 23rd of January, China implemented an extreme lockdown of Wuhan and the Hubei province that lasted for 76 days and affected more than 50 million people. By then, it was already too late to contain the virus, which had spread to Thailand (Jan 13th), Japan (Jan 15th) and the US (Jan 20th)[66]. The new disease, named COVID-19, mobilized international and national disease outbreak responses guided by institutions like the World Health Organization (WHO), Center for Disease Control and Prevention (CDC), National Health Service (NHS) and Norwegian Institute of Public Health (NIPH). Differences in subsequent quarantine rules, lockdowns, and social distancing policies contributed to the success of mitigating the viral spread in some countries[67,68], whereas others rapidly suffered exponential growth of cases suffocating local health care capacities[69]. The WHO declared COVID-19 as the second pandemic of the 21st century on March 11th, 2020. When viral containment became impossible, many countries, including the US, England, and Norway, publicly communicated the importance of "flattening the curve". This strategy was adapted from the Centers for Disease Control and Prevention (CDC, US), with the goal of controlling the spread of the virus to reduce health care burden and allow time for development of specific treatment and vaccines. Although SARS-CoV-2 does not hold the same inherent mutability as influenza, RNA replication errors (substitutions, insertions, and deletions) occur frequently, driving genetic diversity. In addition, evidence of intra-host recombination events has been found, and is a potential source of significant genetic evolution[70]. Billions of viral passages have resulted in the emergence of new variants of concern (VOCs). The main

viral surface protein, called Spike protein, holds important antigenic sites that have changed since the original Wuhan-variant. More advanced variants have demonstrated increased transmission and immune evasion, such as Alpha, Delta, Gamma, and Omicron VOC. In all the different variants, mortality is highest amongst older adults, whereas severe infection in children is rare. However, infection in children has been associated with a rare inflammatory condition named multisystem inflammatory syndrome in children (MIS-C). The risk of short and long-term complications of infection in both children and adults is worrisome[71,72]. No disease-specific treatment was available when the novel virus emerged. Lessons from previous pandemics contributed to international collaborations, public health measures and clinical studies in a collective mobilization to save lives and to stop the pandemic. Thanks to an unprecedented rapid development and roll-out of vaccines, starting in January 2021, deaths by COVID-19 have been prevented in large scale. Rapid point-of-care diagnostics have been developed and adapted for infection control use, both inside and outside health care facilities. Clinical studies have identified useful therapeutics and treatments. Although nobody can predict how and when the pandemic will end, we need to be prepared for different scenarios. Continued efforts to study the new disease, and the implementation of scientifically founded initiatives will help us navigate through the coming pandemic waves.

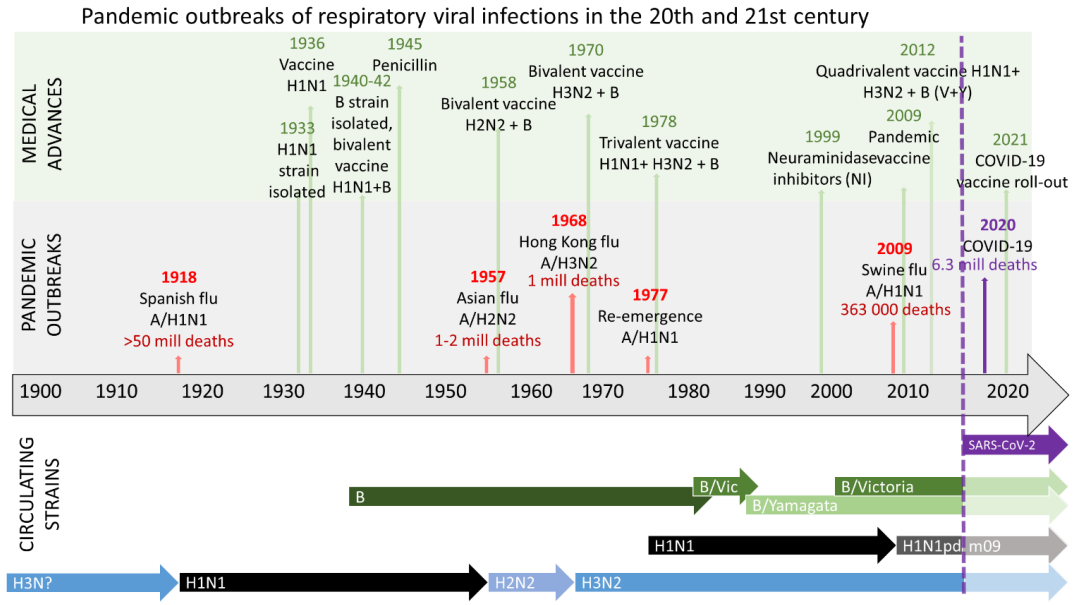

Figure 1. Overview of human respiratory viral pandemic outbreaks, medical advances, and circulating influenza strains from 1918-2022.
The upper part of the figure illustrates the four last influenza pandemics (red vertical lines) the current SARS-CoV-2 pandemic (purple vertical line), and the medical advances during this period (green lines). The lower part of the figure illustrates the circulating strains of influenza, with the addition of SARS-CoV-2 from 2020. The 1977 re-emergence of H1N1 was a mild outbreak compared to other universally acknowledged influenza pandemics and thus not considered a true pandemic. The causing strain was identical to a previously circulating A/H1N1 virus. Drifted influenza strains originating from the pandemic viruses have continued to circulate between pandemics, and since 1977, A/H1N1 and A/H3N2 have continued to co-circulate. Influenza B does not cause pandemics but co-circulates endemically and epidemically with influenza A strains. After the 1918 Spanish flu, the discovery of antimicrobials, antivirals, vaccines, and viral diagnostics have proved advantageous in treatment and infection control for both influenza and COVID-19. Figure was inspired by Hannoun et al[21], Piret et al[40], and Taubenberger et al[73].

Viral characteristica and pathogenicity in the human host

Although human influenza A and SARS-CoV-2 are quite different viruses, they still show important similar characteristics that are hallmarks of pandemic potential. They originated from zoonotic viruses with an animal reservoir, which are transmitted between different species, and they have both made the jump into the human population and adapted to sustained transmission between human hosts through the respiratory route. Both influenza and SARS-CoV-2 are RNA viruses, where different mechanisms

contribute to genetic evolution, host adaption, changing virulence and increase the probability of evading pre-existing immunity.

Both influenza and SARS-CoV-2 bind to receptors that are abundant in human respiratory epithelium, and they commonly cause respiratory disease. Whereas influenza primarily replicate in the respiratory tract, SARS-CoV-2 can replicate in extrapulmonary tissues. These differences are due to different distributions of the target receptors of the two viruses. SARS-CoV-2 binds to Angiotensin-Converting Enzyme 2 (ACE2), which is abundant on both respiratory and gastrointestinal epithelial, and endothelial cells[74]. Influenza binds to cell membrane oligosaccharides containing sialic acid (SA), and human influenza viruses prefer the α2,6-linked SA, which is abundant in the upper airways. Influenza and SARS-CoV-2 outbreaks are similar in their mode of transmission and initial symptomatology, including the significant role of immunopathology in severe disease. The basic reproduction number (R_0), describing the transmissibility of an infectious agent, was initially estimated to be around 2.7 for SARS-CoV-2[75], comparable to R_0 1.8 for the 1918 Spanish flu, but higher than the 2009 H1N1 swine flu pandemic (R_0=1,5)[76]. In seasonal influenza, pre-existing immunity reduces the R_0. Both influenza pandemics and the SARS-CoV-2 pandemic has culminated in repeated pandemic waves.

The clinical course of influenza and SARS-CoV-2 infection depends on both viral and host factors. The host immune response is a major contributor to the final disease outcome as aberrant immune responses significantly increase the risk of disease progression and the development of complications.

Multiple publications have compared the clinical attributes of influenza and SARS-CoV-2, in addition to co-infection rate, severity of infection and complications.

In this thesis, the unique characteristics of the two viruses, but also their similarities will be discussed. It is likely that influenza and SARS-CoV-2 will continue to co-circulate in regular epidemics in the near future. Together they can pose a significant

health care burden. In addition to their individual complications, the current knowledge of the potential effect of viral interference is limited[77]. To reduce the associated health care burden with these viral infections, we must continue to fill the knowledge gaps

about both viruses and their associated host responses. We must improve how we interpret and manage the clinical manifestations of virus-host interactions during infection, to achieve the best possible patient care and infection outcomes.

Influenza

Influenza viruses belongs to the family O*rthomyxoviridae*. Influenza is classified according to the internal proteins, and divided into four main types: A, B, C and D. Influenza C causes mild respiratory disease in humans, influenza A and B causes seasonal influenza epidemics, and only influenza A has pandemic potential. There is a universal nomenclature consensus for the naming of influenza strains: *virus type/place of isolation/strain number/time of isolation/viral subtype*. An example of this is "A/Michigan/45/2015(H1N1)".

By phylogenetic division, influenza viruses are organized into clades and subclades by their genetic resemblance, enabling the surveillance of the rapid viral genetic evolution. Evolutionary surveillance is a useful tool when choosing the strains for the next season's influenza vaccine, and when evaluating pandemic risk.

Viral structure and proteins

The main surface protein of influenza, Hemagglutinin (HA) (**figure 2**), is a trimer with an immunodominant highly variable head-domain and a more conserved stalk domain. The HA head is directly involved in binding to sialic acids (SA) on host cell membranes, leading to endocytosis of virus into the cell. Neuraminidase (NA) is a tetrameric surface protein, possessing a catalytic head domain and a stalk region that can vary in length. The NA is an enzyme that cleaves SAs, which is the binding substrate of HA. This cleaving function both facilitates viral movement through mucus in the respiratory tract, and the budding of newly created virions on host cell membranes. This makes NA an important target for influenza antivirals. 18 subtypes of HA and 11 subtypes of NA have been discovered, 16 and 9 of these are found in wild aquatic birds[78]. M2 is a matrix ion channel involved in maintaining the pH across

the viral envelope. M1 is a structural protein that binds to the lipid membrane and form a multimeric structure. M1 determines the viral shape and is essential for viral assembly and budding. Together with the RNA polymerases and RNA, nucleoprotein form the Ribonucleoprotein complex (RNP) which is essential for RNA replication.

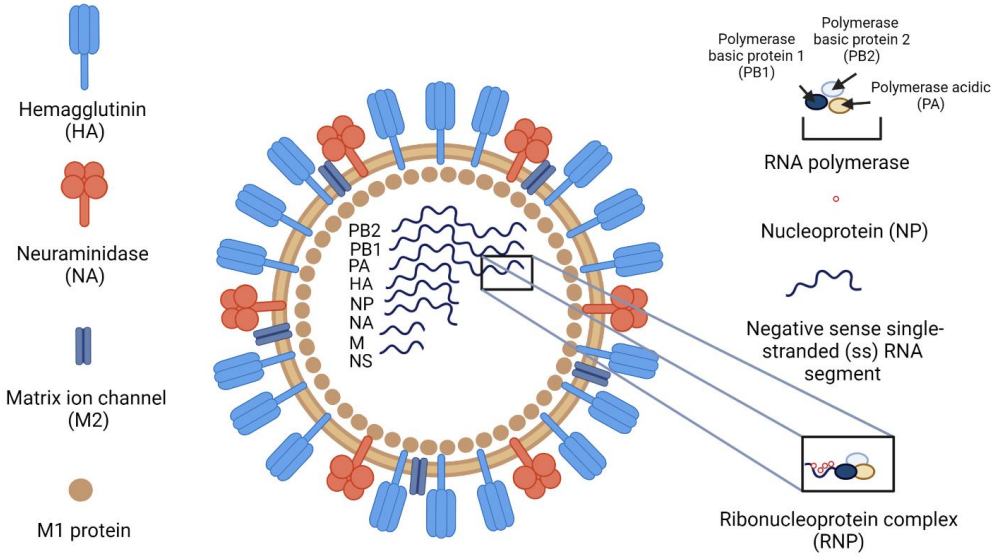

Figure 2. Schematic overview of the Influenza A viral structure.
Influenza is an enveloped virus. The shape of the virus varies, from filamentous to spherical, and the size of a virion (assembled and released viral particle capable of infecting a cell) is about 80-120 nm in diameter (300nm length for the filamentous form). The virus envelope is a lipid membrane layer embedded with important structural and functional proteins. In order of abundance, these include the protruding glycoproteins Hemagglutinin (HA) and Neuraminidase (NA) and Matrix (M2) transmembrane ion channels. The HA and NA proteins are asymmetrically distributed on the membrane surface in order to facilitate mucus penetration[79], and the HA to NA proportion is approximately 4:1[80]. Inside the lipid membrane, M1 forms the viral core, which contains the viral genome organized into eight single strands of negative sense ribonucleic acid (RNA) with associated polymerases and Nucleoprotein, forming the Ribonucleoprotein complexes (RNPs). The eight RNA segments are organized by their size, and according to which protein coding sequences they contain. *Created with BioRender.com*

Viral life cycle, protein function, and viral tropism

Influenza replication cycle

The influenza replication cycle is illustrated in **figure 3**.

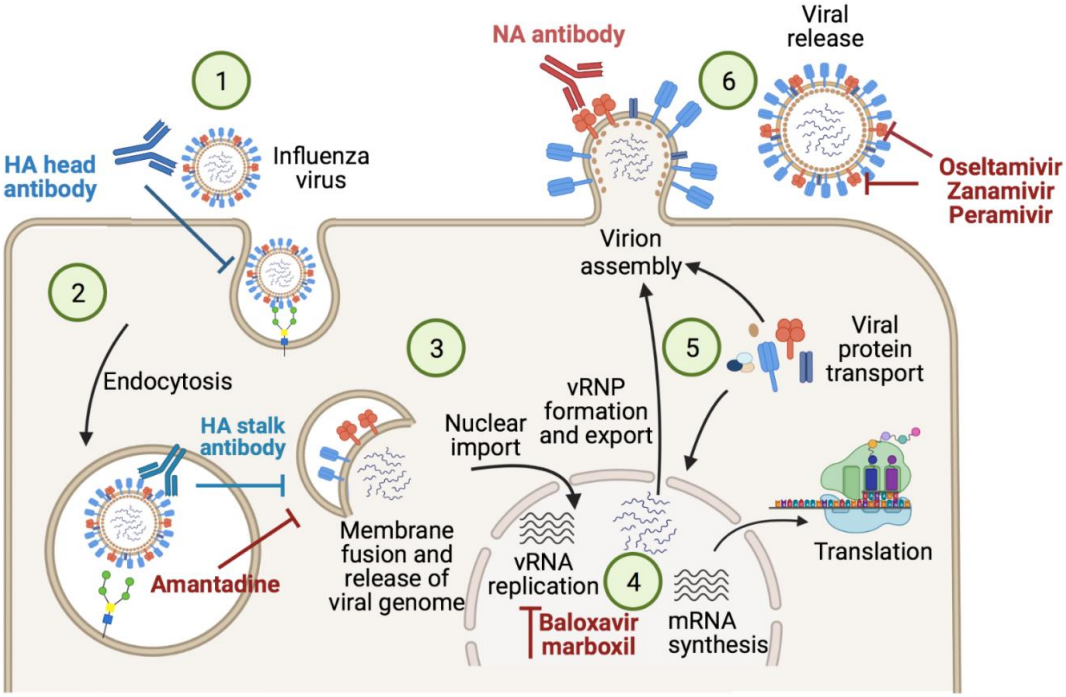

Figure 3. Influenza replication cycle.
1. To enter a host cell, the influenza HA binds to sialic acid-linked receptors in the cell membrane leading to endocytosis of the viral particle. 2. Inside the endosome, low pH opens viral M2 proton channels, and the drop in viral pH lead to a conformational change in the HA, eventually resulting in fusion of the viral and endosomal membrane. 3. Viral RNA (vRNA) is released to the cytosol and transported to the nucleus. 4. Influenza RNA transcription is localized to the cell nucleus. Viral polymerases participate in the transcription of new viral RNA (vRNA) and messenger RNA (mRNA). Protein synthesis takes place on endoplasmic reticulum (ER) associated and cytosolic ribosomes[81]. 5. New viral proteins and vRNA are assembled into new viral particles. 6. M1 proteins are important in viral assembly and formation of budding viral particles. Cleavage of sialic acids by NA is essential for the release of progeny virus from the cell membrane and for aiding movement of viruses through mucus in the respiratory tract. Inhibiting viral entry and release are important immunological and therapeutic targets. *Created with BioRender.com template by Lena Hansen.*

Viral tropism

Viral tropism refers to the ability of a virus to infect and replicate in a particular cell (cell tropism), tissue (tissue tropism) or host (host tropism). The substrate specificity of HA is an important determinant of host and cell tropism. HA of human influenza viruses preferentially binds to α2,6-linked SAs, which are abundant in all parts of the airways including the nasal cavity, but predominantly on non-alveolar cells[82], hence infection generally leads to a mild upper airway disease. In contrast, HA proteins of avian influenza viruses preferentially bind to α2,3-linked SAs found in human bronchioles and alveoli cells, as well as in extrapulmonary tissues like ocular cells. Thus, avian influenza viruses that can infect humans can lead to severe pneumonia. Viral polymerases are also important host tropism factors, as their enzymatic activity is restricted to certain cell types[83]. Reassorted viruses might contain polymerases that are not compatible with human host proteins or transcriptional machinery, and thus such progeny viruses will not have a potential to replicate in human hosts.

Influenza transmission

After replication in the respiratory tract, influenza viruses are expelled by breathing and coughing and is transmitted through respiratory droplets, aerosols, and fomites (surface transmission). The incubation time is short, 1-2 days. In human challenge studies, pre-symptomatic transmission occurs and viral titers peaks at day 2-3 after incubation, corresponding to approximately 1 day after symptom onset[84]. After 6-7 days, viral shedding had normally subsided[85]. Compared to adults, children shed more virus, contributing to the high transmissibility amongst the youngest age groups. Immunocompromised individuals may experience longer periods of viral shedding. Physical distancing, school closures and intensified hygiene and mask mandates initiated during the COVID-19 pandemic resulted in a significant fall in influenza cases, demonstrating their collective influence on influenza virus transmission[86]. This is important knowledge when preparing for the next influenza pandemic.

Influenza acute course and complications

The clinical manifestations of influenza disease ranges from asymptomatic infection to fatal disease. It is estimated that 10-15% of the adult population are infected in annual epidemics, but overall, few patients are hospitalized and develop a severe symptom course. Acute illness typically starts abruptly with high fever, headache, muscle and joint pain, upper airway symptoms and dry cough. The combination of fever and cough is most predictive of influenza disease[17], however, there is significant overlap with symptomatology of the common cold, other influenza-like-illnesses (ILI) and community acquired pneumonia (CAP). Although uncommon, influenza can also present with gastrointestinal, cardiac, and central nervous system (CNS) symptoms. Hospitalization can be due to these extrapulmonary complications, respiratory distress, dehydration, and malaise. Acute disease lasts for approximately 7 days, but patients with moderate to severe disease can experience prolonged symptoms such as fatigue. Respiratory complications of influenza include viral or co- or secondary bacterial pneumonia, septicaemia, and ARDS. Whether the initial viral infection is cleared, and lung homeostasis returns to normal, or the disease progresses to severe pulmonary and/or extrapulmonary complications depend on both viral and host immune response and other risk factors. Timely antiviral treatment has the potential of shortening disease course preventing complications like bacterial pneumonia[87,88] if administered early after symptom debut.

Co- and secondary infection.

Influenza infection can cause primary viral pneumonia, but more commonly predisposes to the development of concomitant or secondary bacterial pneumonia. This is seen in up to one-third of hospitalized patients with severe influenza and associated with increased mortality[89,90]. Patients who receive a diagnosis of bacterial CAP are often found to have a mixed infection with viral pathogens like influenza, illustrating the diagnostic challenge in these patients. Gram-positive bacteria *Streptococcus pneumonia, Streptococcus pyogenes*, and *Staphylococcus aureus* are common colonizers of the upper respiratory tract. Influenza-targeted immune responses

including the suppression of alveolar macrophages, and viral tissue damage, provides a favourable environment for bacterial dissemination to the lower airways, were they can induce a bacterial pneumonia[91].

The differentiation of a co-occurring infection with influenza and another pathogen from secondary- or superinfection, where a second pathogen has resulted in infection after the primary influenza virus infection, is challenging. In some studies superinfection refers to influenza patients that are diagnosed with a bacterial pneumonia after they were admitted to hospital. In the course of a secondary infection upon influenza diagnosis, symptoms will typically develop in a two-phased manner where acute symptom onset represents the initial viral infection, and within a week, secondary deterioration with new respiratory symptoms occurs due to the secondary infecting pathogen. Naturally, it is not always easy to distinguish co-occurring viral/bacterial infection and secondary viral/infection, and for simplicity, both are named co-infection in this thesis. Bacterial co-infection in influenza disease is clinically relevant due to the available treatment options. With the introduction of new, antiviral treatments that are targeted towards other respiratory viruses than influenza, viral co-infection will be of greater diagnostic concern. Natural influenza infection induces tissue damage and antiviral host responses that compromise immune resistance to respiratory bacterial pathogens. Studies have reported of increased mortality in influenza patients presenting with bacterial co-infection[89,92]. The risk of influenza/bacterial co-infection and other influenza complications has been found to increase with early childhood (6 months-5 years old), old age (>65 years old), chronic diseases (including obesity) and pregnancy. These groups are thus recommended annual influenza vaccination by WHO and the NIPH (children only if born prematurely), and even broader vaccine recommendations are given in the US[93]. Most recent studies reporting on the prevalence of influenza co-infection include patients infected during the

A/H1N1pdm09 Swine flu pandemic, and the co-infection rate and influenza severity have varied between epidemic influenza outbreaks depending on population immunity and strain dominance[94]. Influenza patients in intensive care units (ICU) have the

highest incidence of bacterial co-infection, ranging from 17%-30%[95-97]. These and other studies including non-ICU patients and children were included in a 2016 systematic review of influenza co-infection, where the authors concluded the bacterial co-infection rate ranged from 11-35% after heterogeneity adjustments[98].

The most prevalent bacterial pathogen involved in influenza co-infection vary with geographical setting. However, *Staphylococcus aureus*, *Streptococcus pneumoniae* and *Haemophilus influenzae* are of the most common causing agents.

SARS-CoV-2

SARS-CoV-2 is a member of the *Coronaviridae* family, and more specifically belongs to the subfamily *Orthocoronaviridae (***figure 4**). Coronaviruses subdivides into alpha- and betacoronavirus, which only infects mammals, and gamma- and delta coronavirus that infects both animals and birds. The coronaviruses that circulate in humans belong to the alphacoronavirus subfamily and include HCoV 229E and HCoV NL63. HCoV OC43, HCoV HKU1, SARS-CoV-2, MERS-CoV and SARS-CoV are species of the betacoronavirus subfamily. The wide disease spectrum caused by SARS-CoV-2 infection early gained the name *Coronavirus disease 2019*, or COVID-19, by the WHO[99]. Naming convention of SARS-CoV-2 strains resembles influenza nomenclature: *virus type/host/place of isolation/strain number/time of isolation.*

Viral structure and proteins.

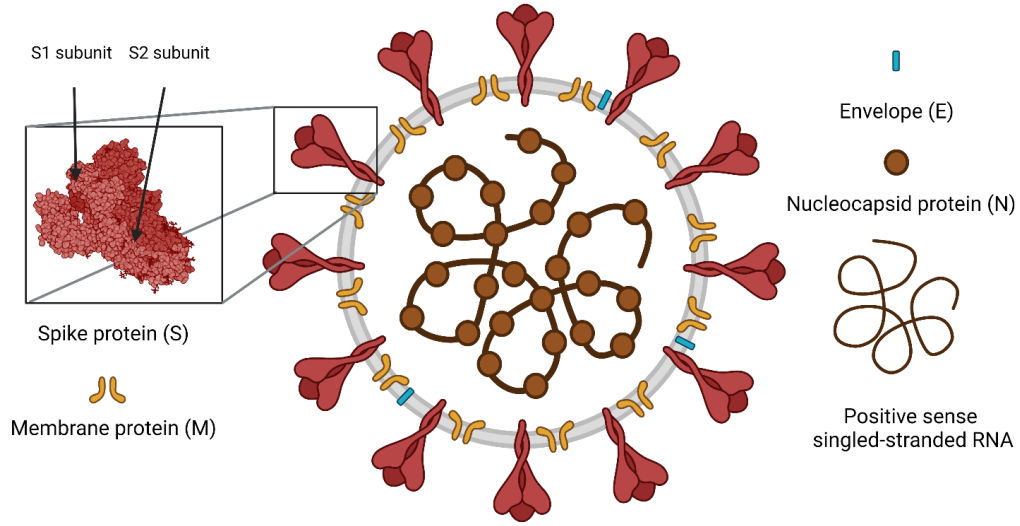

Figure 4 . Schematic overview of the SARS-CoV-2 structure.
SARS-CoV-2 is an enveloped virus, with a size of 60-140nm in diameter[1]. The virus envelope lipid membrane contains structural proteins Spike (S), membrane protein (M) and envelope (E). Knowledge of protein function is based upon studies of other coronaviruses. Spike is heavily glycosylated trimer, which functionally divides into S1, containing the receptor-binding domain (RBD) responsible for the binding to cell receptor ACE2 through the receptor-binding domain (RBD), and S2, which promotes the fusion of viral and host cell membrane. M is the most abundant membrane protein and is important in viral assembly[100]. The E membrane protein is proposed to be a pH regulated cation channel but is also thought to play a role in viral assembly and release[101-103]. The highly conserved nucleocapsid (N) protein coats and protect the large single stranded RNA of 30kb. N is involved in genome packaging and can interfere with host antiviral responses and receptor signaling[104]. In addition, the viral genome encodes several non-structural proteins. *Created with BioRender.com*

Coronaviruses acquired their name due to the crown-like protrusion of spike proteins from their lipid membrane. Spike is a major immunological target with variable antigenic sites, allowing for immune escape. The viral RNA contains open reading frames (ORFs) and encodes structural and non-structural proteins. Non-structural proteins are not incorporated into progeny virions, but are expressed in infected cells.

Viral life cycle, protein function, and viral tropism

SARS-CoV-2 replication cycle

The SARS-CoV-2 replication cycle is illustrated in **figure 5**.

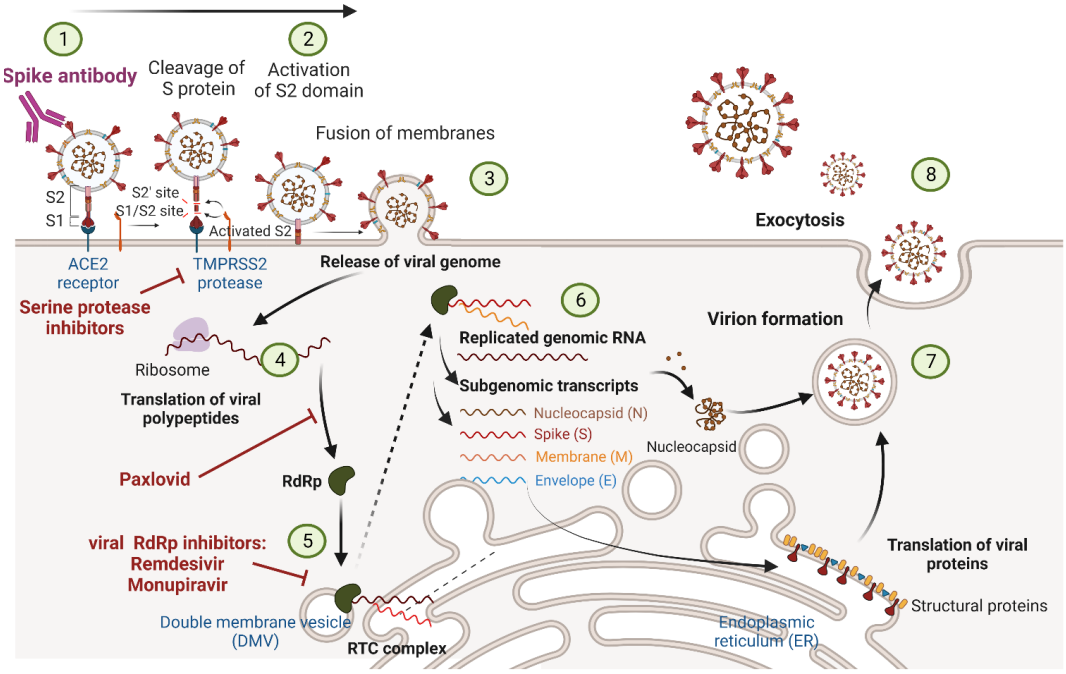

Figure 5. SARS-CoV-2 replication cycle.
Schematic representation of the series of interactions between virus and host cell during the SARS-CoV-2 replication cycle. 1. The receptor binding domain (RBD) of the S1 Spike subunit binds to cell surface ACE2. 2. Host transmembrane serine protease 2 (TMPRSS2) cleaves the fusion site of the Spike protein, promoting S2 subunit fusion of the viral an cellular membrane[105]. 3. Alternative cell-entry by endocytosis involves the endosomal cysteine proteases Cathepsin B and L (CatB/L)[105]. 4. Host ribosome translates two long viral precursor polypeptides that are cleaved by proteases forming essential non-structural proteins like the RNA-dependent RNA polymerase (RdRp). 5. Assembly of a replication-transcription complex (RTC) which associates with double membrane vesicles (DMVs). 6. Subgenomic transcripts of RNA for structural proteins and full-length genomic RNA is synthesized in the DMVs. This is a complex process including steps of replication, proofreading and capping[106]. 7. Structural proteins and genomic RNA assembles into new virus. 8. Progeny viruses are released from the host cell by lysosomal exocytosis[107]. *Created with BioRender, using templates from Dr Benjamin Goldman-Israelow and Glaunsinger lab by Jessica M Tucker, Britt A Glaunsinger et al.*

SARS-CoV-2 tropism

SARS-CoV-2 targets ACE2, a widely present membrane receptor in human tissues[74]. An additional key factor explaining respiratory tract tropism is the co-expression of membrane serine protease 2 (TMPRSS2) in ciliated epithelial and AT2 cells of the respiratory tract, which plays a key role in spike-mediated membrane fusion and cell entry. Viral infection has been identified post mortem in several other cells and tissues expressing ACE2, such as cardiomyocytes, renal tubuli cells, epithelial cells of the gastrointestinal tract, and vascular endothelial cells[108]. Different brain tissue cells express ACE2, and involvement of other cell surface receptors than ACE2, like Neuropilin-1 (NRP1) and CD147 are proposed as alternative mediators of viral infection. Findings of SARS-CoV-2 antigens and RNA in choroid epithelial cells, cortical neurons, and endothelial cells, as well as olfactory epithelium are suggestive of viral replication, but evidence of productive infection is scarce [109,110]. Alternative hypotheses regarding the cause of brain tissue damage observed after COVID-19, includes vascular and immune-mediated damage. To disclose the extent of SARS-CoV-2 neurotropic potential[111,112], more research is needed. Receptor tropism plays a role in explaining difference in infectivity of new VOCs, like Omicron. The Omicron variant is not able to use TMPRSS2 efficiently for cell entry, thus rely on alternative entry routes, which can ultimately alter pathogenicity and cell tropism[113].

SARS-CoV-2 transmission

SARS-CoV-2 is highly transmissible. The virus replicates in cells in the upper and lower airways. The virus is airborne and spreads through aerosols and droplets, in addition to fomites[99]. Close contact increases the chance of being infected, and transmission between family and household members is significant[114,115]. The incubation period has been reported as long as 14 days, and whereas the mean incubation period was found to be approximately 4-5 days in the early days of the pandemic[116,117]. Later variants and recent VOC, such as Omicron, has shown reduced incubation time, reported as short as 3 days[118,119]. Pre-symptomatic viral shedding contributes to the high transmissibility of SARS-CoV-2, complicating contact tracing

and associated mitigation efforts. Viral shedding peaks around symptom onset or the first week thereafter when measured in throat swabs or saliva, respectively[120] [121]. RT-PCR can detect viral RNA for weeks and sometimes months after initial infection, but the infectious period is much shorter. In mild infection, infectiousness normally subsides within 10 days[122]. The period of viable viral shedding is relevant to inform isolation procedures in infected individuals and close contacts. During the pandemic, isolation has been an important national non-pharmaceutical intervention, and in Norway, the isolation period was initially 14 days in the early pandemic but was later reduced to 10 and 5 days. As of February 2022, isolation interventions have been downgraded to advice of staying at home during the symptomatic period of COVID-19.

SARS-CoV-2 acute infection and later complications

The clinical course of SARS-CoV-2 infection can vary from asymptomatic disease to a rapid deterioration and fatal disease. Older age, male gender and pre-existing comorbidities are associated with a higher risk of severe outcome[123]. The general symptom profile of acute disease has interestingly changed since the emergence of the ancestral Wuhan strain to the now dominant Omicron VOC. Initially, typical symptoms of COVID-19 included fever, cough, myalgia, dyspnea, gastrointestinal symptoms, and progressive respiratory failure in severe cases[124]. A distinguishing less critical symptom was the loss of taste and smell[125]. With Omicron, the frequency of upper airway symptoms like runny nose, sneezing and sore throat has increased, and symptoms have currently become more overlapping with other respiratory viral diseases, with fatigue, headaches, and myalgia. Symptoms such as skin rashes, severe confusion and chest pain are less common disease manifestations.

Most symptomatic patients experience acute phase symptoms lasting for about a week, corresponding to the phase of active viral replication. A subset of patient progress to a second stage of disease, characterized by host inflammatory responses. At this stage, signs of progressive pneumonia dominate, and respiratory compromise is present. Disease may progress to ARDS, and multi-organ failure with a dysregulated hyper-

inflammatory stage characterized by systemic vascular endothelial damage with coagulopathy, thromboembolism, end organ damage and death. As clinical knowledge has accumulated during the course of the pandemic, effective symptomatic treatment and targeted therapeutics have improved the prospects for many patients with a severe disease course.

SARS-CoV-2 co-infection

In the early pandemic, the unknowns about the SARS-CoV-2 disease course and complications were dominating. Experience from previous influenza pandemics and epidemics solicited cautiousness due to the possibility of bacterial complications. Radiological imaging of patients admitted with COVID-19 and respiratory failure frequently exhibited characteristics such as peripheral distribution of pneumonia, ground-glass opacities, and vascular thickening. These findings were differed from a "regular" viral pneumonia[126], and inflammatory markers were raised. Thus, at the beginning of the pandemic there was a poor understanding of the occurrence of bacterial co-infections with COVID-19. National and international guidelines recommended the empirical use of antibiotics in severely ill COVID-19 patients, and WHO urged the collection of microbiological specimens before starting treatment[127]. Rapidly, a multitude of studies reported of microbiological findings in SARS-CoV-2 patients, and a meta-analysis published in august 2020 stated that bacterial co-infections were less common than in previous influenza pandemics, only found in 7% of hospitalized patients with COVID-19. There were large regional differenced in co-pathogens, and most commonly were *Mycoplasma pneumoniae*, *Pseudomonas aeruginosa* and *Haemophilus influenzae*[128]. In other studies *Klebsiella pneumonia, Moraxella catarrhalis, Acinetobacter baumannii* and *Staphylococcus aureus were dominating*[129,130]. Geographical factors may be contributing to these discrepancies. In December 2020, a new meta-analysis who distinguished between community acquired (co-infection) and nosocomial (secondary) infection in hospitalized COVID-19 patients found a prevalence of bacterial co-infection of 4% and secondary infection of 14%[131]. A subsequent 2021 update including data from over 30000 patients reported a bacterial co-infection rate of 9%[132]. Importantly, ICU-patients had the highest co-

infection rates. A 2020 US study found a considerable prevalence of viral co-pathogens in SARS-CoV-2 patients[133]. Fungal co-infection also occurs, and reports of diabetic and immune compromised COVID-19 survivors in Indian hospitals suffering from mutilating mucormycosis reached the front pages of global press during spring 2021. Use of corticosteroid and poor glycaemic control probably contributed to this opportunistic infection[134]. We are fortunate that fungal co-infections are rare in the Norwegian health care setting.

Long COVID

Long-term complications after viral infection are not unfamiliar, and both Epstein-Barr virus[135], Ebola virus[136], and influenza subtypes have been associated with symptom sequelae after infection. Chronic fatigue syndrome has been associated with previous viral infections[137].

After the first pandemic wave spring 2020, patients that experienced long-term sequelae after COVID-19 started to emerge. The overall proportion of patient reporting long-term symptoms long after the acute infection has passed, have raised concerns globally. Both asymptomatic patients and patients with mild symptoms can develop long COVID, although the prevalence and severity correlate with increased disease severity[71]. Several names and definitions are used to describe the condition. WHO defines long COVID, or "Post COVID-19 conditions" as persistent or new onset symptoms that is present 3 months after acute infection and lasts for more than 2 months and are associated with a recent SARS-CoV-2 infection. The symptoms are not always present in the acute phase. Long COVID includes a plethora of symptoms, ranging from loss of taste and smell, to debilitating fatigue, neurocognitive symptoms, and respiratory problems[138]. Follow-up studies have found symptom persistence up to two years following acute infection[139,140].

Ongoing studies try to elucidate the pathophysiological mechanisms behind long COVID development. Persistent presence of virus, prolonged elevated cytokine levels, potent antibody responses[71], aberrant T-cellular responses[141] and persistent

endoteliopathy[142] has been suggested to be involved in the pathogenesis of long COVID. The cause is likely to be multifactorial.

Immune responses to influenza and SARS-CoV-2

Much of what we know about the immune responses to SARS-CoV-2 is based on previous research on immune responses after influenza infection and vaccination. The innate immune responses to viral infection are not discriminatory; however, the two viruses use different mechanisms to evade pre-existing immunity which this thesis will address after a more general introduction to the host viral defence.

Innate immune responses

The innate immune system is the first line of defense, reacting rapidly, but non-specific to invading pathogens. In the airways, the innate immune system consists of physical barriers and immune cells adapted for efficient elimination of harmful pathogens, such as alveolar macrophages. Upon infection, chemotactic signals help recruit additional leukocytes from the circulation. Although eradication of respiratory pathogens is crucial for host survival, it is equally important to avoid triggering excessive inflammatory responses in the lungs, as this can be detrimental to the life-dependent alveolar gas-exchange function.

The airway exposure to viral pathogens is significant. However, ciliated epithelial cells counteract the propagation of virus to the lower airways by secreting mucus. The mucus acts as a physiological barrier that inhibits viral passage to the underlying cells. The mucus contains mucins that are heavily glycosylated and rich in terminal SAs, which can function as decoy receptors and immobilizes the influenza virus. The ciliated cells steadily transport the mucus up to the pharynx where it is swallowed. When the infecting virus penetrates these barriers and enters host cells in the respiratory epithelium, the location in the upper or lower airways, with differences in disease resistance and tolerance, will influence infection development[143]. Intracellular innate immune pathogen recognition receptors (PRRs) such as Toll-like receptor 3 (TLR3), retinoic acid-inducible gene I (RIG-I), and TLR7, recognize intracellular viral

components (i.e., ssRNA/dsRNA). This recognition initiates intracellular signaling cascades and activation of several nuclear transcription factors, culminating in the expression of antiviral molecules, pro-inflammatory cytokines, and type I and III interferons (IFN). The type I IFNs function as potent antiviral signals to adjacent infected and uninfected cells, restricting viral replication and promoting viral clearance in the early phase of infection. Type III IFNs can modify host proinflammatory responses, which might be contributing to the susceptibility of secondary bacterial infections in influenza infection[143,144]. Both influenza and SARS-CoV-2 use mechanisms that counteract host cell IFN response[85,145]. The extent of this inhibition may be of particular concern in the development of delayed, excessive lung inflammation and tissue damage seen in severe SARS-CoV-2 infection, which is associated with a delayed, but excessive cytokine release[146]. In SARS-CoV-2, inflammatory cytokines, such as interleukin (IL)-6, correlate with disease severity[147,148], and therapeutic effect of IL-6 antagonist (Tocilizumab (RoActemra®) has been evaluated in clinical studies[149]. Other immunomodulatory agents assessed in clinical trials includes early IFN therapy in severe COVID-19[150,151]. So far, convincing results on hard endpoints are scarce[152]. Innate effector cells including neutrophils, natural killer (NK) cells, and the professional antigen presenting cells (APCs) Dendritic cells (DCs) and macrophages are attracted to the cite of infection by chemokines. They eliminate virus infected cells and activate the adaptive immune system by presenting antigens that can be recognized by T-cells. DCs recognize and engulf viral particles and migrates to the lymph nodes where these antigens are presented to lymphocytes, ensuring activation of the adaptive immune system.

Dysregulated innate immune responses are associated with severe disease in both influenza and COVID-19, and is involved in the development of ARDS, which is characterized histologically by diffuse alveolar damage, pulmonary edema and hyaline membrane formation[153-155]. Different inflammatory mediators can play unique roles in the pathophysiology of ARDS in influenza and SARS-CoV-2, and knowledge of these mediators may help identify potential drug targets.

Adaptive immune responses.

Adaptive immunity constitutes of humoral and cellular immune responses, mediated by B- and T-lymphocytes, respectively. Adaptive immune responses are induced later (days) than innate responses but are pathogen specific. Memory responses ensures rapid reactivation upon secondary encounters with the same pathogen. The role of the humoral immune system is to produce antibodies that eradicate extracellular pathogens and protect from infection upon pathogen re-exposure. Cellular immune responses target intracellular pathogens, and support and navigate humoral immune responses to optimize the targeting of the encountered pathogen. *B lymphocytes, or B cells,* are the cellular mediators of humoral immunity, and proprietary B-cell clonotype antigen receptors (BCR) can recognize a specific antigen, ranging from linear and conformational structures, lipids, fats, and proteins. Encounter with their cognate antigen inside the lymph nodes activates naïve B-cells. Activated B-cells can proliferate into short-lived plasmablasts, responsible for early IgM (and IgD) antibody responses or continue maturation in the lymph node with co-stimulation of $CD4^+$ helper T-cells. B-cells then increase their antibody affinity through somatic mutation, and affinity maturation, and undergo class switching, the process of changing antibody isotype from IgM to IgA, IgG, or IgE[156], with different properties and antibody effector function. Whilst the secretion of IgM reaches its peak early after infection, isotype switched antibody responses peak later, and can be found in the circulation for months to years after infection (**Figure 6**). The longevity of the different antibodies and memory responses are dependent upon the infection by which they were elicited and is not fully investigated in SARS-CoV-2. In influenza patients, seropositivity for neutralizing antibodies to the 1918 virus have been found in survivors over 90 years after the original infection, demonstrating the possible longevity of adaptive immune responses[157]. The fully matured B-cells differentiate into long-lived antibody secreting plasma cells, which home to the bone marrow and continue to produce isotype switched antibodies. Mature B-cells also differentiate into memory B-cells, which can circulate in blood and lymphoid tissue, or take residents in tissues, like in the lungs after influenza infection[158].

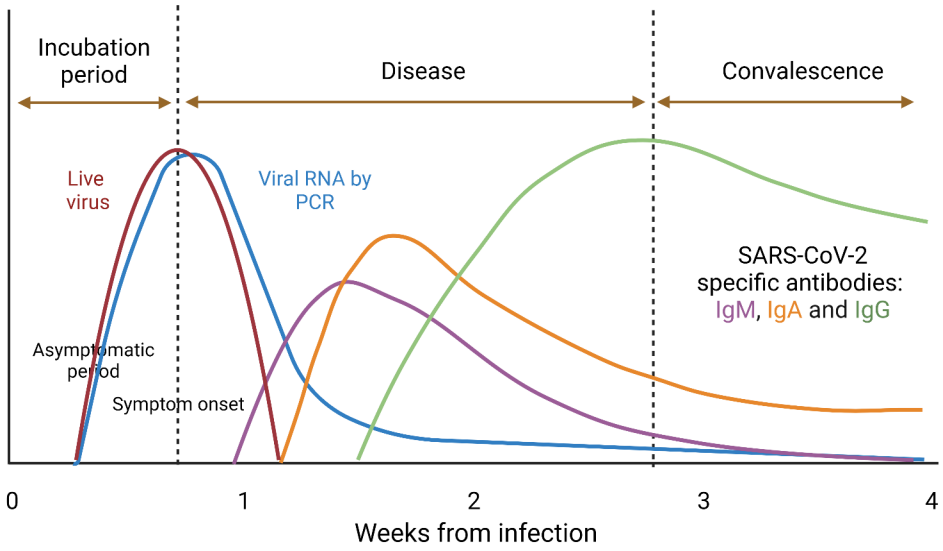

Figure 6. Schematic overview of the SARS-CoV viral and antibody kinetics.
Viral shedding can occur before symptom onset. The earliest antibody responses consist of IgM. Later, IgA, and IgG provide durable antibody responses. IgG targeting RBD, as well as neutralizing antibodies (Nabs) have been detected up to 16 months post SARS-CoV-2 infection [159]. *Created with BioRender.com. Inspired by Azkur et al*[160].

Antibody responses to influenza and SARS-CoV-2 infection appear to follow the same antiviral pattern; they are initially dominated by shorter-lived IgM responses, followed by IgA and IgG. IgA is predominant in upper airways, whereas IgG levels are higher in serum and lower respiratory tract. The antibody response after natural influenza infection is broadly studied. Most influenza antibodies target the surface antigens HA and NA. Antibodies can be strain-specific when targeting the HA head domain, or more cross reactive if they target more conserved stalk domain. Antigen epitopes on the HA head are immunodominant to the stalk region. Antibodies with neutralizing or hemagglutination inhibition effect correlates with protection from disease[161,162]. The dominating strain-specific properties of influenza antibody responses pose a challenge to lasting immunity from infection and vaccination. However, researchers have also found broadly neutralizing antibodies targeting conserved regions of the surface proteins, providing a strategy for universal influenza vaccine design[163]. The first childhood exposure to influenza by infection influence the subsequent antibody responses to later infections or vaccinations, a phenomenon described as imprinting or

original antigenic sin[164-166]. This particularly refers to birth cohorts and has been used to explain why some age groups are better protected against novel subtypes of influenza A. Irrespective of birth year, repeated seasonal influenza vaccinations may also alter antibody responses later in life, blunting vaccine effectiveness (VE) by negative antigenic interaction. We might observe a similar phenomenon for SARS-CoV-2, as it continuous to circulate in the population with different patterns of both infection and vaccination.

The antibody response to SARS-CoV-2 targets several structural proteins (e.g., M, N, S). The spike protein is a key target for inducing antibodies, and neutralizing antibodies mainly target the RBD and N-terminal domain (NTD)[167]. The ongoing genetic evolution of SARS-CoV-2 is causing rapid mutational changes in the Spike protein, including the RBD, resulting in increased virulence and significantly limiting pre-existing immunity elicited by previous infection or vaccination[168,169]. Knowledge gained from studies on antigenic drift and vaccine responses to influenza virus may inform future vaccine strategies to overcome the important level of immune evasion that has been observed for new SARS-CoV-2 variants.

T–lymphocytes, or T-cells, are the mediators of cellular immunity. T-cells are grouped based on their expression of Cluster of Differentiation 8 (CD8) and CD4. $CD8^+$ and $CD4^+$ T-cell have inherently different T-cell receptors (TCRs), which can bind peptide antigens that are presented on cell surface molecules called Major Histocompatibility Complex (MHC) I and II, respectively (**figure 7**). MHC class I is found on all nucleated cells and interact with $CD8^+$ T-cells, whereas MHC class II is mainly found on professional APCs and B-cells and interact with $CD4^+$ T-cells. When MHC molecules present intracellular pathogen-derived peptide antigens on the cell surface, this can activate naïve $CD8^+$ and $CD4^+$ T-cells who recognize the foreign antigen. Naïve $CD8^+$ and $CD4^+$ T can differentiate into various effector cells, and memory T-cells. Inter-individual differences in MHC molecules determines the range of antigens that they can present to the T-cells. Activation of naïve T-cells takes place in lymph nodes. $CD8^+$ T-cells are also called cytotoxic T-cells (CTLs), because of their lytic properties, and

are important for viral clearance and play a role in protection in both influenza and COVID-19[170,171]. In response to antigen stimulation, $CD8^+$ T-cells migrate to the site of infection where they efficiently kill infected cells by the release of granzymes and perforins and activate macrophages by secreting IFNγ.

Depending on the specific cytokine milieu, activated $CD4^+$ T-cells differentiate into several T-helper cell lineages including T helper (Th) 1, Th2, Th17, T follicular helper (fh), and T regulatory (reg) cells, all with distinct functions and cytokine profiles. $CD4^+$ Tfh cells are essential for efficient stimulation of B-cell maturation and antibody production. Type 1 interferons promote the differentiation of naïve $CD4^+$ T-cells to the Th1 subtype. $CD4^+$ T-cells migrate to the site of infection where they promote macrophage and $CD8^+$ T-cell activation by the release of cytokines such as IFNγ, IL-2 and tumor necrosis factor-α (TNFα).

Like B-cells, T-cells can differentiate into subset of memory cells that can reside in lymph nodes and tissues for a long time after viral clearance. As T cells recognize peptide antigens that can be conserved between viral strains, they have the potential of inducing important cross-protective immunity.

A common characteristic of SARS-CoV-2 infection is the development of acute phase lymphocytopenia, correlating with disease severity. This phenomenon can occur in other viral infections, as seen during the A/H1N1pdm09 swine flu pandemic[172,173]. Different mechanisms can be involved in lymphocytopenia, whether it is a sign of T-cell exhaustion, or a compartmental shift where cells migrate to the infected tissues[174].

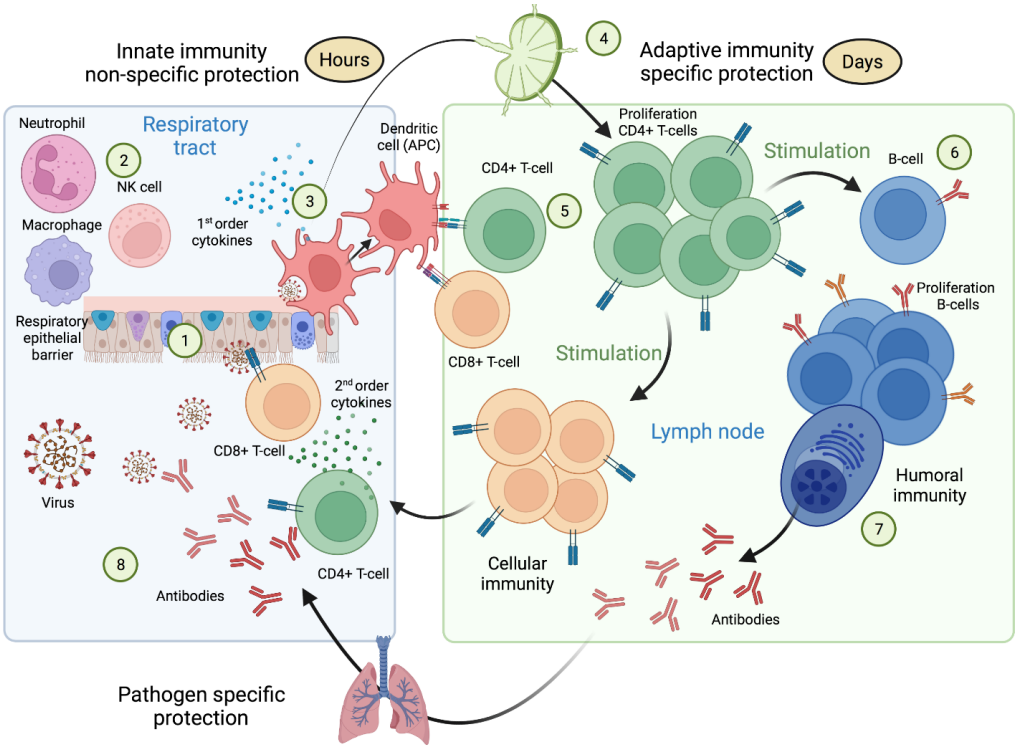

Figure 7. Overview of innate and adaptive immune responses in viral respiratory infection.
1. The innate immune system is the first line of defense against microbes and mediates early reactions to pathogen exposure, the first barrier being the mucous surface of the respiratory epithelial layer. Infected cells release interferons with antiviral activity (type I and III) and other inflammatory cytokines, attracting other immune cells and initiating local antiviral responses. 2. Innate immune cells have pathogen recognition receptors (PRRs) that can recognize foreign pathogens. Neutrophils are phagocytic cells that respond rapidly to inflammatory signals and migrate to the site of infection. Macrophages both phagocytose and present antigens to $CD4^+$ and $CD8^+$ T-cells. NK-cells kill virus infected cells by cytolytic granules and cytokine release. 3. Dendritic cells (DCs) phagocytose viral pathogens. 4. DCs then migrates to the T-cell zones in lymph nodes where they interact with adjacent lymphocytes. 5. By recognition of its cognate antigen, and CD28:B7 co-stimulation, naïve T-cells activates and proliferates. 6. B-cells that are activated upon antigen-recognition, is co-stimulated by activated $CD4^+$ T-cells to mature and proliferate. 7. Proliferated mature B-cells can turn into antibody secreting plasma cells that will continue to produce antibodies after the initial infection is cleared. 8. Activated $CD4^+$ and $CD8^+$ T-cells can migrate to the site of infection to mediate helper and cytotoxic T-cell support. Both cell types can differentiate into a resting memory phenotype that persists after infection is cleared. Antibodies can be actively secreted into the respiratory tract, as well as circulate in blood, binding extracellular virus particles and use different effector functions to clear infection. *Adaptation of figure by K G-I Mohn and G Johansen, inspired by Ozbiosciences and A.Abbas[175]). Created with BioRender.com*

Prevention

With the encounter of highly contagious viral pathogens like Influenza and SARS-CoV-2, the first step in combatting infection is to prevent the viral spread. The extent

of initial mitigation measures depends upon multiple factors, including politics and economy, and knowledge about the virulence and reproduction number (R_0) of the pathogen involved, as well as possible pre-existing cross-immunity.

When novel pandemic virus SARS-CoV-2 started spreading in country after country, the basic R_0 was high, indicating that unless countries were sealed off, contact tracing and infection control measures could only slow down viral transmission. Non-pharmaceutical infection control measures lead to R_0 decrease, and temporal dispersion of patients in need of hospital care relieved health care services. However, due to transmission in asymptomatic individuals, and short incubation time, contact tracing was a challenging task. Several measures have been important contributors to reduction of viral spread, including social distancing, mask mandates, home schooling, home-office and hand disinfection. The unprecedented availability of rapid test for screening and diagnostics has played a significant role in controlling transmission, both in hospital and in society.

Vaccination is a key measure in the prevention of highly contagious infectious diseases like influenza and SARS-CoV-2 in the epidemic and pandemic setting. An additional potential benefit is the preservation of antibiotic treatment[176,177]. In Norway, annual influenza vaccination is recommended for selected occupational groups including health care workers (HCWs) and for people with predefined risk factors such as older age, pregnancy, obesity and chronic illnesses.

Facilitation of rapid large-scale production of pandemic vaccines has become a key priority in pandemic preparedness. During the A/H1N1pdm09 swine flu pandemic, vaccine manufacturing was rapidly initiated, but time-consuming due to dependence on virus propagation in embryonated chicken eggs, resulting in a 6 month gap from the onset of the pandemic, to available vaccines. In 2009, inactivated pandemic virus vaccines were adjuvanted to elicit protective responses, and the AS03-adjuvanted Pandemrix vaccine, primarily used in Europe, became associated with an increase of childhood narcolepsy[178]. A similar vaccine formulation, Arepanrix, only demonstrated small risk increase[179]. The mechanisms behind the association between Pandemrix and

narcolepsy, and the potential role of wild-type virus infection in a proposed two-hit model, remains disputed. Nonetheless, the findings significantly contributed to vaccine hesitancy. In Norway, there is a high population confidence in governmental advice, and despite voluntary COVID-19 vaccination, the vaccination coverage is high (80%). In other countries, vaccine hesitancy has seriously hampered the pandemic response.

The speed of the SARS-CoV-2 vaccine development was unprecedented. The first vaccination outside clinical trials was on December 8th 2020, and a recent modelling study claims that vaccination averted over 19 million COVID-19 deaths within the first year of use[180]. However, global vaccine distribution inequities and inadequate manufacturing capacity are remaining challenges that hampers the life-saving potential of COVID-19 vaccines[181]. Hundreds of COVID-19 vaccines are currently in pre-clinical and clinical trials, and currently only four are available in Norway, including two mRNA vaccines, one subunit vaccine and one replication-incompetent virus vector vaccine (**Figure 8**). So far, mRNA vaccines targeting the Spike protein have demonstrated robust humoral and cellular immune responses and are in widespread use. Unfortunately, some of the vaccines came with unanticipated side effects, like the ChAdOx1 nCoV-19 adenovirus vector vaccine, which have been associated with a potentially fatal vaccine induced immune thrombotic thrombocytopenia (VITT)[182]. New VOCs challenge vaccine efficacy, and repeated vaccination appears to be a requirement for infection prevention, at least in risk groups. Strategies to update vaccine composition to better target new and future variants are under consideration.

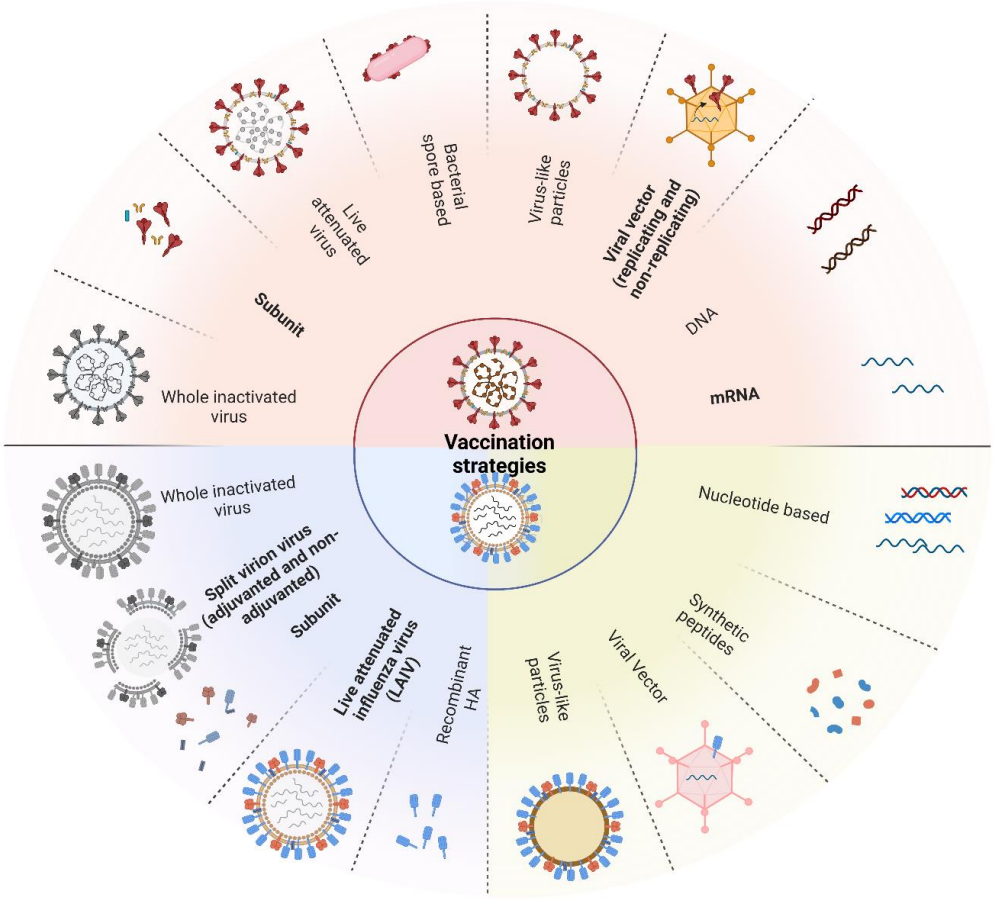

Figure 8. Influenza and SARS-CoV-2 vaccine strategies.
Upper half circle*:** SARS-CoV 2 vaccines, both licensed and in development. Vaccine types that are currently approved for use in Norway are marked in bold. At the moment only mRNA vaccines from Pfizer/BioNTech and Moderna are part of the national corona vaccination program. In Norway, vaccines were initially prioritized for old adults, patients with severe comorbidities and selected occupational groups, like HCWs, and has later become available for everyone from the age of 5 years. Around 80% of Norwegians have received at least one dose of COVID-19 vaccine (NIPH, ourworldindata.org). ***Lower half circle, blue background: Licensed seasonal influenza vaccines includes inactivated influenza virus vaccines (IIV), live attenuated influenza vaccines (LAIV) and recombinant HA vaccines (blue background). Vaccines types available in Norway are marked in bold. LAIV is administered intranasally and suitable for vaccination of children. IIV is recommended for immunocompromised individuals and risk groups, and are most commonly used. IIVs are either trivalent or quadrivalent, adjuvanted, or non-adjuvanted. The vaccines elicit systemic humoral immune responses targeting surface proteins HA and NA, that are mostly strain-specific. LAIV elicit systemic and mucosal immune responses. In ***yellow background***: next generation influenza vaccine platforms aim to generate universal influenza vaccines that are cross-reactive to different influenza strains. In addition to the illustrated strategies, these include T-cell vaccines, NA targeted vaccines, chimeric HA-based vaccines, and computationally optimized broadly reactive antigen (COBRA) vaccine approaches. In the coming years, a vaccine-combo, including both influenza and SARS-CoV-2 may become reality. *Inspired by template from Prof. Akiko Iwasaki and Prof. Ruslan Medzhitov. Created with BioRender.com*

Studying the responses to natural infection is necessary to understand vaccine responses. Although both innate and cellular immune responses contribute to the protection from influenza disease, antibodies play a key role in the host immune response and resistance to re-infection. The breadth of the antibody response is greater after natural influenza infection compared to after vaccination. Natural infection induces Abs that targets mainly HA, and to a lesser degree NA and internal proteins. Seasonal influenza split virus vaccines mainly elicit systemic antibodies targeting HA, but also NA. Live attenuated influenza vaccines (LAIV) can elicit both systemic HA responses and mucosal IgA antibody responses, particularly in children[183]. Whereas current influenza vaccines are inducing narrow range immune responses, the next generation influenza vaccines aim to induce universal and long-lasting protectiveness. By studying infection rate in vaccinated versus non-vaccinated individuals in clinical trials and in the general population, we can calculate vaccine efficacy and effectiveness.

There are several documented immune correlates of protection for influenza. Most influenza vaccines mainly target the surface glycoproteins HA and NA. Antibodies that bind to the HA head and inhibit agglutination of red blood cells in vitro can be readily measured in the Hemagglutination-inhibition (HI) assay. A HI titer > 40 correlate with 50% protection from influenza disease[161]. Other correlates of protection with less established cut-offs include neutralizing antibodies targeting HA and NA, HA-stalk antibodies measured by ELISA, influenza specific $CD8^+$ T-cells [184-187] and $CD4^+$ T-cells[188].

Although no universal correlate of protection from SARS-CoV-2 has been established, several immune responses are associated with a level of protection. Neutralizing antibodies after vaccination appear to be a potential correlate of protection, in addition to spike or RBD-binding Abs[189,190]. In clinical trials, vaccine efficacy in developed COVID-19 vaccines ranged between 50-95% against symptomatic infection with the Wuhan type virus, and repeated vaccinations have elicited durable antibody responses[191]. Immune escape is a challenge with the new VOCs. Although Delta and Omicron variants have demonstrated significantly reduced vaccine effectiveness,

vaccination still provides important protection from hospitalization and severe acute disease.

Diagnostics

Reliable tools for pathogen identification are cornerstones in both screening and symptom targeted diagnostics for infectious diseases such as influenza and COVID-19. As with vaccines, diagnostic tools are not readily available in the beginning of a pandemic involving a new pathogen, but rapid development and distribution of accurate and rapid diagnostics are a key measure to contain viral spread. Previous studies have demonstrated that the accuracy of clinical influenza diagnosis is poor[17,192], and it is safe to assume that we could expect the situation to be similar for COVID-19. Lessons from epidemic and pandemic influenza diagnostics have been useful to inform the application of SARS-CoV-2 diagnostics.

As influenza and SARS-CoV-2 replicate in the respiratory tract, most rapid diagnostic tools rely on adequate and easily available sampling sites such as the nasal cavity, nasopharynx, oropharynx, or saliva.

Serological detection of antibodies is an epidemiologically important diagnostic tool, which can detect immune responses to ongoing or past infection. As antibodies take time to elicit, they are not present early in the case of a primary infection and do not fulfil the requirements of a reliable diagnostic tool in the acute setting.

Influenza antigen immunoassays, rapid influenza diagnostic tests (RIDTs), have been available since the 1990s, and has been associated with improve targeted antiviral and antibiotic use[193,194]. During the 2009 Swine flu pandemic, RIDTs were used to distinguish pandemic influenza A/H1N1pdm09 cases from patients with other circulating respiratory viral diseases. However, limitations of RIDTs include their low sensitivity. A 2012 meta-analysis of 159 studies evaluating 26 RIDTs found a pooled sensitivity of 62% (58%-67%) and a pooled specificity of 98% (97%-98%), concluding that "*influenza can be ruled in, but not ruled out through the use of RIDTs*"[195]. The

widespread use of antigen tests seen during the current pandemic, is unprecedented, and requires improved standards for test sensitivity.

SARS-CoV-2 antigen tests commonly detect the abundant nucleoprotein or the less conserved spike protein (**Figure 4**) and have become a useful supplement to molecular diagnostics of COVID-19 in professional health care settings. In smaller Norwegian hospitals, with limited laboratory capacity, antigen tests have played a key role to rapidly assess patients for COVID-19. More importantly, antigen rapid diagnostic tests (Ag-RDTs) have also gained new ground as an over-the-counter tool for home diagnosis and community screening later during the pandemic, and as availability increased. In Norway, the roll-back of social distancing and mandatory quarantine rules correlated with the mass distribution of Ag-RDTs to the public for screening and symptomatic self-testing purposes. Antigen tests are cheap and easy to distribute compared to commercial molecular tests, and they also provide a useful alternative in the low-resource setting were nucleic acid amplification tests (NAATs) are not available. Ag-RDTs are more likely to detect infection when the viral load is high, and a positive test indicates that viral replication is ongoing, as proteins from non-infectious viruses degrade rapidly in the body. Upon a positive home test, infection control can be achieved by self-isolation for a defined time-period, or until testing negative after an initial positive test. There are hundreds of COVID-19 ag-RDTs, and their performances varies. In general, the sensitivity is lower than in NAATs, and both viral load and sampling technique can influence the test result. This has led to advices on serial testing - using multiple tests for confirmation of results. In the case of SARS-CoV-2, the ag-RDTs must be versatile enough to detect mutated variants of the virus, and the WHO minimum standard requires >80% test sensitivity and >98% test specificity among symptomatic individuals[196]. However, test performance needs to be reevaluated as new variants emerge. As widespread Ag-RDT use is a new phenomenon in the pandemic setting, there are still important knowledge gaps regarding optimal application of this diagnostic resource. Testing strategies should be adapted to the current disease burden, in addition to national or local resources and priorities.

NAATs such as the reverse transcription polymerase chain reaction (RT-PCR) are the gold standard diagnostics of influenza and SARS-CoV-2. At the beginning of the SARS-CoV-2 pandemic, diagnostic challenges included lack of available validated tests, and low testing capacity, resulting in strict testing criteria. As an example, our hospital in Bergen, Norway used a central hospital laboratory in-house developed SARS-CoV-2 RT-PCR, to compensate for the lack of rapid and validated diagnostic tools, as did other laboratories globally. Health care workers screened symptomatic patients for COVID-19 upon hospital admission, and cohort isolated them until test results were ready, with the risk of infection transmission to test-negative patients due to analysis delay. Rapid upscaling of test capacity and pooled testing was implemented to meet the challenges of an overwhelming diagnostic demand[197]. As previously mentioned, smaller hospitals that did not have the same 24-hour availability of RT-PCR testing, greatly benefited from the later development of rapid antigen-based tests for SARS-CoV-2.

RT-PCR is an extremely sensitive analysis, which can detect the presence of a virus, from scarce sample material. Developed for influenza detection in 1991[198], it is a sophisticated and time-demanding method, requiring trained laboratory personnel. Briefly, RT-PCR generates billions of DNA copies of the original viral RNA, using nucleic acids as building blocks and DNA polymerases as constructors. To detect viral RNA in a sample, the test applies the enzyme reverse transcriptase to first synthesize a complementary DNA (cDNA) strand from RNA. Raising the temperature separates the two strands (denaturation). By reducing the temperature again, chosen primers attach to specific parts of the single stranded DNA (annealing). Then, a DNA polymerase amplifies the primer-associated DNA (extension). Repetition of this cycle, now using both the old and new DNA strand as templates, results in exponential amplification of target DNA, presuming available reaction components. Real-time RT-PCRs register the accumulated copies of DNA over time, using a predefined threshold for detection of a positive test. A limitation of RT-PCR is that by detecting RNA presence, there is no distinction between infective and non-infective virus, for which viral culture is the most reliable diagnostic tool, and to which antigen tests are better targeted.

Real time RT-PCR, and other molecular tests using isothermal amplification technology, are suitable for use at the point-of-care (POC). These assays have gained an important role in rapid influenza diagnostics during seasonal epidemics and are in increased use for SARS-CoV-2 detection. Although cost-efficient at the patient level, they necessitate use of electricity and expensive equipment, unfortunately making them less useful in limited resource settings[199]. Molecular platforms are designed for laboratory or POC settings and differ in capacity and complexity depending on area of use. Molecular tests may have turnaround times within 5 minutes to a couple of hours. Their sensitivity profiles are favorable compared to antigen tests, and in the health care setting they can be easily incorporated into hospital logistics and medical records.

According to FIND, the global alliance for diagnostics (www.finddx.org), over 600 RNA-based tests are now available for the detection of SARS-CoV-2. By autumn 2020, several new molecular platforms had established POC diagnostics for SARS-CoV-2 detection[199]. To describe the different technologies that are available is beyond the scope of this thesis. Multiplex respiratory platforms also include SARS-CoV-2 in their new panels. In line with pre-pandemic studies on rapid molecular POCTs for influenza and other respiratory viruses, the implementation of these tests has been associated with shorter test turnaround times and improved patient flow in the hospital setting[200,201]. However, test sensitivity is still suboptimal in some platforms, and more studies are needed to establish their overall clinical benefits[202].

The future holds promise for the advances in rapid diagnostics. However, there are important global inequities in test availability that are important to address. Diagnostic tools are crucial to sustainable health care services in all countries, and these viral diseases know no borders.

Influenza treatment

Antiviral therapy

Several antivirals have been developed for use in influenza, targeting essential proteins in the viral replication cycle (**Figure 3**). Most relevant are the neuraminidase inhibitors (NAIs) which were first licensed in 1999. The two broadly available NAIs are oseltamivir (Tamiflu) and zanamivir (Relenza), whereas NAIs peramivir and laninamivir have limited approvals[203]. Antivirals targeting the M2 protein (amantadine, rimantadine) are no longer preferred due to side-effects and broad resistance development. Other antivirals target the RNA polymerase complex (ribavirin, favipiravir, baloxavir). During the A/H1N1pdm09 pandemic, hospitalized patients received stockpiled NAIs, and most evidence of the clinical benefit of NAIs is derived from observational studies from this period. A large 2015 meta-analysis including 4328 influenza patients from nine trials showed that NAI reduced time with influenza symptoms by one day and reduced the risk of hospitalization and lower respiratory tract infections[204,205]. It also has side effects including nausea and vomiting. Another meta-analysis of 29234 hospitalized patients from 78 studies concluded that NAIs were associated with reduced mortality when given within 48 hours of symptoms, and when given at any time in pregnant women[206]. These studies contrast a Cochrane review in only found a significant time to reduction of symptoms of 16.8 hours in patients receiving NAI treatment outside hospital[207]. The use of NAIs and alternative antivirals varies from country to country. Current guidelines for the use of NAIs in Norway recommend NAIs within 48 hours for patients outside hospital who are at risk of severe complications and in hospitalized, and/or severely ill patients at any time, as well as exposure prophylaxis in risk groups. If influenza is suspected, and there is a treatment indication, treatment should not await diagnostic confirmation. In Norway, oseltamivir is not broadly used in the outpatient setting.

Globally, surveillance is implemented to track the antiviral resistance development[208]. Although currently low, NAI resistance is a matter of concern[209]. New antivirals like Baloxavir currently provide a treatment option in NAI resistant strains[210] .

Other treatment

Most symptomatic influenza patients experience mild disease, and manage well at home. Symptomatic care includes antipyretic and mildly analgesics such as paracetamol (acetaminophen) and oral rehydration. Patients with severe disease may require sophisticated supportive care, as symptoms may include respiratory failure, dehydration, septic shock, and seizures. Various therapies target organ failure and complications, including hydration therapy, non-invasive or invasive oxygenation and pressors. Bacterial co-infections necessitate antibiotic therapy and are estimated to affect 1/3 of patients with severe influenza and several guidelines (Norwegian, CDC, WHO) recommend the use of empirical antibiotic therapy based upon knowledge of local resistance patterns and common pathogens. Corticosteroids are usually not recommended due to increased risk of superinfections, but can be considered in the ICU-setting ARDS[211]. Intravenous immunoglobulins (VIG) are not standard care for influenza patients. Immunomodulatory treatment is under investigation for use in severe influenza.

Co-infection treatment

Viral-bacterial interactions increase the risk of bacterial complications in influenza[212], and co-infections must be treated appropriately. At the same time, increased antimicrobial resistance (AMR) is a silent pandemic of global concern[213], which is strongly correlated with antibiotic use[214,215]. There is currently a mismatch between the prevalence of influenza co-infections reported in studies, and the frequency of antibiotic prescribing in influenza patients[216]. In Norway, public guidelines recommend empirical antibiotic treatment for suspected bacterial pneumonia in hospitalized patients. This includes the use of intravenous (IV), primarily narrow spectrum penicillin for mild to moderate disease and broader spectrum antibiotic in more severe disease and in ICU patients. Due to the low prevalence of antimicrobial resistance in Norway, these therapy guidelines are restrictive compared to international literature. According to antimicrobial stewardship recommendations, reassessment of initiated treatment should occur within 48-72 hours[217].

There are some controversies regarding the use of antibiotics in influenza patients. Some studies suggest that macrolides are associated with improved outcome in severe influenza[218,219], and one study found association between antibiotic use and reduced risk of hospitalization or complications[220]. Balancing these claimed benefits against the risk of drug side-effects, increased cost, and importantly, accelerated antibiotic resistance development that is associated with widespread antibiotic use, is critical. In the pandemic setting, this strategy could however be relevant in selected patient groups.

SARS-CoV-2 treatment

In the ongoing SARS-CoV-2 pandemic, we have experienced that the idea of prophylactic antibiotic use has been revisited in the initial stages of the pandemic.

Targeted and supportive treatment for SARS-CoV-2 is beyond the scope of this thesis and will be discussed briefly. Rapid research advances lead to frequent changes in COVID-19 treatment recommendations. Severe respiratory failure in COVID-19 requires advanced supportive care in the ICU[221]. COVID-19 associated coagulopathy is treated with anticoagulants in hospitalized patients. According to the WHO, antibiotic therapy is not indicated in mild to moderate COVID-19. In severe COVID-19, the WHO recommends antibiotics based on clinical judgement, and daily assessment for de-escalation is advised. Several new targeted drugs have received emergency approval prior to solid documentation of their clinical benefits. Thanks to pre-pandemic framework preparation, a global body of clinical trials have been performed in the search of efficient therapeutics for COVID-19, including efforts to repurpose well-known drugs with potential immunomodulatory or antiviral properties[222-225]. This has led to the establishment of dexamethasone as treatment for severe COVID-19, as studies have indicated reduced mortality and improved clinical outcomes in COVID-19 patients with respiratory failure[226,227]. Other drugs that initially were flagged as promising, including malaria drug hydroxychloroquine and broad-spectrum antibiotic azithromycin, have been rejected due to lack of clinical efficiency in COVID-19 through WHO led global clinical trials[228,229]. New therapeutics include antivirals that target essential elements of the SARS-CoV-2 replication cycle (**Figure**

5) and monoclonal antibodies with neutralizing abilities. In addition, new and old drugs that target the host immune response to SARS-CoV-2 infection are currently in clinical trials. Drugs that are now recommended for selected patient groups include IL-6 receptor blocker Tocilizumab, inhibitor of IL-6 signaling pathway Baricitinib, RNA polymerase inhibitor Remdesivir and protease inhibitor Paxlovid™. The latter is recommended for use in non-hospitalized COVID-19 patients at risk of developing severe complications, and should be administered within 72 hours of symptom onset[230].

Recommendations for SARS-CoV-2 prophylaxis and treatment should be based on evidence-based medicine (EBM). Preprint of study-, industry-financed research, different trial designs and the competitive environment in COVID-19 research challenge the interpretation of studies and selection of patient groups that will benefit from treatment.

The last literature search was performed in July 2022

Aim and objectives

The aim of the thesis was to investigate clinical management, outcomes, and immunological characteristics of well-known and novel highly contagious respiratory viral infections, and to identify potential for improvement of clinical practice.

Primary objective

To investigate diagnostic, clinical, and immune correlates of disease in patients with influenza and SARS-CoV-2 infections and their relationship to clinical outcomes.

Secondary objectives

- Compare hospital admission length and clinical management of acutely admitted patients with suspected influenza in 2018/2019 following two different rapid influenza point-of-care or laboratory-based tests (**Paper I**).

- Compare antibiotic treatment and associated risk factors in patients hospitalized with influenza in 2018/2019 and COVID-19 in 2020 (**Paper II**).

- Study long-term symptoms and their associations with the adaptive immune responses 12 months post-infection in non-hospitalized COVID-19 patients infected during spring 2020 (**Paper III**).

Methods

Overview of study populations and study design (papers I, II, III)

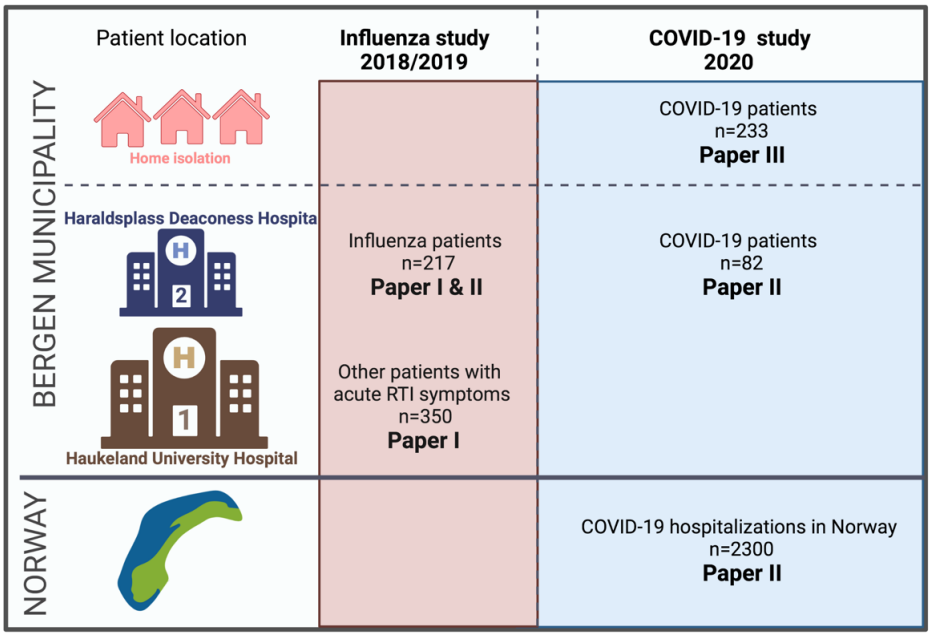

Figure 9. Overview of the studies conducted during this thesis and their connection to paper I, II and III. Patients were recruited at Bergen Municipality Emergency Clinic, Haraldsplass Deaconess Hospital, Haukeland University Hospital and the Norwegian Intensive care and Pandemic Register (NIPaR). Bergen Municipality Emergency Clinic is a 24-hour open public outpatient emergency clinic in Bergen, Norway. The municipality of Bergen has a population of around 270 000 people. Haukeland University Hospital is a university referral hospital situated in Bergen, Norway. The hospital has 12 000 employees and 40 0000 visits to emergency department (ED) per year. Haraldsplass Deaconess Hospital is a private hospital with a public operating agreement and University affiliation. The hospital has around 1000 employees, and 9000-10 000 ED visits per year. The two hospital serves as local hospitals with acute care functions for separate geographical regions of the city of Bergen. Hospital admission is either direct or through consultation with a family doctor or through Emergency Clinics. *Created with Biorender.com*

This thesis and the presented papers are based on a series of prospective observational studies from Bergen, Norway (**Figure 9 and 10**). **Paper I** was based on the first observational study, referred to as *the Influenza Study*. The study recruitment took place in two hospitals in Bergen, Norway, December 2018-March 2019. We refer to

Haukeland University Hospital as “hospital 1” or the “intervention hospital”. This was due to a change in routine influenza diagnostic pathway in hospital 1 when our study was conducted during the influenza season of 2018/2019. The other hospital, Haraldsplass Deaconess Hospital, is referred to as “control hospital” or “hospital 2”. In the Influenza Study we compared patient outcomes in the two different hospitals using either an ultrarapid molecular point-of-care-test (POCT), (hospital 1, n=400) or a rapid laboratory-based test for influenza (hospital 2, n=163). In addition, we compared patients with a confirmed influenza diagnosis (n=217) to non-influenza patients (n=350).

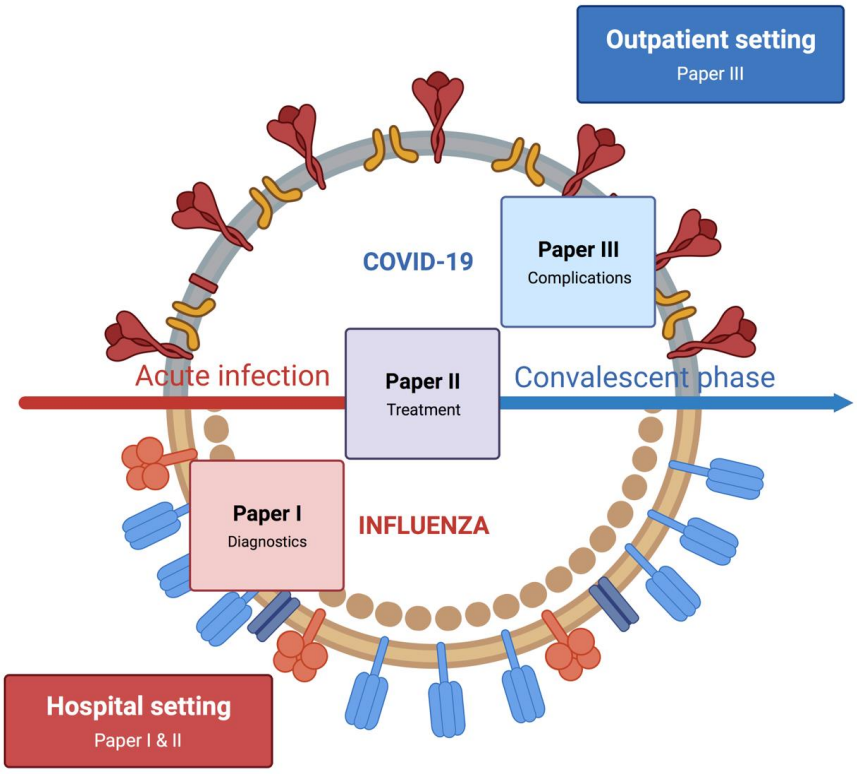

Figure 10. Overview of the thematic relationship between the studies conducted in patients with two different respiratory viral infections of pandemic potential. Studies conducted concerning rapid diagnostics in the acute phase, through treatment while in hospital to novel long-term complications in the convalescent phase. *Created with BioRender.com*.

Secondly, we conducted an observational study in 2020-2021, referred to as *the COVID-19 study.* This study included hospitalized patients infected with the novel SARS-CoV-2 during the first and second pandemic waves (March 2020 to September 2020) in the same two hospitals where patients were recruited for the Influenza Study the year before. The COVID-19 study also included home-isolated cases infected with SARS-CoV-2 from the Municipality of Bergen, and their family members. **In paper II**, we compared the hospitalized COVID-19 patients to influenza patients recruited in the Influenza Study the year before. To our knowledge, the Influenza Study is the largest single data collection including prospective information on clinical characteristics and hospital management of influenza patients in Norway. We believed this would provide valuable information to investigate clinical practices in patients hospitalized with viral respiratory tract infection before and after the pandemic outbreak. We also used data from the Norwegian Intensive care and Pandemic Register (NIPaR), which provided a national comparison group. Lastly, in **paper III,** we followed-up home-isolated COVID-19 patients from the COVID-19 study (n=233) for 12 months, and compared their prevalence of persistent symptoms to a control group, consisting of seronegative household members (n=26) and adults who were prioritized for vaccination in January-March 2020 due to age, occupation or other risk factors (n=163). Their baseline data were collected contemporarily with the 12-month follow-up of home-isolated COVID-19 patients (REC nr 218629). A subgroup of patients was additionally followed for 18 months (n=149).

The Influenza study (papers I and II).

The purpose of the Influenza study was to conduct clinical and immunological studies on hospitalized influenza patients, and to investigate the impact of a diagnostic molecular POCT for influenza on patient outcomes. The Influenza study design was a prospective controlled observational study of patients with influenza and other respiratory illnesses, as reported in **paper I**. Patients were enrolled upon admission in the Emergency Department (ED). Patients who had a rapid influenza molecular POCT performed in the ED were recruited from Hospital 1, and patients who had a rapid laboratory-based influenza test performed in the Emergency Care were recruited at

Hospital 2 during the influenza season of 2018/2019. The study conducted during the influenza epidemic started in December 2018 and lasted until the end of March 2019. To be eligible for inclusion, patients had to be 18 years or older, and able to provide written informed consent in person or by next-of-kin. At ED presentation, maximum symptom duration was set to 7 days. More specifically, 2 or more of the following symptoms had to be present: temperature >37.5ºC, malaise, exacerbation of asthma or chronic obstructive pulmonary disease, dyspnea, sore throat, cough, myalgia, arthralgia, vomiting or/and diarrhea. Dedicated study personnel ensured patient inclusion on Monday to Friday between 08:00-20:00. Due to limited time in the ED, a limited number of patients were included at the ward within the following days of admission. Upon inclusion, patients were briefly interviewed, and demographic and clinical data were prospectively registered. We collected additional data on antibiotic prescription, isolation, medical complications, culture results, radiological imaging and clinical outcomes from hospital data records.

The informed consent included a separate invitation to donate biological samples which were only applicable to influenza patients in Haukeland University Hospital. The donation included blood and nasopharyngeal sampling in the acute phase of influenza, and blood sampling once in the convalescent phase (4-6 months) with the purpose of still ongoing immunological studies.

The COVID-19 study

The COVID-19 study was initiated to conduct clinical and immunological investigations of mild, moderate, and severe SARS-CoV-2 infections. Hospital management and clinical characteristics of patients with moderate/severe SARS-CoV-2 infection were investigated in **paper II**. This included all hospitalized COVID-19 patients in the COVID-19 study. Inclusion criteria were the ability to provide informed consent and a confirmed SARS-CoV-2 positive RT-PCR. SARS-CoV-2 patients were included from both Hospital 1 and Hospital 2 during admission. Upon inclusion, data regarding demographics, presenting symptoms, laboratory-investigations, radiology, complications, and treatment were registered. In **paper II**, we only used data collected

from the acute phase of COVID-19, where comparable data were available from the Influenza Study.

In **paper III**, we investigated long-term clinical outcomes of patients with asymptomatic and non-severe/mild SARS-CoV-2 infection. The study was a prospective case-control study. Cases with a mild SARS-CoV-2 infection included patients that were not in need of hospital care during their acute disease course and excluded patients that were admitted to hospital. Thus, the home-isolated COVID-19 patients, and their later confirmed seropositive family members, belonged to this category. Patients were telephoned by study personnel upon a positive SARS-CoV-2 RT-PCR nasopharyngeal swab taken at the Bergen Municipality Emergency Clinic. They received information about the COVID-19 study and were asked to participate. Permission to contact their family members was also requested. After receiving oral and written information about the study, all participants provided written informed consent. For participants under the age of 16, parents provided written informed consent. Household members that consented to participation were invited to a follow-up 6-8 weeks after SARS-CoV-2 infection was confirmed in their household. At the follow-up, participants were interviewed by study personnel and provided clinical and demographical data that were recorded into electronic case report forms (CRFs). We collected blood samples at 6-8 weeks to detect initially unidentified SARS-CoV-2 infected household members by seroconversion and categorized them as cases in our study. Onwards, participants met for follow-ups at 4, 6 and 12 months for repeated convalescent blood samples, and reported clinical symptoms at 6 and 12months. A subgroup of patients (149 adults, four children) met for an additional follow-up at 18 months. We registered the following clinical symptoms: fever, headache, dizziness, palpitations, tingling, gastrointestinal upset, dyspnea, sleep-, concentration- and memory problems and fatigue. We used the validated Chalder Fatigue Scale (CFS) questionnaire to assess fatigue-associated symptoms. Consistently seronegative household members were categorized as controls, under the added conditions that they had never had a positive SARS-CoV-2 RT-PCR or antigen test and did not exhibit COVID-19 symptoms at the time when the index-person in their household was

infected. These precautions were taken to assure the true SARS-CoV-2 naïve status of controls.

Vaccine study

We recruited seronegative controls amongst adults eligible for COVID-19 vaccination during spring 2021, at the same time-period as the 12 months follow-up in the COVID-19 study. These individuals were included as part of a novel study to compare clinical and immunological responses to COVID-19 vaccination and infection. Vaccine-eligible individuals who were SARS-CoV-2 naïve at baseline, were used as controls for the infected cases. Controls provided demographical and clinical data, reported on respiratory symptoms, and responded to the CFS questionnaire contemporarily with the 12-month follow-up of infected cases.

The laboratory methods were developed and optimized in-house as in the start of the pandemic there were not validated assays available.

Biological sampling and immunological assays (paper III)

Blood samples

Blood samples were collected for immunological studies. Trained study personnel collected blood samples and transported them to the research laboratory the same day. For analysis reported in **paper III**, 1-2 serum tubes and 1 EDTA tube were collected from all participants at each time point. For separation of serum, whole blood was clotted at room temperature for min 30 minutes, or at 4°C for 2-4 hours before centrifugation at 2000 rpm at 4°C. Separated serum was given a unique identification number and aliquoted before storing at -80°C until use. Before use in serological assays, serum was thawed and heat-inactivated at 56°C for one hour. EDTA tubes were frozen directly at -20°C or -80°C until future use.

Enzyme-linked immunosorbent assay (ELISA) (paper III)

This immunoassay measures SARS-CoV-2 antigen (RBD and spike protein)–binding serum antibodies, based on published methods[191]. Serum antibodies bound to the target antigen are quantified by colorimetric analysis. A secondary horseradish peroxidase (HRP)–conjugated anti-human IgG antibody is added, which binds to the antigen-associated serum antibodies. The addition of 3,3',5,5'-tetramethylbenzidine (TMB) substrate results in color development catalyzed by the HRP enzyme and the optical density (OD) is measured by a spectrophotometer. The highest reciprocal serum dilution resulting in an OD value equal to the mean of negative controls plus 3 standard deviations was considered as the antibody titer. When antibodies were not detectable, the sample was appointed a titer of 50 for calculation purposes.

The ELISA was done in a two-step fashion, first by screening for the presence of RBD-binding antibodies, and secondly, by testing against the full-length spike protein to determine the anti-spike IgG titer. On day 1, 96-well ELISA plates were coated with recombinant SARS-CoV-2 RBD, or spike protein diluted in PBS (2µg/ml and 50µl/well), covered and incubated at 4°C over night. On day 2, the plates were washed 6 times using PBS with 0.05% Tween-20 and blocked with blocking buffer (PBS with 0.1% Tween-20 and 3% milk, 200µl/well) for 2 hours at room temperature (RT) before removal. Sera diluted 1:100 in sample buffer (PBS with 1% milk and 0.1% Tween-20) were added to the plate and further 5-fold serially diluted and incubated for 2h at RT. The secondary HRP-conjugated anti-human IgG antibody was diluted in sample buffer (1:15000). The plates were washed 6 times and secondary antibody was added (50 µl/well) and incubated for 1 hour at RT. Following incubation, the plates were washed 6 times and TMB substrate was added (100µl/well) and incubated for 10 min in the dark. The reaction was stopped by adding 0.5M HCl (100µl/well) and the OD was measured immediately at 450 and 620 nm using a plate reader.

Microneutralization assay (paper III)

This assay was used to quantify the neutralizing capacity of SARS-CoV-2-specific antibodies in patient serum *in vitro*. Neutralizing antibodies bind antigens so that infection is inhibited. The assay was performed in a certified Biosafety level 3 facility by a trained operator, due to the use of replication competent virus.

Briefly, Vero cells were seeded in 96-well plates 24 hours before inoculation and incubated at 37ºC with 5% CO_2. Sera were heat-inactivated at 56ºC for 1 hour before use. Sera were serially diluted from 1:20, mixed with 100 x 50% tissue culture infectious dose of a local SARS-CoV-2 isolate collected in March 2020 (hCoV-19/Norway/Bergen-01/2020, GISAID accession ID EPI_ISL_541970) and incubated at 37°C for one hour. The mixture was added to the Vero cells and incubated for 24 hours at 37ºC. The next day, cells were fixed and permeabilized using methanol and 0.6% hydrogen peroxide. The plates were first incubated with anti-SARS-CoV-2 nucleoprotein rabbit IgG, followed by incubation with a biotinylated secondary goat anti-rabbit IgG (H+L) antibody (Southern Biotech) and lastly with an ExtrAvidin-peroxidase (Sigma-Aldrich) *o*-phenylenediamine dihydrochloride (OPD) substrate (Sigma-Aldrich) was used for color development. The microneutralization (MN) titer was the reciprocal of the serum dilution giving 50% inhibition of virus infectivity.

T-cell receptor sequencing (paper III)

Previously, SARS-CoV-2-specific T-cells have been identified using experimental mapping of T-cell receptor antigen specificity. By broad stimulation with SARS-CoV-2 peptides in COVID-19 cases and non-infected controls[192], T-cells binding antigens have been sorted and further sequenced. With high throughput immune sequencing and subsequent mathematical modeling, CDR3 regions of rearranged TCRβ-chains associated with T-cell binding of SARS-CoV-2 antigens have been identified. This technology can be used for the identification of SARS-CoV-2 specific cellular immune responses after infection or vaccination. Each identified TCR sequence holds

a unique signature of a clonal lineage of T-cells. The number of copies of each TCR signature reflects the magnitude of clonal expansion of the specific clonal subtype.

In this analysis, genomic DNA was extracted from blood that had been stored in EDTA tubes, amplified in a multiplex PCR, followed by high-throughput TCR sequencing and sequence analysis, all performed by Adaptive Biotechnologies (Seattle, WA). The outcome is presented as the *clonal breadth,* which is defined as the fraction of SARS-CoV-2 specific unique T-cell clones in the overall T-cell repertoire, and the *clonal depth,* defined as the relative expansion of SARS-CoV-2 specific T-cells related to the overall TCR repertoire **(figure 11**). The clonal breadth and depth were further categorized into SARS-CoV-2 specific spike or non-spike associated TCRs and human leukocyte antigen (HLA) class I or HLA class II associated TCRs.

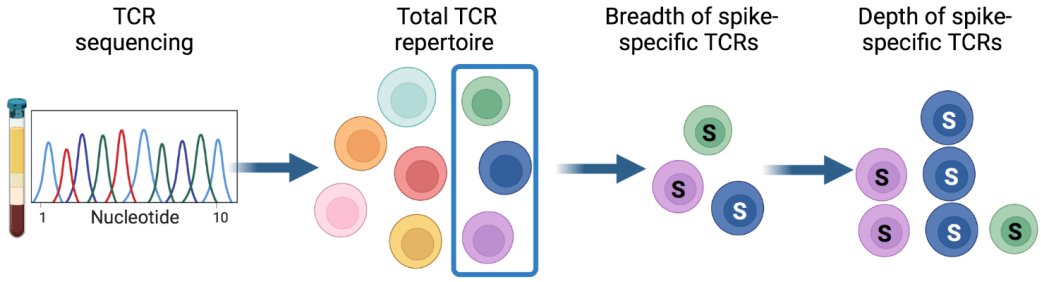

Figure 11. TCR breadth and depth.
High throughput immune sequencing is used to identify the total T-cell repertoire, and the SARS-CoV-2 specific TCR sequences. The magnitude of clonal expansion may differ between individual clonal subtypes. The clonal depth reflects the SARS-COV-2 specific clonal expansion related to the whole T-cell repertoire, whereas the clonal breadth reflects the number of unique clonal subtypes specific to SARS-COV-2. *The figure is created by Lena Hansen with BioRender.com, with permission to reuse.*

Diagnostic assays

Rapid test (Abbot ID NOW Influenza A&B)

Figure 12. POCT platform situated in the ED of Haukeland University Hospital.
Photo by Rune Sævig, with permission to reprint.

The Abbot ID NOW™ Influenza A&B 2 test and two platforms (previously known as Alere I Influenza A&B 2) were placed in the ED at Haukeland University Hospital in the 2018/2019 influenza season (**figure 12**). The ID NOW™ Influenza A&B 2 is an isothermal nucleic acid amplification test (NAAT), using proprietary enzymes and nicking enzyme amplification reaction (NEAR)[231] to replicate predefined segments of RNA. The templates used for the amplification process are the polymerase basic 2 (PB2) gene for influenza A and polymerase acidic (PA) gene for influenza B. Finally, a fluorescent tag, or molecular beacon, attached to the replicated genomic segments, is registered. The signal hence grows in proportion to the amplified product. When a predefined threshold level is reached, a positive test result is shown. Consequently, test samples with a high viral load could turn positive earlier than a sample with lower viral loads. Compared to conventional RT-PCR methods, isothermal amplification is significantly time saving. Another advantage of the Abbot ID NOW™ platform was the direct integration of test results into electronic patient records.

The producer advertised that the test provided molecular influenza results in less than 15 minutes (more precisely within 5-13 minutes). As it was not possible to extract the

exact turn-around-times (TATs) of the individual tests performed, we set a constant TAT for the test at 15 minutes, to make sure that we did not skew our results to overestimate the POCT performance. The test platform ran one sample at a time. The test material used was direct nasal swabs (CLIA waived). Briefly, the test contained seven manual steps, and a hands-on time of approximately 5 minutes. The test was performed by ED nurses and doctors after a formal introduction and training by certified laboratory-personnel. When available in the ED, study personnel assisted in conducting the test upon request.

Cepheid GeneXpert® II (California, US)

In Haraldsplass Deaconess Hospital, the two-module configuration of the GeneXpert System was used. The GeneXpert platform uses real-time RT-PCR technology and integrates the necessary steps to provide qualitive detection of nucleic acids in an automated fashion. Hence, the use is associated with little hands-on time. The system used in the hospital laboratory had the capacity to run two samples at the time, and the pathogens that could be detected by the test platform included a range of respiratory and non-respiratory pathogens. In our study, the laboratory used two different analytical cartridges, depending on availability: Xpert Xpress Flu/RSV and Xpert Flu. Samples were collected from the nasopharynx and/or oropharynx. The test was performed by trained laboratory-personnel in the hospital main laboratory. The machine provided a test result within 20-75 min according to the manufacturer, depending on which of the test cartridges were used. The genes targeted for amplification were Matrix protein (M), Polymerase Basic Protein 2 (PB2) and Polymerase acidic protein (PA) for influenza A, and Matrix protein and non-structural proteins for influenza B.

Data management, analysis and statistical methods.

In the influenza study, data were registered on paper case report forms (CRFs) and transferred to electronic format in SPSS. Randomly selected registration (10% of total registrations) was double-checked to ensure the compliance of information in paper and electronic format. CRFs were stored in a locked area, only available for study personnel. In the COVID-19 and Vaccine studies, data were registered in electronic CRFs by RedCap (Vanderbuilt, US). Access to data was restricted and limited to central study personnel.

Statistical analysis

Normality distribution of continuous variables was assessed using the Kolmogorov–Smirnov test, Shapiro–Wilk test, histogram, and Q-Q plots. Student's t-test was used to compare the means of normal distributed variables. The Mann-Whitney U-test (Wilcoxon rank-sum test) was used to compare continuous variables between two groups when distribution was non-parametric. The association between two categorical variables was assessed by the Chi square test when >80% of cell counts were ≥5, and the Fisher's exact test when ≥20% of cell counts were <5. Significance level (α) was set to 0.05 for all statistical analyses.

In **paper I**, Kaplan-Meier survival estimates were generated to assess the length of antibiotic treatment and length of hospital stay. To mitigate the effect of extreme values, admission that exceeded 30 days was censored at 30 days. The log rank test was used to compare the difference in survival between two groups. To assess the associations between length of stay (survival time) and multiple predictor variables, we used Cox proportional hazard regression. Predictor variables that were included in multivariable analysis had a 2-sided p-value <0.05 in bivariate analysis. In **paper II**, univariate and multivariate binomial logistic regression was used to assess the associations between predictor variables and the binary outcome variable "antibiotic treatment." Multivariable analysis included covariates with a 2-sided p-value <0.05 in univariate analysis. Non-parametric correlation analysis was performed using

Spearman's rho. In **paper III**, crude risk differences with 95% confidence intervals (CI) were calculated, and multivariable binomial logistic regression models were used for binary outcome variables, and negative binomial logistic regression was used to assess count data with non-negative integers. To assess data with repeated measurement, generalized estimation equations were used. Predefined predictor and confounder variables were included in the regression models. Missing data were excluded from analyses. Data analysis and visualization were performed in IBM SPSS Statistics version 24-26 (SPSS, Inc., Chicago, IL) Prism for Mac version 8.1.2 (GraphPad Software Inc., La Jolla, CA) and R 4.2.1 (R Foundation for Statistical Computing, Vienna, Austria)

Ethical considerations:

Patient recruitment, data collection and sample handling in the studies included in this thesis were conducted in accordance with the declaration of Helsinki and Good Clinical Practice (GCP). The individual studies and the combination of our observational studies with data from NIPaR were approved by the Regional Ethical Committee of Western Norway (REC number 2018/1772, 118664 and 218629).

Our observational studies involved the recruitment of acutely ill patients in infectious outbreak situations. Although challenging both logistically and ethically, it is important to conduct prospective studies in these acute situations, as retrospective analysis could have caused loss of important data on clinical symptoms and diagnostic timelines not typically registered in hospital charts, as well as the introduction of recall bias.

In the Influenza Study, hospitalized patients with influenza or ILI were asked to participate after ED admission during the influenza season of 2018/2019. Significant efforts were made to ensure that study participation did not cause an extra burden to the sick patients, and that participation was voluntary. The patients did not receive any monetary compensation. Study personnel did not participate in active treatment decisions, and this was communicated to the patients. Patients were not approached repeatedly when acutely ill. Only adult patients (≥18 years old) were included. To avoid exclusion of critically ill patients, eligible patients were prospectively recruited in the

ED (preferably) or within the first two days of hospitalization, and inclusion by next-of-kin, was permitted in patients that where comatose or by other means unable to provide informed consent. With this prolonged inclusion window, we avoided influence of patient treatment, and delays of critical treatment procedures in the ED when necessary. Dedicated study personnel included most patients during working hours. Study personnel provided oral and written information about the study when patients were interested in joining the study. Patients provided written informed consent before inclusion in study. Written information and consent forms where available in the ED during the inclusion period. Patients admitted outside working hours or in weekends were either asked to participate by the attending doctors on night duty, or the following day, when information about the eligible patients was forwarded to study staff. This contributed to ensuring a good informed consent process. For most patients, participation in the Influenza Study included responding to a short questionnaire. Although outside the scope of this thesis, consenting patients with influenza recruited at Haukeland University Hospital also provided one blood sample for immunological analysis upon inclusion and were asked for a follow-up at 6 months. Whenever feasible, blood sample collection was arranged to be performed at the time of routine blood investigations, or by study personnel at patient´s convenience.

The home-isolated patients and household members recruited in the COVID-19 study were not approached physically during acute illness but contacted by phone upon the positive RT-PCR test. Written informed consent from eligible participants was provided at first physical follow-up at 6-8 week after acute illness, and guardians or parents consented for their household children. The hospitalized COVID-19 patients in Bergen were asked to participate in the study when they were admitted to hospital, where they gave informed consent.

Due to the extraordinary pandemic situation in 2020, there was a crucial need to collect data systematically to improve understanding of SARS-CoV-2 epidemiology and characteristics of COVID-19. As a pragmatic approach to ensure quality data from all of Norway, informed consent was waived for the national registration of NIPaR data on hospitalized patients with COVID-19. Information about the possibility of register withdrawal was readily available to patients.

Withdrawal of consent:

Influenza study: One patient from hospital 2 withdrew consent before the data analysis, and was excluded from the study before paper I, and additional two patients withdrew their consent before the second manuscript, and were excluded from analysis in paper II.

Methodological considerations

Randomized controlled trials (RCTs) are the gold standard study method to assess the impact of an intervention. However, circumstances do not always allow this type of desired study. RCTs are costly, and require logistical procedures that are not always, readily available. In addition, when the outcome of interest depends upon factors that are not possible to randomize for (e.g. getting infected), RCTs are no longer suitable. This was the case in our studies of infection in the epidemic and pandemic settings. As an example, the recommendation for oseltamivir treatment in influenza is based on evidence derived from observational studies. A pre/post-intervention observational study was not possible for the influenza POCT, as there were no available data from the pre-intervention period. Also, a randomized controlled trial was not feasible as the hospital wanted rapid influenza testing to be available for all patients. A prospective controlled observational study allows for examining effects in a "real-life" setting and is an alternative method to investigate an outcome of interest. However, it is important to be aware that observational studies are more sensitive to bias and confounders.

In all our studies, we compared groups with differences in diagnosis or treatment to assess outcomes of interest. However, we addressed potential confounders and other limitations in each paper. In **paper I**, there are limitations to our study design. Patients were recruited in two different hospitals. There are only two hospitals in Bergen with public emergency care services, and the patient´s home address decides which hospital they will be admitted to in the case of an emergency. Thus, besides geographic circumstances, the patients in our study, were recruited from an unselected population of acutely admitted patients with influenza like illness during the influenza season. In

both hospitals, 38% of recruited patients had a diagnosis of influenza. This meant that our study was biased towards recruiting influenza positive patients, since the proportion of patients presenting in the ED with respiratory symptoms and ending up with a diagnosis of influenza was lower. As the study focused on influenza, study personnel were more likely to be contacted about influenza patients, particularly if recruited outside working hours. Overall, the number of patients recruited that did not have an influenza diagnosis was significantly larger, and eventually this inclusion bias allowed for a more robust subgroup analysis of influenza positive patients, which were an important target of interest. However, we are aware that although we tried to unselectively recruit as many patients as possible, situations with simultaneous presentation in the ED of several eligible patient might systematically lead to prioritization of some patient types and add additional selection bias to our cohort. Furthermore, patients presenting with severe symptoms, both influenza and non-influenza, were difficult to recruit from the intensive care setting. We did put an important effort into recruitment of critically ill patients, and we managed to include some of these patients. Inclusion of these patients was time-consuming and dependent on the collaboration with the ICU-staff. When patients were not able to provide informed consent, next-of-kin consent was difficult to achieve, as relatives only visited the ICU for limited time periods each day, and we were reliant upon ICU staff to inform next of kin about this study and contact the study staff about potential patients, which were not always the case. Although our study does not include all critical cases, they did account for a minority of admissions.

We assessed 625 patients for the influenza study. Of these, 57 were excluded from the study. The inclusion of patients at night or during weekends, by ED doctors, led to inclusion of some patients that did not fulfil all study criteria, either due to symptoms lasting over 7 days, or because influenza testing was performed as pre-operative screening, and not due to influenza specific symptoms, illustrating the benefit of trained study personnel for assessing eligibility.

Patients in both hospitals were treated by different doctors that were educated to use the same national guidelines for antibiotic prescription. But, according to a national

report, the overall prescription rate of resistance driving antibiotics were >30% in hospital 1 versus <20% in hospital 2, thus inherent differences in prescription practices were present. Our study design did not allow us to adjust for these and other non-tangible differences between patient care in the two hospitals.

However, we used multivariate analysis to assess the independent effect of predictors and known confounders on our outcome of interest.

Overall, our findings can only state that we found significant associations between the intervention and outcomes of interest, but we cannot establish causation. However, by addressing known confounders the association is strengthened. The additive summarized knowledge from multiple observational studies will bring us closer to accepting a possible cause-effect relationship between a predictor and an outcome when RCTs are not feasible.

In **paper II** we compared the rate of antibiotic prescription in influenza and COVID-19 patients. Less than hundred COVID-19 patients were admitted to the two local hospitals during the recruitment period, which limited the possibility to address rare events and to generate precise effect estimates. Therefore, we included national register-data on COIVD-19 admissions from the whole country. However, results from the smaller local influenza cohort may not be generalizable to the whole country, which affects the validity of the comparison between local and national data. Nonetheless, there is valuable information in the data that we present, which could motivate larger interventional studies of antibiotic treatment in respiratory viral infections. If we had the chance to redesign the study, information on antibiotic duration in and outside the hospital along with key biochemical and clinical parameters at admission should be registered in all patients.

In the national register (NIPaR), patient admissions were registered, and some patients were admitted twice (<2% of total cohort). Due to anonymized data, it was not identifiable which the first and last admission was, and it is possible that some COVID-19 patients admitted during the first pandemic wave returned home before deterioration and were readmitted in the proximate future. In the beginning of the pandemic, the typical clinical deterioration of COVID-19 patients in the second week of disease was

not recognized, which may have led to premature discharge of some patients. We chose to include these hospitalizations in our overall cohort, as we considered these cases to be clinically relevant. However, a separate analysis excluding these patients with possible readmission did not change the results of the analysis or the significance of results.

In **paper III** we compared the long-term symptoms in SARS-CoV-2 infected cases to seronegative controls. All infected cases were defined as having non-severe COVID-19 illness. Long COVID is more prevalent after severe COVID-19 in hospitalized patients, thus it would have been preferable to be able to categorize the severity of the acute phase symptoms in more detail. For a large RCT for Remdesivir, Beigel et al defined an eight-category ordinal scale for COVID-19 severity[232]: Our definition of mild disease included categories 1, 2 and 3 according to this scale. We did not collect objective data on respiratory compromise in our home-isolated cohort, thus it is possible that some patients had hypoxia during their disease course, indicating more severe disease. Although patients may not have perceived dyspnea, the pathophysiological effects of lower oxygen levels may have influenced the development of long COVID symptoms.

Our study had a small size of age-stratified groups, and the lack of gender-matching in the control group is a limitation. Also, more cases had comorbidities compared to controls. Hence, we added gender and comorbidities as covariates when we compared cases to controls. Due to limited information on smoking habits and body-mass index of controls, we chose to not include these variables in analysis of case symptom development. However, BMI has been shown to be associated with more severe acute COVID-19 and would be relevant to add in multivariable analysis if available.

As this was a longitudinal study, we experienced some loss to follow-up from the baseline inclusion. At 12 months the study still included 77% of all patients diagnosed with COVID-19 during the first pandemic wave from February 28th to April 4th, 2020. However, at the 18-month follow-up there was a further decrease, with 53% of the original patient cohort providing information. Hence, our findings could be biased towards patients experiencing more symptoms, as these people are more likely to

continue in the study. Also, reinfection, and vaccination could have influenced symptom prevalence, but in comparison with the overall cohort symptom prevalence at 6 and 12 months the sub cohort followed for 18 months did not diverge significantly.

Summary of results

Paper I

"Point-of-Care Influenza Testing Impacts Clinical Decision, Patient Flow, and Length of Stay in Hospitalized Adults."

In this paper, we hypothesized that a novel, rapid influenza POCT in the ED would improve length-of-stay and overall logistics, improve targeted antivirals and reduce antibiotic use. We conducted a prospective 2-center clinical observational study (n=567) comparing patient outcomes with the use of different rapid influenza diagnostic tests in two hospitals, one point-of-care in the ED (hospital 1, n=400), and the other laboratory-based (hospital 2, n=167). The proportion of influenza positive patients was 39% and 38% in hospital 1 and 2, respectively. Use of POCT resulted in shorter turnaround-times from initial patient triage to test results (median 69 vs 269 min). We found that early targeted isolation of influenza patients was superior in hospital 1, using POCT (91% vs 80%, p=0.025). Shorter length-of-stay (LOS) was associated with POCT in both univariate ($p<0.0001$) and multivariate analyses ($p<0.0012$). Encouragingly, NAIs were similarly prescribed to >80% of influenza positive patients in both hospitals, within a median of 4 and 5 hours in hospital 1 and 2, respectively. Antibiotic treatment was given to >70% of patients in both hospitals, but rapid antibiotic discontinuance in influenza positive patients was superior in hospital 2 than hospital 1 (median treatment duration of 3.5 versus 7 days, p=0.002). In conclusion, POCT was associated with shorter LOS, better isolation priorities, but not reduced antibiotic use. Although ED-admitted patients in the two hospital were unselected, inherent differences between the two hospitals, like the larger size of hospital 1 (12000 employees versus 1000 employees in hospital 2) including more specialized departments, could have influenced continuity and quality of patient follow-up.

Paper II

"Lower antibiotic prescription rates in hospitalized COVID-19 patients than influenza patients, a prospective study."

This study was a consequence of the findings in **paper I.** We addressed expressed concerns of potential excessive antibiotic use in COVID-19 patients and hypothesized that COVID-19 patients received more antibiotics than clinically comparable influenza patients did the previous year. We compared timing and use of broad and narrow-spectrum antibiotics in the combined cohort of hospitalized influenza patients described in **paper I,** to COVID-19 patients admitted to the same two hospitals, and patients registered nationally during the first and second COVID-19 pandemic waves of 2020. Surprisingly, we found that despite the novelty of SARS-CoV-2 infection, and initial uncertainty of the bacterial co-infection potential, overall antibiotic prescription rates in influenza patients significantly exceeded those of local COVID-19 patients (69% vs 49%, $p<0.001$) and national COVID-19 patients overall (53%, $p<0.001$) independent of other risk factors. The odds of influenza patients receiving narrow-spectrum, but not broad-spectrum antibiotics, were significantly higher than in COVID-19 patients. Early antibiotics (prescribed within 24 hours) accounted for most antibiotic treatment in influenza patients (96%), and local COVID-19 patients (90%). We observed a significant drop in antibiotic use in COVID-19 patients nationally from the first to second pandemic wave (65% to 42%, $p<0.001$). This was importantly due to reduced prescription of broad-spectrum and early antibiotics, the latter decreasing from 76% in the first wave to 65% in the second wave. In conclusion, COVID-19 patients received fewer courses of antibiotics than influenza patients overall, but a larger proportion received broad-spectrum antibiotics. Antibiotic use decreased from the first to second pandemic wave of the SARS-CoV-2 pandemic as increased understanding of the disease led to more targeted clinical treatment.

Paper III

"Symptom burden and immune dynamics 6 to 18 months following mild SARS-CoV-2 infection - a case-control study."

Long COVID is defined as a variety of new, recurring, and persisting symptoms that are present at or persist beyond 3 months following infection with SARS-CoV-2. Presently, the condition is poorly characterized. Reports of novel and durable symptom sequelae seen in young patients post COVID-19 led to the investigations in **paper III**. Long COVID symptoms in patients who were home-isolated with acute COVID-19 during spring 2020 (n=233) were compared with symptoms in age-matched seronegative controls (n=189). We further studied the kinetics of SARS-CoV-2 specific immune responses and their association with long COVID symptom development. After 12 months follow-up, we found that compared to non-infected controls, home-isolated patients reported a significantly higher burden of fatigue (Odds Ratio (OR) 5.86, 95% confidence interval (CI) 3.27-10.5), memory problems (OR 7.42, 95%CI 3.51-15.67), concentration problems (OR 8.88, 95%CI 3.88-20.35) and dyspnea (OR 2.66, 95%CI 1.22-5.79). Efforts to reduce the risk of confounders were addressed by including controls which had experienced the same pandemic circumstances and public infection control interventions as the infected patients, and who lived in the same geographical area, as well as being matched for age. We found it worrisome that cognitive symptoms were overrepresented in young adults after COVID-19, compared to their age-matched non-infected controls. At 12 months, 46% of home-isolated patients had one or more of eleven specific symptoms assessed. There was no significant symptom improvement from 6 to 18 months after acute COVID-19. We found a relationship between elevated peak (2 months) and longitudinal spike-specific IgG titers and persistent dyspnea 12 months after COVID-19. SARS-CoV-2 specific TCRβ sequence analysis served as a marker of T cell activation induced by SARS-CoV-2 infection. The outcome was presented as the *clonal breadth*, corresponding to the SARS-CoV-2 specific fraction of the total T-cell repertoire, and the *clonal depth*, corresponding to the relative frequency of the SARS-CoV-2 specific T-cell clonotypes present (**Figure 12**). We found that spike-specific $CD4^+$ T-cell clonal depth at 6 months, adjusted for gender, comorbidities, and reciprocal breadth, was

associated with dyspnea, and increasing number of symptoms at 12 months. In conclusion, 46% of home-isolated patients had persistent symptoms at 12 months follow-up. Non-infected controls had significantly lower prevalence of fatigue and cognitive symptoms than home-isolated patients did. Higher spike-specific IgG responses were associated with persistent dyspnea, and 6 months spike-specific $CD4^+$ T-cells depth were associated with persisting dyspnea and increased number of symptoms at 12-month. Our findings adds knowledge to the long COVID symptom burden after non-severe COVID-19, and provides evidence of a potential role of adaptive immune responses in long COVID.

Discussion of major findings

In this thesis, the work conducted investigated several important steps in the chain of care of Influenza or SARS-CoV-2 infected patients; from bedside diagnostics to long-term complications. The thesis encompasses studies on the effect of rapid point-of-care influenza diagnostics in the ED, antibiotic use in hospitalized influenza and COVID-19 patients, and finally long-term sequelae after COVID-19 amongst home-isolated patients.

Prior to the COVID-19 pandemic, the use of rapid point-of-care diagnostics for respiratory viral infections were limited to health care facilities. Since the beginning of the pandemic, there has been a revolution in the use of rapid point-of-care antigen testing for COVID-19, making diagnostics available both domestically and in the professional health care setting. This increased use has dramatically changed our perception of the utility of rapid diagnostic tools and will impact the future handling of infectious diseases, and the development of future rapid diagnostics.

The use of rapid diagnostics in the ED has dramatically improved the possibilities of patient triage and isolation in the COVID-19 pandemic compared to the 2009 Swine-flu pandemic situation. In smaller health care facilities and hospitals in Norway, antigen-based tests, although with inferior accuracy compared to molecular testing, have been used to inform ED handling of patients with suspected COVID-19. This suggests that where other nucleotide-based diagnostics have not been readily available, rapid antigen-based tests have had an important function also in specialized health care. Furthermore, recent experience with molecular POCT including influenza and multiplex panels has been useful to inform the implementation of novel tests that now include SARS-CoV-2 in their diagnostic panels.

In Norway, overall antibiotic use is under public surveillance through the National Institute of Public Health, but there is no specific surveillance of antibiotic use in respiratory viral infections. The use of empirical (based on experience) versus directed (targeted towards a recognized pathogen) antibiotics is adding a burden to the silent pandemic of antimicrobial resistance [233]. By highlighting differences in empirical

antibiotic prescriptions for influenza and COVID-19, this thesis hopes to contribute knowledge which can help improve antimicrobial stewardship strategies in virus-associated respiratory infections.

Finally, it is important to map infection complications beyond the acute phase, and although much has been learned, there are still significant knowledge gaps concerning long-term complications of COVID-19, as well as in other respiratory viral diseases. Longitudinal observational studies of long COVID are important to increase understanding and to motivate translational research on the underlying pathogenesis and the potential contributors to the risk of such sequelae.

To conduct research in an outbreak setting is a challenging task. In the ED setting, complex logistics, the urgency of management of patients and interactions between professionals from many different specialties provide challenges. Therefore, great demands are placed on studies that intervene in these interactions. Nonetheless, it is vital to provide evidence-based knowledge from the real-life setting to improve conditions for both ED staff and patients, fulfilling the goal of this thesis to help improve the chain of patient care.

POCT Turn-around-times

This thesis describes the effect of implementing an influenza POCT in the ED during a regular influenza season, one year prior to the SARS-CoV-2 pandemic. When compared to RT-PCR results, the precision of clinical influenza diagnosis has been found to be poor (diagnostic sensitivity between 36%-38% and specificity between 78%-91%)[234,235]. Others have investigated the benefits of molecular POCT on influenza management in the ED, including assessment of length-of-stay in hospital and ED facilities, and targeted antiviral, antibiotic and isolation use[236-238]. However, study designs are heterogeneous, and results are mixed. This may be due to differences in local and national treatment guidelines, study inclusion criteria, or characteristics of the molecular POCTs and their respective comparison tests. The ED organization may differ between countries, as can the level of experience in doctors working there. In Norway both junior doctors and interns work shifts in the ED. During busy work hours,

there is minimal time for thorough supervision, which could lead to mis- or delayed treatment of patients.

In Paper I, we found that the time from ED presentation (triage) to influenza testing was shorter in the hospital using POCT and laboratory-based influenza testing. How fast sample collection was prioritized upon admission, affected the total time until test results were available. An observation made while the study was conducted, was that the availability of the influenza POCT changed the behavior of the ED staff so that the POCT often became one of the first diagnostic procedures upon ED triage. This was reflected in a mean time from triage to test results of only 69 minutes, compared to 269 min for the laboratory-based test. Even with a longer intrinsic test turn-around -time for the laboratory-based test than the POCT (119 min vs 15 min), the largest difference in the time from triage to test result was the mean time before sample collection was performed (54 min vs 150 min for the POCT and laboratory-based test, respectively). Although we do not know the exact waiting time from ED presentation to triage, this logistic procedure is of high priority in all EDs when there is a high influx of patients, and we expect it to be similar in the two hospitals. Thus, this finding indicates that the proximity of the test station in the ED could positively influence the priority of performing the influenza test. This is supported by a pre/post influenza care pathway intervention study, which found that a PCR-based POCT in the ED strongly decreased time from ED presentation to sample collection (47min vs 194min) and time from ED presentation to result (62min vs 1094min)[239]. Other studies have found significantly reduced time spent in the ED after implementation of a POC NAAT (617min vs 772 min and (255 vs 366min)[236,240]. Although differences in patient influx and ED capacity will influence such results, they indicate that molecular POCT can improve ED patient flow, a critical element in hospital infection control. The prioritization of the POCT observed in our study may also indicate that the test was perceived as a useful tool by all ED staff, since the POCT results were communicated to relevant staff managing the patient, and not later to just the responsible doctor.

Test performance

The performance of molecular POCTs varies between tests performed under stringent laboratory control with trained operators, and those performed in a hectic real-life ED environment with less experienced operators. A limitation for the use of influenza molecular POCT is the increased likelihood of false-negative results compared to standard RT-PCR. The performance of the two test platforms used in our intervention and control hospitals have been compared in previous studies. When compared to the laboratory-based GeneXpert/Flu system or RT-PCR, sensitivity of the Abbot ID NOW influenza POCT ranged from 80%-95% for Influenza A and 45%-100% for influenza B, when performed on samples in virus transport medium (VTM). When nasal swabs were used, the sensitivity was 92% and the specificity 100%[237,241-243]. In the influenza season we conducted our study, influenza B circulation was unusually low, representing <1% of influenza cases nationally, and the contribution of influenza B to the test performance in our study was minimal, as we only found three cases of influenza B. The year our influenza study was conducted, the Department of Microbiology calculated the diagnostic accuracy of the influenza POCT compared to the in-house laboratory PCR at our intervention hospital. They reported an influenza A sensitivity of 89% and a specificity of 98%. Low viral loads can reduce sensitivity of the influenza POCT[244,245], which is of clinical relevance, especially when a person is admitted long after symptom debut. Failing to correctly isolate patients that shed infectious viruses can lead to nosocomial (health care associated) transmission and delayed antiviral treatment. While a false positive test can lead to initiation of antiviral treatment with potential adverse effects and unnecessary isolation use. Thus, in both influenza, and SARS-CoV-2 diagnostics, it is important to be aware of these limitations to molecular POCT. Nonetheless, considered better than clinical evaluation alone, both antigen-based and rapid molecular-POCT are in widespread use in the ED setting.

Isolation use

Importantly, in **Paper I**, we found better targeted initial isolation in the ED of the intervention hospital using POCT. Besides reduced ED patient transit time, rapid and accurate isolation use are important mitigation strategies for hospital infection control. National guidelines are clear on their recommendation of droplet precaution isolation of symptomatic influenza patients[246]. The association between isolation use and influenza diagnosis found in both study hospitals, suggests adherence to these guidelines, but that using POCT ensures an even higher degree of initial targeted isolation use. Only 7% of non-influenza patients in the POCT hospital and 12% of non-influenza patients in the laboratory-based hospital were isolated upon admittance, indicating that unnecessary isolation of test-negative individuals was largely avoided by POCT. Several studies have found that the use of influenza POCT improves isolation use[239,247,248]. One study reported successful influenza patient cohort-isolation based on influenza POCT diagnosis in the ED. This lead to better single-room capacity as well as improved hospital infection control[249]. Improved targeted isolation use and reductions in hospital acquired influenza have also been associated with the use of very rapid laboratory-based influenza testing[250,251].

Without access to POCT, other strategies are used to reduce the risk of nosocomial spread of respiratory infection in hospital. When there are plenty of available isolation rooms, all patients admitted with respiratory symptoms could be isolated until the return of the laboratory PCR test result. This scenario was probably the case in some of the admitted patients in our control hospital, without available influenza POCT. However, in an epidemic or pandemic situation, this is not a sustainable solution, due to mismatches between ED isolation capacity and patient numbers. In the beginning of the ongoing SARS-CoV-2 pandemic, other means, such as temporary separated ad hoc triage areas when awaiting PCR test results were implemented for patients with suspected COVID-19. This practice was conducted in our local hospitals as well as internationally, offering some protection from nosocomial spread of infection in the hospital. Consequently, non-infected patients with similar symptoms risk being infected with COVID-19 while awaiting test result in the triage area[200]. Pandemic ED

triage interventions for COVID-19 have gradually been discontinued in our hospital, and rapid and accurate molecular SARS-CoV-2 POCT have become increasingly available. Experiences from our first POCT study concerning influenza, contributed to implementation of improved ED logistics in our hospital during the SARS-CoV-2 pandemic, with separate handling of patients with suspected versus non-suspected COVID-19. In other smaller hospital settings, in Norway, antigen tests were used broadly to aid ED logistics when they became available. As test accuracy is a high priority in the hospital setting, studies of influenza, and emerging evidence from COVID-19, suggest molecular POCT has a huge potential to target the use of isolation and initial clinical management in the ED.

Length-of-stay

In Paper I we assessed patient and hospital predictors of hospital LOS during influenza illness. LOS is used as a measure of hospital efficiency. It is a fine balance between providing sufficient specialized patient care and reducing unnecessary time spent in hospital. In multivariable analysis we found that the use of POCT was significantly associated with reduced LOS (median 3 vs 4 days), whereas broad-spectrum or prolonged antibiotic treatment, diabetes and cancer were significantly associated with prolonged LOS. These patient and treatment related factors could indicate more severe disease at admission, or a higher risk of nosocomial infection, requiring prolonged treatment measures. The association of molecular POCT and reduced LOS is supported by several studies from different countries. This includes comparing multiplex molecular POCT within 24 hours of admission to routine care in a randomized controlled trial (LOS 5.7 vs 6.8 days)[252] and three pre/post intervention comparisons of molecular influenza POCT in the ED vs laboratory testing which all found a reduction in LOS of around 1.5 days or more[237,239,247]. In contrast, another quasi-randomized study comparing multiplex molecular POCT to laboratory-PCR failed to show a significant difference in LOS between intervention and control group (4.1 in the POCT arm vs 3.3 days in the controls)[238]. However, this study had long turn-around-times (TATs) from admission to results in the POCT group (19 hours), compared to only 4.5 hours (269 min) in our laboratory-based influenza test control group, thus the POCT

did not qualify as truly rapid. As for the turn-around-times, these results indicate that the relative reduction in time to test result using POCT influences the hospital LOS. Reduced LOS in both test-positive and test-negative individuals[253], indicated that POCT result aided the clinical decision-making process in the ED. Moreover, faster informed treatment decisions may eventually favor patient outcomes and time to hospital discharge, both by rapid ED evaluation and discharge and by implementation of efficient patient care pathways.

The results from our study suggest that the shorter LOS in the POCT hospital was influenced by a greater proportion of patients discharged directly from the ED or associated short-term ward. The study data however does not have information on in-hospital ward transfer logistics, but more patients in the POCT hospital were discharged within 24 hours. Although known confounders were adjusted for (gender, age, comorbidities, clinical characteristics), differences in hospital practices may influence our analysis. Besides discharge policies, this may include other differences in patient management affiliated with destination wards, and day-to-day ward capacity. The cost-savings of reducing hospital LOS are important. Consequent financial savings are likely to compensate the cost of liberal use of POCT, in both influenza outbreaks, and the ongoing SARS-CoV-2 pandemic. Future studies will need to highlight these benefits to influence priorities in support of rapid diagnostic testing in the ED.

Antiviral treatment

Prompt use of NAIs is crucial in treatment of severe influenza infections as they reduce mortality and provide earlier symptom relief, particularly when given within 48 hours of symptom onset[205,254,255]. Both local and international clinical guidelines advise starting NAI treatment before diagnostic confirmation of influenza, if suspicion of infection is sufficiently strong, and the patient is at risks of a severe disease course or in hospital[256,257]. The only antiviral drug used to treat influenza infection in our study was oseltamivir (Tamiflu). In paper I, oseltamivir prescription was high in both the intervention and control hospitals (83% and 81% of influenza positive patients in hospitals 1 and 2, respectively). Unnecessary antiviral prescription was negligible as

only 3% of non-influenza patients in both hospitals received oseltamivir. This finding suggests a high confidence in the accuracy of the respective diagnostic tests to guide antiviral treatment. The time to treatment was short in both hospitals, with a median of 4 and 5 hours in the POCT and laboratory-based test hospital, respectively. Although not explicitly studied, the time to NAI treatment and the strong correlation between the test result and NAI prescriptions, suggest the initiation of NAIs were delayed until after test results were obtained. Additionally, a delay in NAI initiation could be related to the storage of this medicine, which was outside the ED. Identification of this delay, demonstrated in our study, should be used to further optimize hospital routines concerning storage of medication commonly prescribed in the ED on site. Studies that include adults[240,248,251,253] and children[258] in hospital ED, as well as in outpatient populations[259] agree that molecular POCT improve time to administration of antivirals and increase the proportion of influenza patients receiving antiviral treatment. Although the current knowledge concerning antiviral treatment for COVID-19, and the access to their use is limited, viral replication is clinically important at the time of symptom debut in both influenza and COVID-19 patients. Hence, it is reasonable to suggest that early antiviral treatment is beneficial in treating both diseases, and that molecular POCT also provides a diagnostical benefit in COVID-19. Trials for antivirals in COVID-19 are ongoing, and the global demand for targeted treatment is significant. Increased use of home-testing could also have important implications for the use of antivirals outside the hospital setting.

Antibiotic treatment for influenza

Antimicrobial resistance (AMR) has been termed the next pandemic threat, now accounting for an estimated 1.2 million lost lives annually, according to the WHO, defining AMR as one of ten global health threats that call for immediate action. Due to the rapid spread of antimicrobial resistance, it is imperative to reduce unnecessary antibiotic use. Since 2020, AMR is specifically mentioned under the United Nations 3rd Sustainable Development Goal, adopted by all member states. In Norway, the government developed a strategic plan for achieving a 30% reduction of antibiotic use in humans from 2012-2020. This goal was achieved in 2020. Most antibiotics are

prescribed in general practice (GP), and respiratory tract infections are one of the leading causes of antibiotic prescriptions[260]. In the early phases of the pandemic, the coinciding of social restrictions, and thus a significant reduction of GP consultations due to respiratory tract infections, may have contributed to these encouraging results. At the same time, the use of broad-spectrum antibiotics has increased in the hospital-setting, demonstrating the need for continued attention towards antibiotic prescription practices. In this thesis, we have investigated the current antibiotic use in two respiratory viral infections that are commonly managed in the hospital setting, Influenza and SARS-CoV-2.

Diagnostic uncertainty of etiology in acute respiratory tract infections contributes to unnecessary antibiotic prescription[261]. There is a paucity of rapid diagnostics that can confidently exclude bacterial infection. According to a 2013 study assessing the microbial yield of the diagnostic FilmArray Biofire panel, bacterial co-infections were associated with approximately 40% of all viral respiratory tract infections, and >90% of hospitalized patients with viral infections received antibiotics[262]. In a local study from our hospital in 2019, which implemented POCT, 33% of Community-Acquired Pneumonia (CAP) patients had a viral-bacterial co-detection in samples from the lower respiratory tract at the time of admission[263]. Due to missed diagnosis, the overall prevalence of bacterial co-infection in influenza is unknown. According to one review, bacterial co-infection was estimated to occur in 0.5% of influenza infections in healthy young people, and in over 2.5% of influenza infections in the oldest age groups[89]. Mortality during previous influenza pandemics has been linked to bacterial co-infection, particularly in the pre-antibiotic era[89]. Bacterial co-pathogens were also found in 25% of autopsy specimens from the A/H1N1pdm09 pandemic, according to a meta-analysis[264]. Co-infection is thus a severe complication of influenza. In seasonal influenza infection, bacterial co-infection occurred in 11-35% of hospitalized influenza patients according to a systematic review, and 16% of ICU admitted patients[98,265].

In **paper I**, we found that POCT was not associated with reduced antibiotic use, contrary to our expectations. There are studies from the ED and outpatient settings indicating that Rapid influenza diagnostic tests (RIDTs) are associated with fewer

antibiotic prescriptions in adults[266-268] and in children populations results are mixed[194,269]. Similarly, retrospective studies with larger differences in TATs between comparison groups have found associations between molecular POCT in the ED and reduced antibiotic use[270,271], both in the ED and in total antibiotic use during hospital admission in both test-positive and test-negative patients[239]. With antibiotic use as a primary outcome, a large RCT with patients randomized to routine-care or multiplex molecular POCT within 24 hours, found POCT to be associated with more single-dose and short (<48h) antibiotic courses, but not overall reduced proportion of antibiotics, as many patients were started on antibiotics before the POCT result was ready. Another observational study did not find an association between POCT and reduced antibiotic use but did not specify the TATs involved in the different tests[236].

We did not find lower antibiotic prescription levels in our intervention hospital, using POCT, compared to the hospital using the laboratory-based influenza test, suggesting that the difference in TATs between the two tests was not large enough to influence the decision to initiate antibiotic therapy. Additional clinical signs and investigations, or clinician's considerations, which we did not assess may have a stronger influence on whether patients are given antibiotics. In the hospital using laboratory-based testing, the duration of antibiotic treatment in influenza patients was significantly shorter than in the hospital using POCT.

Local antibiotic treatment policies may have an important impact on the antibiotic stewardship and antibiotic reassessment routines, affecting antibiotic use in the destination wards. In a 2020 national report published by NORM (Norwegian Surveillance of Antibiotic Resistance in Microbes) the control hospital using laboratory-based hospital was listed as one of the best performing hospitals in the country in terms of use of preferred antibiotics (narrow-spectrum instead of resistance driving). The hospital which implemented POCT used more resistance-driving antibiotics. Although there were differences in bed-capacity and specialized wards between the two hospitals, they both had public EDs serving an unselected population within similar geographic areas of the city of Bergen. The hospitals are therefore expected to provide similar ED services to the public. Compared to the non-influenza group, influenza patients reported more symptoms upon admittance, which could be

interpreted as a sign of co-infection, making decisions to refrain from antibiotics difficult. Still, the overall antibiotic use found in our study was surprisingly high with 72% of influenza patients in the intervention hospital using POCT group and 62% of patients in the hospital with laboratory-based testing receiving antibiotics. Rapid POCT with broader respiratory panels including both viral and bacterial pathogens may be more informative in guiding antibiotic use and other treatment choices than a single pathogen test, and should be considered in future molecular POCT platform solutions[272]. However, careful clinical interpretation is warranted as it becomes more complex once several pathogens are detected. Training of personnel can be necessary to distinguish upper from lower respiratory tract results, presence from prior infection, and colonization from actual infectious disease, requiring treatment.

Comparison of antibiotic use in influenza and COVID-19.

In the northern hemisphere, SARS-CoV-2 emerged during the expected 2019/2020 influenza season. Rapid global spread of the novel pandemic virus led to the registration of the first Norwegian patient with COVID-19 on February 26th, 2020, and a national lockdown starting from the 12th March 2020. Subsequently, reports of overuse of antibiotics in COVID-19 patients and few confirmed bacterial co-infections outside of the ICU raised concerns of accelerated development of antimicrobial resistance[273]. This motivated us to investigate whether the new pandemic setting changed adherence to national antibiotic guidelines. We wished to assess whether antibiotic treatment assessments that were used in SARS-CoV-2 compared to influenza, another respiratory tract infection with similar symptomatology. The observational investigations, presented in **paper II**, were based on the hypothesis that hospitalized COVID-19 patients would receive more antibiotic treatment than clinically comparable influenza patients. As cases of influenza dropped drastically after implementation of strict infection control measures on the 12th of March 2020, a prospective parallel comparison of Influenza and COVID-19 hospital admissions was not feasible. Thus, we conducted a prospective comparison in the same two hospitals in two consecutive years and compared the rate of early antibiotic prescriptions (prescription within the first 24 hours of admission for influenza or COVID-19) and

later antibiotic prescriptions (started after 24 hours of admission) in influenza and COVID-19 patients. To our surprise, our initial hypothesis on more antibiotic use in COVID-19 patients was incorrect. Overall, influenza patients received significantly more antibiotic treatment than COVID-19 patients, (69% versus 53%) in the study hospitals. Furthermore, 49% nationally registered COVID-19 patients were treated with antibiotics. Furthermore, we found that antibiotics were more frequently prescribed within 24 hours of admission in influenza patients. However, COVID-19 patients received more broad-spectrum antibiotics, which could be due to early reports recommending broad empirical antimicrobial treatment. For instance, the macrolide antibiotic azithromycin was suggested as a possible treatment for COVID-19, and although proven inefficient in later studies[274,275], the use of this resistance driving antibiotic increased significantly early in the SARS-CoV-2 pandemic, particularly in the US[132,276]. Globally, studies reporting of antibiotic resistance and antibiotic prescribing in COVID-19 patients have been heterogeneous and dependent on geography and health care setting, but high-level antibiotic use have been a consistent finding[277,278]. In our study, very few COVID-19 patients received azithromycin, and mainly in the beginning of the pandemic. The restrictive practice regarding broad-spectrum antibiotics that has been implemented by the Norwegian guidelines for years, and advice of adherence to national antibiotic guidelines, unless COVID-19 patients were recruited into clinical trials, and a low level of antibiotic resistance in our hospital, could have contributed to this finding. Two early reviews found bacterial co-infection frequency in hospitalized COVID-19 patients at 7-8%[128,279] while a more recent systematic review and meta-analysis distinguishing co-infection from superinfection found a pooled bacterial co-infection prevalence of 8% (95% CI 5%-11%) and bacterial superinfections in 20% (95% CI 13%-28%)[280]. Comparative studies early in the pandemic indicated that in-hospital mortality and pulmonary complications were more frequent in COVID-19 than seasonal influenza[281,282]. In a Swedish retrospective study using data from 2011-2020, 4% of patients admitted with COVID-19 had co-infection at admittance, compared to 27% of influenza and 29% of RSV patients[283]. Furthermore, the most common bacterial finding was *S. pneumonia* in all three viral infections, followed by *S.aureus* in COVID-19. Norway has a very low prevalence of

antimicrobial resistance in clinical isolates from humans[217], hence narrow-spectrum penicillins would be the recommended first line empirical choice in CAP. Findings from our study showed that in the second pandemic wave, antibiotics were prescribed less frequently to COVID-19 patients in Norwegian hospitals compared to patients in the first wave. It is remarkable, that despite the recognition of the severity of the new viral disease, the reduction in antibiotic prescriptions after the onset of the first pandemic wave were rapid and in concordance with national antibiotic guideline modifications and reports of the infrequent incidence of bacterial co-infections in COVID-19. In Norway, the trust in governmental advice have been high throughout the pandemic, resulting in high compliance to social restrictions, isolation, and quarantine rules. The specialized health care services have retained capacity to treat severe COVID-19 cases, keeping low mortality rates, which might have contributed to lower perception of subjective risk amongst the population. In a study including over 4571 Norwegians, only 10.5% reported hesitancy towards vaccination, which is considerably lower than in most other countries[284,285]. Likewise, confidence in national antibiotic guidelines and medical treatment updates regarding COVID-19 has been mostly adhered to by health care professionals.

Although bacterial co-infections appear to be more common in influenza than COVID-19, they are not present in most influenza patients. Bacterial complications are considered more likely 5-7 days after acute influenza illness debut, whereas patients included in our study from the 2018/2019 influenza season had a mean time from symptom onset to admission between 2 and 3 days. We also excluded patients with symptoms duration over 7 days, expecting to reduce the number of patients with bacterial co-infection upon admission in our patient cohort. Another Norwegian retrospective study reported of stable antibiotic prescribing rates in Norwegian hospitalized influenza patients from 2014-2018[216], thus there has not been a trend of temporal improvement of prescribing practices. Findings from our comparison study indicate that in a novel and uncertain pandemic situation where there is limited knowledge of favorable treatment, policy makers and medical professionals are attentive to the latest literature to adjust recommendation and clinical practices. Lessons learned from the management of COVID-19 should be taken into

consideration to improve antibiotic prescribing practices in hospitalized patients with influenza and other respiratory viruses. Although we adjusted for clinical differences between influenza and COVID-19 patients in **paper II**, unidentified factors could also greatly influence on antibiotic treatment choices. Vital signs, the time of radiological examination and the results of blood tests for inflammatory markers may strongly influence the decision on antibiotic treatment in the ED. Lessons learned from our stringent antibiotic policy during the second wave of the pandemic, will hopefully aid us in future influenza seasons.

Long-term sequelae of COVID-19

Our prospective observational studies of acute influenza infection and COVID-19 included assessments of hospital complications and 30-day mortality. However, our subsequent pursuit to investigate broader clinical and immunological consequences of respiratory viral infections, necessitated longer follow-up time. From 2020 to 2022, long-term complications of COVID-19 have gained global attention. Due to early recruitment and a strong motivation in COVID-19 affected individuals suffering long term complications to engage in clinical observational studies, we were able to conduct a longitudinal follow-up study with high follow-up rates. The patients were recruited in the first pandemic wave, the spring of 2020, at the time of their acute SARS-CoV-2 infection. In **paper II** we reported on clinical characteristics and antibiotic treatment in hospitalized COVID-19 patients, whereas **paper III** presents clinical and immunological results from home-isolated COVID-19 patients in Bergen, Norway. Centralized diagnostic SARS-CoV-2 PCR testing in the municipality of Bergen made it possible to recruit a near-complete cohort of individuals diagnosed with mild COVID-19 during the first pandemic wave, and not in need of hospitalization. Due to limited testing capacity in the first pandemic wave, commonly only a single member of a family was prioritized for testing. Thus, we recruited household contacts of positive index cases to participate in our study, as they were of substantial risk of infection, being close contacts. We assessed the presence of anti-SARS-CoV-2 antibodies in all included participants, and household contacts who had seroconverted at 2 months follow up were included as cases. Hence, we had a valuable cohort that

included both children and adult COVID-19 cases. We investigated clinical and immunological correlates of long COVID in home-isolated patients 12 months after initial infection and compared symptom prevalence 12 months after infection to non-infected controls. In a sub cohort of patients, we investigated symptom trajectories up to 18 months post-infection. Controls were age-matched, and consisted of seronegative household contacts, and SARS-CoV-2 naive individuals that were prioritized for COVID-19 vaccination early in 2021, coinciding with the 12-month follow up of the home isolated COVID-19 cases. The study had several novel contributions to research on long COVID. To our knowledge, this was the longest (18-month) prospective follow-up of non-severe COVID-19 patients that included a confirmed SARS-CoV-2 seronegative comparison group, thus controlling for the general pandemic effect on the individuals and society[286]. In comparison, a previously published study reported of 2-year follow-up results from a smaller and primarily non-hospitalized patient population, without the inclusion of controls[140], and a large study with 2 years follow-up that did include controls, only assessed health outcomes in hospitalized patients[139]. Previous studies that included non-hospitalized COVID-19 patients and non-infected controls had been conducted with a follow-up time up to 8 months[287-290]. Of three relevant studies, only one measured antibody titers to assess seroconversion in the control group, which is important to show that controls have not had SARS-CoV-2 infection [287]. The largest of the three studies was a Norwegian online survey, where significant health deterioration in the last year was reported more frequently 3-8 months after COVID-19 (36%) compared to controls (18%). A 4 month follow-up study, including only health care workers (HCW), found significantly more anxiety and depression in mildly infected HCWs than healthy HCWs, but no objective general cognitive impairment in COVID-19 patients, evaluated by the Mini-Mental State Examination (MMSE)[288]. The last study reported higher incidences of gustatory and olfactory symptoms in infected HCWs compared to healthy controls 6 months post-infection[287]. In paper III we found that the prevalence of fatigue (37% vs 9%), memory (26% vs 5%), concentration problems (24% vs 4%), and dyspnea (15% vs 5%) were significantly higher in adults 12 months after COVID-19 (n=220) compared with contemporary non-infected controls (n=182). In total, we evaluated 11 long COVID

symptoms (fever, tingling, dizziness, palpitations, gastrointestinal upset, headache, fatigue, sleep-, memory-, and concentration problems). We found that almost half of our SARS-CoV-2 infected group reported at least one of these 11 symptoms after 12 months. Markedly, this was without assessing for reduced taste or smell anosmia, which could have increased the number of symptomatic patients at 12 months. Our study supports previous findings of frequent symptom persistence 12 months post-infection in both hospitalized and non-hospitalized patient populations[291,292]. Furthermore, in **paper III** we observed that in a sub cohort of patients followed for 18 months, the pooled frequency of patients with symptoms present did not improve from 6-12-18 months post-infection. Notably, within each specific symptom assessed, there were patients that reported the symptom first at later follow-ups, suggesting that unidentified factors triggered symptom development or termination, or that long COVID symptoms fluctuate by nature, as proposed in other longitudinal studies with multiple patient assessments[293,294]. In hospitalized patients, Huang et al found that the proportion of patients reporting to be symptom free increased from 6 to 12 months (32-51% respectively). Whereas they reported that most symptoms improved, dyspnea, anxiety and depression were more frequently reported at 12 months[295]. According to a recent meta-analysis which included 63 individual studies in time intervals of 3-, 6-, 9- and 12-months post-infection, there were large variations between studies, and accumulated symptom prevalence differed between early and late follow-ups. Fatigue was the dominant symptom overall, with increased prevalence over time (32% at 3-6 months, 37% at 9-12 months, and 41% >12 months follow up)[296].

The fluctuation of symptoms observed in many studies, including ours, makes the pursuit of a long COVID pathogenesis even more challenging. The University of Cincinnati Medical Center for COVID-19 sequelae have acknowledged the different clinical phenotypes and proposed a 5 category scale that distinguishes between symptom trajectories of persisting and relapsing symptoms[297]. The heterogeneous burden of long COVID warrants long-term observational and interventional investigation of symptom burden combined with objective examinations of organ function.

Immune correlates of long COVID

Several hypotheses have been proposed to explain the pathophysiology of the plethora of long COVID symptoms affecting patients who have survived both mild, moderate, and severe acute disease. Evolving data indicate that multiple factors are involved in long COVID development. Similarities to other post infectious conditions suggest that aberrant host inflammatory responses could be an important contributor to a prolonged inflammatory state affecting lungs, neuronal tissue, and vasculature. Persistent viral infection in trophic tissues (e.g. adipocytes) have also been proposed in the pathogenesis of long COVID. This could lead to durable immune stimuli due to circulating antigens. Different mechanisms of autoimmunity that could be involved include molecular mimicry and spillover effects of SARS-CoV-2 specific immune responses. Direct viral invasion as well as immunological driven procoagulant and inflammatory effects could cause endotheliopathy and vascular dysfunction [142]. To address the existing knowledge gap, we investigated associations between SARS-CoV-2 specific humoral and cellular immune responses and long COVID symptoms. Previous studies on our cohort have suggested an association between peak convalescent spike IgG titers, neutralizing antibodies, and long COVID symptoms at 6 months[71]. At 12 months follow-up, higher spike IgG titers, and longitudinal IgG responses were significantly associated with dyspnea that persisted at 6 and 12-months after infection, but not with other long COVID symptoms. Our findings support a multifactorial pathogenesis of long COVID. More severe disease has been associated with more potent SARS-CoV-2 antibody responses. More pronounced initial viral inflammatory responses in lung tissue, resulting in higher antibody responses, could confer with more long-term damage to the lung tissue, and thus dyspnea. Spike-specific antibody responses measured in the systemic circulation were not associated with fatigue or central nervous system (CNS) symptoms like memory problems or cognitive impairment. The potential role of antibodies in long COVID is currently unknown. Antibodies do not flow freely over the blood-brain barrier, but the barrier permeability to antibodies increases with inflammation. Circulating factors associated with long COVID CNS pathology could be better assessed in CNS fluid, where researchers previously have found both increased cytokine levels and abnormal antibody banding

patterns associated with cognitive sequelae after COVID-19[298,299]. As a measurement of the magnitude of spike-specific immunity, we used T-cell Receptor sequencing, and the reciprocal SARS-CoV-2 specific T-cell breadth (relative frequency of different T-cell clonal subtypes) and depth (the relative quantity of each clonal subtype). As part of an exploratory hypothesis generating investigation, we found associations between the SARS-CoV-2 spike-specific $CD4^+$ T cell depth at 6 months and both dyspnea and a higher number of persisting symptoms at 12 months follow-up. These finding could lend support to other studies which have found aberrant T-cell responses associated with long COVID[300]. The spike-specific clonal depth may reflect the extensive immune stimulations that drives T-cell proliferation, generating a higher number of circulating T-cells over time. Through antigen-driven cell-interactions and cytokine stimulation this may cause tissue damage and disruption in cell signal homeostasis. We did not adjust for initial disease severity in our analysis due to lack of information in our home isolated cases, but it may be associated with the magnitude of T-cell response, as this relationship has been demonstrated by others, and thus a confounder for the relationship between T-cell responses and long COVID[301].

Recognizing the causes of long COVID may have implications beyond COVID-19 related sequelae. Long-term complications after viral infection are not a new phenomenon [302,303]. A large body of evidence exists for the role of the viral infection in chronic fatigue syndrome development[137]. Under normal circumstances, it is difficult to identify the potential associations between mild viral respiratory infection and background fatigue symptoms in the population due to extensive confounding and few efforts to investigate the underlying pathophysiological mechanisms behind symptom development. A massive focus on long COVID research, may contribute to findings that are applicable to long term fatigue that is not linked to COVID-19.

Also, inevitably, due to the focus and new knowledge, an increased focus on long-term complications will be important whenever we find ourselves in the next pandemic.

Conclusion

This thesis covers important aspects in the chain of care of patients infected with seasonal influenza and SARS-CoV-2.

By comparing a molecular POCT for influenza with bed-side localization in the ED, to laboratory-based diagnostics, we found that POCT was associated with more rapid diagnosis, correct prioritization of isolation rooms and shorter hospital length-of-stay compared to laboratory-based testing. We found that overall, antiviral prescribing was reserved to patients with a positive influenza test, and that half of patients who received antivirals commenced treatment within 6 hours of admission regardless of testing procedure used.
Use of POCT was not associated with less antibiotic use as hypothesized. This may be due to differences in hospital practices for which we could not adjust, or that the two tests compared where both rapid. Our findings suggest that there are clinical and logistical benefits of using influenza POCT in the ED, but that other measures should be considered to counteract antibiotic overuse.

Furthermore, by comparing antibiotic use amongst hospitalized COVID-19 patients in 2020 to the hospitalized influenza patients one year prior, we found that less antibiotics were prescribed to COVID-19 patients. Although COVID-19 patients received more broad-spectrum antibiotics, prescription practices became more restricted from the first to second pandemic wave. Our findings suggest that it is possible to change antibiotic prescriptions practices rapidly when the focus is on novel diagnostics and treatment. We propose that clear communication of antibiotic guidelines and attentiveness amongst clinicians towards changing treatment and knowledge of the new viral disease, could be important contributors to this change. This could have implications for the development of antibiotic stewardship strategies for other respiratory diseases.

When studying long-term complications after home-isolation for COVID-19 during spring 2020, we found that long-term symptoms affected nearly half of infected cases 12 to 18 months after acute infection. At 12 months post-infection, COVID-19 cases had significant excess risk of fatigue, memory problems, concentration problems and

dyspnea, compared to seronegative time-period- and age-matched seronegative controls. Few cases described their symptoms as severe. Higher peak spike IgG antibody titers were associated with increased chance of dyspnea and multiple symptoms at 12 months. Regarding cellular immune responses, we found that spike-specific clonal depth of $CD4^+$ T-cells at 6 months, was associated with dyspnea at 12 months, suggesting that infection induced immune responses are linked to long COVID pathogenesis. Our findings illustrate the slow resolution of long COVID symptoms, and the need for long-term follow-up after COVID-19.

Future perspectives

Historically, millions of people have succumbed to influenza during pandemics. However, most fatalities occur during the regular inter-pandemic influenza seasons. Currently, SARS-CoV-2 is responsible for the deadliest infectious disease outbreak during the last century.

Evidence suggests that SARS-CoV-2 will continue to circulate in the human population and find ways to escape newly established immunity. This year we have observed a small, but delayed influenza outbreak in Norway, and increased occurrence of other respiratory viruses coinciding with the removal of social restrictions used to control the ongoing pandemic. Co-circulation of SARS-CoV-2 and other viral pathogens such as influenza, rhinovirus, other coronaviruses, and respiratory syncytial virus (RSV) will pose new clinical challenges in terms of diagnostics and treatment, and we must combine pre-pandemic experience with newly gained knowledge when dealing with a novel virus. Our comparisons and investigations of clinical management in influenza and COVID-19, suggest that important lessons from the COVID-19 pandemic are transferrable to influenza patient care and vice versa.

Prior to the SARS-CoV-2 pandemic, we have demonstrated clinical and patient management benefits associated with the use of influenza POCT in the ED for diagnosis of influenza, in patients hospitalized with respiratory symptoms during a regular influenza epidemic in Norway. When passing the current state of emergency, it is important to emphasize the relevance of these findings when health care services return to their pre-pandemic activity levels. Rapid hospital diagnosis of respiratory viral infections has huge clinical relevance in terms of prompt treatment and isolation interventions, and distinguishing between influenza, COVID-19 and other ILI respiratory viral infections will be crucial to guide future patient care. The use of COVID-19 antigenic tests has revolutionized possibilities of patient home-management and provided a rapid test solution for primary health care and rural areas. However, the added logistical benefit of molecular platforms connected to medical e-

records are important in the hospital setting, and facilitates communication of results, multiple pathogen testing and the surveillance of recorded cases. With the possible co-circulation of a plethora of respiratory viruses and newly developed targeted treatments, future studies should focus on the impact of multi-pathogen molecular POCT including respiratory viral pathogens with clinical implications, like SARS-CoV-2, influenza and RSV.

We found less early antibiotic use in COVID-19 patients than in influenza patients, despite rapid influenza diagnosis, and timely decrease of antibiotic use in COVID-19 patients from the first to second pandemic wave. This suggest that inter-pandemic factors, such as increased attention towards current guidelines, few detections of co-pathogens outside ICU, and clinical experience, have contributed to reduced antibiotic prescriptions in patients with COVID-19. Acknowledging this unique position of increased attention to the SARS-CoV-2 virus and novel diagnostics and treatments, may inform strategies to improve awareness of antibiotic stewardship in other respiratory viral infections.

There is still a knowledge gap on how the use of molecular POCT can aid in optimizing antibiotic use in the hospital setting. Future research should evaluate the dual effect of molecular POCT and diagnostic biomarkers or algorithms that are helpful to rule our bacterial pathogens, to improve accuracy of antibiotic treatment.

Optimizing treatment for COVID-19 patients may have implications beyond the acute disease course, as long-term sequelae spanning months after the original infection can occur after both severe and non-severe acute infection. At present, we have only just begun to understand the pathophysiological mechanisms behind these complications, commonly termed long COVID. Multiple factors are involved, and both host inflammation, and direct consequences of viral damage are suggested to play a role in the development of long COVID. Our study indicates a role of specific immunity in the pathogenesis of long COVID. The high proportion of non-severe COVID-19 cases that develop long-term symptoms highlights the need for treatment intervention outside

hospital. More particularly, data is urgently needed on the role of vaccination in protection against long COVID. Treatments that target viral replication could supplement immunomodulatory agents and future studies should investigate the effect and safety of acute phase antivirals, immunomodulatory agents, or both, in home-isolated as well as hospitalized COVID-19 patients to prevent the development of long COVID.

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Papers I-III

I

The Journal of Infectious Diseases

MAJOR ARTICLE

Point-of-Care Influenza Testing Impacts Clinical Decision, Patient Flow, and Length of Stay in Hospitalized Adults

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Background. Influenza is difficult to distinguish clinically from other acute respiratory infections. Rapid laboratory diagnosis can help initiate early effective antiviral treatment and isolation. Implementing a novel point-of-care test (POCT) for influenza in the emergency department (ED) could improve treatment and isolation strategies and reduce the length of stay (LOS).

Methods. In a prospective, controlled observational cohort study, we enrolled patients admitted due to acute respiratory illness to 2 public hospitals in Bergen, Norway, one using a rapid POCT for influenza (n = 400), the other (n = 167) using conventional rapid laboratory-based assay.

Results. Prevalence of influenza was similar in the 2 hospitals (154/400, 38% vs 38%, 63/167; $P = .863$). Most patients in both hospitals received antiviral (83% vs 81%; $P = .703$) and antibiotic treatment (72% vs 62%; $P = .149$). Isolation was more often initiated in ED in the hospital using POCT (91% vs 80%; $P = .025$). Diagnosis by POCT was associated with shorter hospital stay; old age, diabetes, cancer, and use of antibiotics, particularly broad-spectrum antibiotics, were associated with prolonged stay.

Conclusions. POCT implementation in ED resulted in improved targeted isolation and shorter LOS. Regardless of POCT use, most influenza patients received antivirals (>80%) and antibiotics (>69%).

Keywords. influenza; point-of-care test; hospitalized adults; molecular assay; length of stay; antibiotics; isolation; neuraminidase inhibitor.

Acute (lower) respiratory tract infections are a leading cause of morbidity and mortality worldwide [1]. Influenza is one of the most commonly recognized viral pathogens [2, 3], and globally responsible for a significant burden on health care resources both in primary care and in hospitals. Influenza infection alone is estimated to cause up to 650 000 deaths annually [4–6]. Influenza may also pave the way for secondary bacterial pneumonia by reducing the effectiveness of alveolar macrophages [7, 8].

Clinically, influenza is difficult to distinguish from other respiratory tract infections of viral and bacterial origin [9]. Studies on the etiology of community acquired pneumonia (CAP) in hospitalized patients have found viral etiology to be common, as well as viral-bacterial coinfection, the last accounting for up to one-third of CAP infections [3, 10–14]

Initial misdiagnosis in hospital negatively impacts early treatment. In severe influenza disease, early onset of treatment with neuraminidase inhibitors (NAIs) is essential, as it reduces mortality, influenza-related pneumonia [15], and length of stay (LOS) in hospital [16–20]. Influenza diagnostics by laboratory-based reverse transcriptase polymerase chain reaction (RT-PCR) have long turn-around times (TATs) [21–23], limiting early NAI treatment. Antigen detection-based tests are limited by their low sensitivity. New point-of-care tests (POCTs) based on molecular assays like RT-PCR or similar nucleic acid amplification technologies generate results with high sensitivity and specificity in less than 30 minutes and the analysis can be performed at the bedside [24]. Their simplicity makes new POCTs easy to use in the emergency department (ED), outside laboratory facilities. Rapid tests in hospitals have logistical benefits and could potentially reduce the use of antibiotics [25]. Compared to traditional RT-PCR tests, studies suggest that POCT influenza diagnosis improves use of isolation, antibiotic stewardship, and antiviral use, reduces LOS, and results in overall health care savings [26–31]. However, these results need comparison to rapid laboratory-based influenza diagnostics. Upon the reorganization of the influenza diagnostic pathway in our hospital we hypothesized that the introduction of a novel

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POCT for influenza would improve logistics, NAI prescription, and overall antibiotic use during an influenza epidemic.

METHODS

Study Design

We conducted a prospective controlled observational clinical cohort study in 2 referral hospitals in Bergen, Norway, during the influenza season of 2018–2019. The 2 neighboring hospitals used different rapid influenza tests, Haukeland University Hospital used a novel POCT (hospital 1) and neighboring Haraldsplass Diaconess Hospital served as a control using a laboratory-based test (hospital 2). The inclusion period was December 2018 to March 2019, during the peak of influenza activity in Norway. The study was approved by the Regional Committee for Medical and Health Research Ethics (REK) in Western Norway (REK number 2018/1772), and the data collection conducted in accordance with the Declaration of Helsinki's principles of Good Clinical Practice (GCP). All enrolled patients provided written, informed consent. Adult patients fulfilling inclusion criteria were prospectively enrolled in the ED when admitted to hospital. The 2 study hospitals are cooperating teaching facilities providing equal services within the field of general surgery and internal medicine. They serve the unselected public in predefined geographical areas of Bergen. The hospitals differ in size and subspeciality expertise with hospital 1 being a referral and local hospital, and hospitals 1 and 2 serving public emergency care services for 500 000 and 145 000 people, respectively.

Inclusion Criteria

Eligible patients were adults (aged ≥ 18 years) referred to the ED, and able to provide informed consent. Next of kin could provide consent, enabling inclusion of severely ill patients and elderly patients with cognitive impairment. Patients were prospectively included from the time of admission or within 2 days if ED inclusion was not feasible. Inclusion criteria were symptoms of acute respiratory illness lasting ≤7 days and 2 or more of the following symptoms: temperature ≥ 37.5°C, malaise, exacerbation of chronic obstructive pulmonary disease or asthma, dyspnea, sore throat, cough, myalgia, arthralgia, headache, or gastrointestinal symptoms.

Acute respiratory illness was defined as an episode of influenza-like-illness or upper or lower respiratory tract infection including CAP. Exclusion criteria was previous inclusion in the study.

Molecular Diagnostic Assays

In hospital 1, the available influenza POCT was Abbott ID NOW Influenza A and B 2, an isothermal nucleic acid amplification-based assay targeting the polymerase basic gene 2 (PB2) for influenza A virus and polymerase acidic gene (PA) for influenza B virus. Test samples were obtained from the nostril. The manufacturers TAT was reported to be less than 15 minutes. The control, laboratory-based influenza test in hospital 2 was the Cepheid GeneXpert II, using the Xpert Xpress Flu/RSV and Xpert Flu test kit, real-time RT-PCR–based assays targeting influenza A matrix protein, PB2 and influenza A acidic proteins (PA), and influenza B matrix and nonstructural (NS) proteins. The assay provided results within 20 minutes with the Xpress test kit and 75 minutes for negative results with the ordinary Flu test kit using a nasopharyngeal swab for sampling. The producers report high sensitivities (81.6% and 94.9%, respectively, for POCT and the Xpert assay) and specificity (94.0% and 100%, respectively) when compared to reference standard RT-PCR [32, 33]. Between 10 and 18 March 2019 there was a shortage of the GeneXpert influenza/RSV tests (n = 12), and the Eplex Respiratory pathogen panel from GenMark Dx was performed instead.

Research Staff

GCP-trained medical staff and students identified and included study patients admitted in the ED during the study period Monday to Friday 09:00–18:00. Outside these hours, consultants with ED duty included a small number of patients.

Study Procedures

Patients received standard clinical care, with the responsible ED physician deciding if a nasopharyngeal test and a POCT influenza test was indicated, making the patient eligible for study inclusion. In hospital 1 the influenza POCT was generally supplemented by a laboratory-based RT-PCR including a broader respiratory panel (available after 24–48 hours; Supplementary Table 2). This was the exception in hospital 2. Baseline clinical and demographic characteristics were collected upon inclusion; subsequent clinical data was collected retrospectively from hospital records.

Narrow-spectrum antibiotics included phenoxy- and benzylpenicillins, aminopenicillins, and aminoglycosides. Broad-spectrum antibiotics included extended-spectrum agents such as piperacillin-tazobactam, second- and third-generation cephalosporins, quinolones, and carbapenems [34]. Resistance-driving antibiotics also included clindamycin, glycopeptide antibiotics, macrolides, and linezolid [35].

Statistical Analysis

Proportions of patients were compared by χ^2 test or Fisher exact test, while continuous variables were compared across groups using Wilcoxon rank-sum test (Mann-Whitney) or Student *t* test as appropriate. A *P* value ≤ .05 was considered significant. Multivariable analyses of explanatory factors associated with POCT was done using binary logistic regression. Outcome variables duration of hospital stay and duration of antibiotic use were assessed by Kaplan-Meyer survival analysis, log-rank tests, and Cox proportional hazards regression. Data analysis was performed in R (R Core Team; http://www.R-project.org/), IBM SPSS statistics version 24, and Prism version 8.1.2 (GraphPad Software).

RESULTS

Baseline Characteristics

Between December 2018 and March 2019, 625 patients were recruited (Figure 1A). Of these, 442 and 183 patients were recruited at hospitals 1 and 2, respectively. One patient withdrew from the study, and 57 patients (not fulfilling inclusion criteria) were subsequently excluded from analysis.

The age distribution of patients was similar at the 2 hospitals (Table 1 and Figure 1B), although there was a small but significant median age difference of 4 years, with older patients in hospital 2. Influenza was confirmed in 154 (38%) and 63 (38%) of patients in hospitals 1 and 2, respectively. The majority of patients had one or more comorbidities (85% and 90% in hospitals 1 and 2, respectively; Table 1). The most common

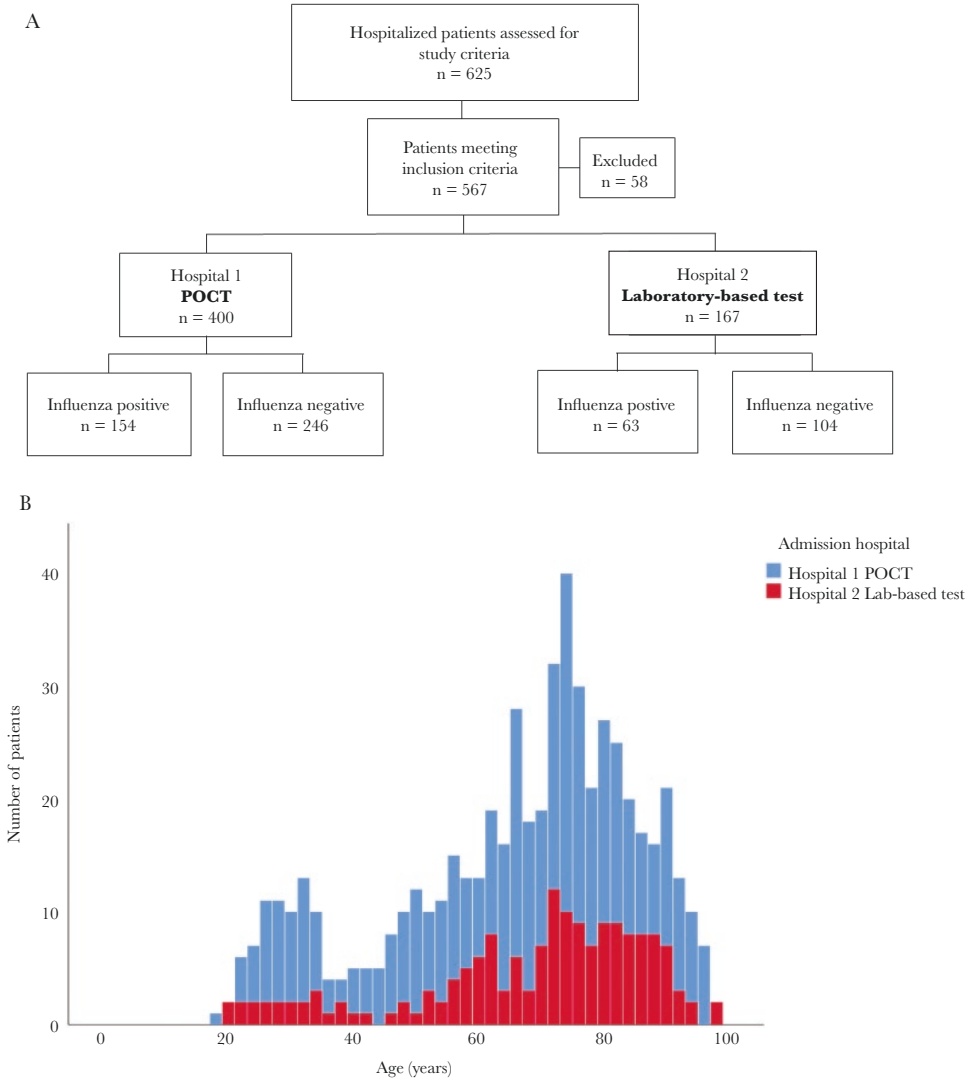

Figure 1. *A*, Study design. The study was designed as a prospective observational controlled study. All patients were tested for influenza upon admission. Participants were enrolled from 2 university referral hospitals in Bergen, Norway, between December 2018 and March 2019. The 2 hospitals differed in their rapid influenza diagnostic pathways. Forty-two patients at hospital 1 and 15 patients at hospital 2 were excluded as they did not fulfil inclusion criteria. One patient at hospital 2 withdrew from the study. *B*, Age distribution was similar in intervention hospital 1 and control hospital 2, with a peak of patients with ages between 65 and 70 years. Abbreviation: POCT, point-of-care test.

Table 1. Baseline Patient Characteristics

Characteristics	Hospital 1 POCT (n = 400)	Hospital 2 Laboratory-Based Test (n = 167)	*P* Value
Age, y, median (IQR)	68 (51–79)	72 (60–82)	**.040**
Sex			
Female	193 (52)	86 (52)	.956
Male	207 (48)	81 (48)	
Influenza vaccine			
2018	185 (47)	91 (56)	.051
Last 5 years	256 (65)	115 (71)	.183
Triage score upon admittance, mean (SD)[a]	1.6 (0.73)	1.6 (0.87)	.795
Need for respiratory support			
Oxygen therapy	160 (40)	77 (46)	.186
Noninvasive	49 (12.3)	10 (6.1)	**.029**
Invasive	7 (2)	0 (0)	.085
Comorbidities			
None	61 (15)	16 (10)	.072
Cardiovascular disease	156 (39)	81 (49)	**.032**
Respiratory disease	179 (45)	87 (52)	.110
Diabetes mellitus	60 (15)	35 (21)	.083
Hypertension	137 (34)	72 (43)	**.046**
Renal disease	65 (16)	26 (16)	.840
Liver disease	12 (3)	0 (0)	**.024**
Neurological disease	92 (23)	45 (27)	.317
Obesity (BMI > 30)	86 (22)	30 (18)	.341
Active cancer	49 (12)	21 (13)	.895
Immunocompromised[b]	60 (15)	24 (15)	.869
Pregnancy	6 (3)	1 (1)	.366
Other comorbidities[c]	122 (31)	57 (34)	.407
Current smoker			
Yes	66 (17)	30 (18)	.708
No[d]	330 (83)	137 (82)	
Additional diagnostics			
Influenza test	400 (100)	167 (100)	NS
Positive test	154 (39)	63 (38)	.863
Respiratory panel	325 (81)	34 (20)	**<.001**
Positive pathogen other than influenza	51(16)	14 (41)	**.002**
Blood culture	321 (81)	152 (91)	**.002**
Positive culture	24 (7)	10 (7)	.724
Urine pneumococcal antigen	165 (42)	-	
Positive culture	16 (10)	-	-
Chest X-ray	341 (86)	155 (93)	**.021**
Positive infiltrate	118 (35)	48 (31)	.426
Duration of symptoms upon admittance, d, median (IQR)	3 (1–4)	2 (1–4)	**.011**

Data are No. (%) except where indicated. *P* values are based on the χ_2 test for differences in proportions for binary data and Mann-Whitney *U* test or Student *t* test as appropriate for continuous data. Bold font indicates a significant difference as defined by *P* value < .05.

Abbreviations: BMI, body mass index; DMARD, disease-modifying antirheumatic drug; IQR, interquartile range; POCT, point-of-care test; SATS, South African Triage Scale.

[a]Triage score: the Norwegian SATS emergency prioritization score is based on SATS and additional investigation. The score is presented as a color code. For calculation purposes, green = 0, yellow = 1, orange = 2, and red = 3

[b]The definition of immunocompromised patient includes:

1. Patients on regular oral prednisolone from 5 mg/d or prolonged courses (>10 d of elevated doses equivalent to 20 mg oral prednisolone or more), n = 28.
2. Patients treated with prednisolone in combination with DMARDs or biologic DMARDs, n = 16.
3. Patients receiving chemotherapy, n = 11.
4. Patients with organ transplants and immunosuppressive treatment, n = 7.
5. Patients on immune suppressive drugs for inflammatory bowel disease, n = 3.
6. Patients with acquired or innate immunodeficiencies, n = 8.
7. Other causes, n = 11.

[c]Other autoimmune diseases, rheumatological diseases, drug addiction, etc.

[d]Includes previous smokers.

were respiratory disease, cardiovascular disease, and hypertension, the latter 2 significantly more prevalent in hospital 2 (Table 1). While influenza-positive patients had less frequently comorbidities (79.6% vs 90.8%, $P < .001$) and fewer concomitant comorbidities (mean 2.0 vs 2.7, $P < .001$, Student t test), they reported a higher symptom load than the influenza-negative patients (mean 6.4 vs 5.2 symptoms, $P < .001$). The most common symptoms were cough, temperature >37.5°C, malaise, and dyspnea (Supplementary Table 1).

Influenza-positive patients had shorter LOS than influenza-negative patients in both hospitals. Interestingly, intervention hospital 1 had shorter LOS (3 versus 4 days; Table 2), despite patients having a longer duration of symptoms before hospitalization (3 vs 2 days; Table 1). Oxygen therapy was provided to 40% and 46% of patients in hospitals 1 and 2, respectively ($P = .176$). The proportion of patients receiving noninvasive respiratory support was significantly higher in hospital 1 (12.3%) than in hospital 2 (6.1%, $P = .029$; Table 1), regardless of influenza status. Overall, only 7 patients needed ventilator treatment, all in hospital 1. Of these, 4 were influenza positive and all had comorbidities. None were pregnant and only one had received influenza vaccination.

Both hospitals use the Norwegian adaptation of South African Triage Scale (SATS) to assess patients according to severity of symptoms and signs in the ED. Patients are scored with a color code upon arrival with increasing severity from green, yellow, orange, to red (Supplementary Figure 1). The proportion of patients with combined mild (green, yellow) versus moderate/severe (orange, red) SATS scores were equal between the 2 hospitals.

Additional nasopharyngeal RT-PCR diagnostics for respiratory pathogens was performed in 81% and 20% of patients in hospitals 1 and 2, respectively. In hospital 1, the laboratory-based in-house RT-PCR yielded results within 24–48 hours (Supplementary Table 2), and detected 9 additional influenza cases. Altogether, 16% of conducted RT-PCR tests in hospital 1 detected respiratory pathogens other than influenza; comparably, hospital 2 detected other pathogens in 41% of patient samples. However, sampling in hospital 2 was restricted to those with a negative influenza test and suspicion of viral etiology.

Table 2. Clinical Outcomes of the Patients

Clinical Outcomes	Hospital 1 POCT (n = 400)	Hospital 2 Laboratory-Based Test (n = 167)	2-Sided *P* Value
Length of hospital stay, d, median (IQR)	3 (1–5)	4 (2–7)	**<.001**
Influenza positive	2 (1–4)	3 (1–6)	.075
Influenza negative	3 (2–5)	4 (2–7)	**<.001**
Initial isolation	159 (40)	59 (37)	.507
Influenza positive	140 (91)	47 (80)	**.025**
Influenza negative	18 (7)	12 (12)	.175
30-Days mortality	13 (2)	4 (3)	.204
Influenza positive	3(2)	1 (2)	.512
Influenza negative	10 (4)	3 (3)	.327
Antibiotics all treatment	303 (76)	122 (73)	.469
Influenza positive ($n^a = 154$, $n^b = 63$)	110 (72)	39 (62)	.149
Influenza negative ($n^a = 246$, $n^b = 104$)	193 (79)	83 (80)	.777
Antibiotics, broad spectrum and resistance driving	131 (43)	41 (34)	**.047**
Influenza positive ($n^a = 110$ $n^b = 39$)	40 (36)	14 (36)	.958
Influenza negative ($n^a = 193$, $n^b = 83$)	91 (47)	26 (32)	.015
Antibiotics, all treatment, duration, d, mean (SD)	7.8 (5.3)	6.9 (5.6)	.120
Influenza positive ($n^a = 110$, $n^b = 39$)	7.3 (4.9)	4.5 (4.2)	**.002**
Influenza negative ($n^a = 193$, $n^b = 83$)	8.1 (5.4)	7.9 (5.9)	.877
Antibiotics, all treatment, duration, d, median (IQR)	7 (5–10)	6 (3.5–9)	.120[c], **.046**[d]
Influenza positive	7 (5–9)	3.5 (1–8)	**.002**[c,d]
Influenza negative	8 (6–10)	7 (6–10)	.877[c], .621[d]
NAI treatment total	136 (34)	54 (32)	.673
Influenza positive ($n^a = 154$, $n^b = 63$)	128 (83)	51 (81)	.703
Influenza negative ($n^a = 246$, $n^b = 104$)	8 (3)	3 (3)	.847
Time from triage to NAI treatment, h, mean (SD)	6.2 (7.9)	6.2 (6.0)	.985[c], .189[d]
Time from triage to NAI treatment, h, median (IQR)	4 (2–7)	5 (3–7.5)	.933[c], .189[d]

Data are No. (%) except where indicated; median (IQR) or mean (SD) as appropriate according to the distribution of data. *P* values were calculated using appropriate comparison: χ^2 for binary categorical variables and Mann-Whitney test or Student t test for continuous variables. Bold font indicates a significant difference as defined by *P* value < .05.

Abbreviations: IQR, interquartile range; NAI, neuraminidase inhibitor; POCT, point-of-care test.

[a]Hospital 1.

[b]Hospital 2.

[c]t test *P* value.

[d]Mann-Whitney *P* value.

Use of Isolation

In both hospitals a positive influenza test result was strongly associated with patient isolation. Nonetheless, a significantly higher proportion of influenza-positive patients were isolated immediately in the ED in hospital 1 using POCT (91%) than in hospital 2 (80%, $P = .025$). Isolation was largely restricted to influenza-positive patients, with only 7% and 12% of influenza-negative patients being isolated in hospitals 1 and 2, respectively. These patients were commonly isolated upon exhibiting gastrointestinal symptoms, not because of suspicion of contagious respiratory viral illness.

Antibiotic Treatment

Similar percentages of patients received antibiotics in the 2 hospitals, 76% (n = 303) in hospital 1 and 73% (n = 122) in hospital 2 ($P = .469$; Table 2). Overall, significantly fewer influenza patients compared to noninfluenza patients were prescribed antibiotics (69% vs 79%, $P = .008$). Interestingly, the length of antibiotic treatment in influenza patients was significantly shorter in hospital 2 compared to hospital 1 (median 3.5 vs 7 days, $P = .002$; Figure 2). Rapid antibiotic discontinuance (termination of initiated treatment the following day) was observed in 42.1% of influenza patients in hospital 2 compared to only 15.6% in hospital 1 ($P = .001$). In the influenza-negative patients, antibiotic treatment was terminated the following day in only 8% and 6% of patients in hospitals 1 and 2, respectively ($P = .651$). Of the 65 patients with a positive RT-PCR for respiratory pathogens other than influenza, 79% received antibiotics and no trend of antibiotic discontinuance upon other viral diagnosis was observed.

Neuraminidase Inhibitor Treatment

Importantly, the majority of influenza patients received NAI treatment (83% and 81% in hospitals 1 and 2, respectively). Mean treatment duration was 4.5 days and was comparable between the hospitals. Influenza patients were more likely to receive NAIs if symptom duration did not exceed 48 hours prior to hospitalization (89% vs 77%, $P = .023$). The use of NAI treatment in influenza-negative patients was low (3%) and treatment duration shorter (mean 3.3 days), suggesting that treatment was ended upon conclusive laboratory diagnostics. The mean time from triage in the ED to NAI treatment in influenza patients was equally rapid, 6.2 hours in both hospitals ($P = .985$; Table 2), with 69% receiving early NAIs (within 6 hours).

Mortality

The overall 30-day mortality rate was 2% among the influenza-positive patients in both hospitals, and 4% versus 3% for the influenza-negative patients in hospitals 1 and 2, respectively.

Turn-around Times

The mean time from swabbing to test result was 15 minutes for the POCT and 119 minutes (102–136 minutes) for the laboratory-based influenza test, and the mean difference between the time from test to result was 104 minutes (95% confidence interval [CI], 87–121 minutes) between the 2 hospitals. Furthermore, the effective TATs from triage to test result was 69 minutes (SD, 190 minutes) in hospital 1 for the POCT and 269 minutes (SD, 308 minutes) for the laboratory-based influenza test in hospital 2. To conclude, our results showed a mean time difference from triage to test result of 200 minutes (95% CI, 146–254 minutes).

Duration of Hospital Stay

We found that patients diagnosed with POCT, that is those admitted to hospital 1, had significantly shorter median duration of hospital stay than those admitted to hospital 2 (3 days vs 4 days, $P < .001$; Table 2 and Figure 3). Diagnosis by POCT was the only factor associated with shorter duration of hospital stay in both multivariable and univariable analysis (Table 3).

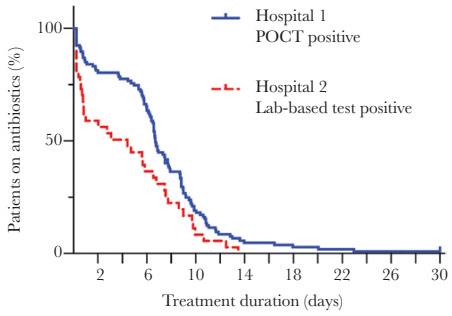

Figure 2. Duration of antibiotic treatment. Kaplan-Meyer curve demonstrating the duration of antibiotic treatment in influenza-positive patients admitted to hospitals 1 and 2. Log-rank test *P* value = .012. Antibiotic treatment length was set to a minimum of 0.25 days and prolonged antibiotic treatment >30 days was censored after 30 days for calculation purposes.

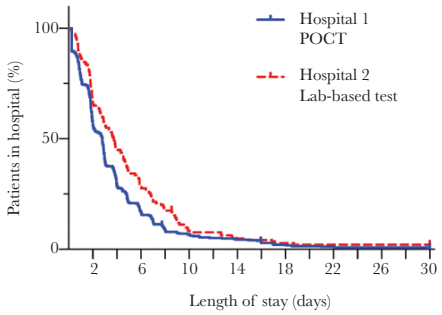

Figure 3. Length of hospital stay. Kaplan-Meyer curve demonstrating the overall length of stay of patients in hospitals 1 and 2. Log-rank test *P* value = .002. Hospital length of stay was set to a minimum of 0.25 days and prolonged hospital stay >30 days was censored after 30 days for calculation purposes.

Table 3. Risk Factors for Prolonged Hospital Stay

Predictors	n	Length of Stay, d, Median (IQR)	Univariable Analysis *P* Value, Wilcoxon[a]	Multivariable Analysis *P* Value, Cox[c]
Overall	566	3 (2–5)	NA	NA
Demographics				
Age				
Older, ≥ 70 y	281	4 (2–6)	**<.0001**	.0975
Younger, < 70 y	285	2 (1–4)		
Sex				
Female	274	3 (2–5)	.9155	.1967
Male	292	3 (2–5)		
Vaccination				
Influenza vaccine				
Vaccinated any time	371	3 (2–5)	**.0355**	…
Never vaccinated	183	3 (1–5)		
Influenza vaccine 2018				
Vaccinated 2018	276	3 (2–6)	**.0032**	.7753
Not vaccinated 2018	278	3 (1–5)		
Influenza vaccine 2017				
Vaccinated 2017	251	3 (2–5.5)	**.0497**	…
Not vaccinated 2017	300	3 (1–5)		
Influenza vaccine 2016				
Vaccinated 2016	229	3 (2–5)	.0621	…
Not vaccinated 2015	322	3 (1–5)		
Influenza vaccine 2015				
Vaccinated 2015	201	3 (2–6)	**.0130**	…
Not vaccinated 2015	348	3 (1–5)		
Risk factors				
Any underlying disease				
Present	486	3 (2–6)	**<.0001**	…
Absent	76	1 (0–3)		
Cardiovascular disease				
Present	236	3.5 (2–6)	**<.0001**	.4948
Absent	329	2 (1–5)		
Hypertension				
Present	207	3 (2–6)	**.0015**	.1483
Absent	359	3 (1–5)		
Respiratory disease				
Present	266	3 (2–6)	**.0026**	.9522
Absent	300	2 (1–5)		
Smoking				
Current	95	4 (2–8)	**.0001**	.9249
Previously or never	467	3 (1.5–5)		
Obesity, BMI >30				
Present	177	3 (2–5)	.1993	…
Absent	300	3 (1–5)		
Diabetes mellitus				
Present	95	4 (2–8)	**.0001**	**.0107**
Absent	471	3 (1.5–5)		
Renal disease				
Present	90	3 (2–6)	.0576	…
Absent	476	3 (2–5)		
Liver disease				
Present	11	3 (1–5.5)	.6187	…
Absent	555	3 (2–5)		
Neurological disease				
Present	136	3 (2–6)	**.0276**	.3749
Absent	430	3 (1.25–5)		
Immunodeficiency				
Present	86	3 (2–5)	.2199	…

Table 3. Continued

Predictors	n	Length of Stay, d, Median (IQR)	Univariable Analysis *P* Value, Wilcoxon[a]	Multivariable Analysis *P* Value, Cox[c]
Absent	479	3 (2–5)		
Cancer				
Present	68	4.5 (2.75–7)	**<.0001**	**.0153**
Absent	497	3 (1–5)		
Other comorbidities				
Present	177	3 (2–6)	.2779	…
Absent	388	3 (2–5)		
Status on admission				
Duration of symptoms				
≥ 3 d	285	3 (2–5)	.8563	…
< 3 d	192	3 (2–5)		
Triage score				
2–3	313	3 (2–6)	**.0004**	.7793
0–1	215	2 (1–5)		
Diagnostics				
Use of POCT				
POCT, hospital 1	399	3 (1–5)	**<.0001**	**<.0012**
Laboratory-based test, hospital 2	167	4 (2–7)		
Influenza test result				
Positive	217	2 (1–5)	**<.0001**	.7549
Negative	349	3 (2–6)		
Blood culture				
Pathogen recovered	34	4 (2.25–7.75)	**.0333**	.2215
No pathogen recovered	530	3 (2–5)		
Urine pneumococcal test				
Positive	16	3 (2–7.5)	.2414	…
Negative	547	3 (2–5)		
Urine culture				
Pathogen recovered	15	3 (2–4)	.9722	…
Negative or contaminated	174	3 (2–5)		
Chest X-ray				
Infiltrate	166	4 (2–6)	**<.0001**	.4300
No infiltrate	397	3 (1–5)		
Interventions				
Antimicrobial treatment				
Received	424	3.5 (2–6)	**<.0001**	<.2727
Not received	141	1 (1–3)		
Longer antimicrobial treatment				
>1 d	362	4 (2–6)	**<.0001**	**.0002**
≤ 1 d	51	2 (1–4)		
Broad-spectrum antibiotics				
Received	172	5 (2.75–8)	**<.0001**	**<..0001**
Not received	394	2 (1–4)		
Oseltamivir				
Received	191	3 (1.5–5)	.3695	…
Not received	372	3 (2–6)		
Steroids				
Received	217	3 (2–6)	**<.001**	.0781
Not received	348	3 (1–5)		

Potential risk factors for prolonged hospital stay assessed in univariable analysis using Wilcoxon rank-sum test, and in multivariable analysis by both Poisson regression and Cox proportional hazards analysis. n = 566 (1 patient excluded due to missing data regarding comorbidities). Bold font indicates a significant difference as defined by *P* value < .05.

Abbreviations: BMI, body mass index; IQR, interquartile range; NA, not applicable; POCT, point-of-care test.

[a]Wilcoxon rank-sum test.

[b]Poisson regression.

[c]Cox proportional hazards analysis.

Comorbidity with diabetes or malignancy, use of broad-spectrum antibiotics, and duration of antibiotic use >1 day was associated with prolonged duration of hospital stay. In univariable analysis, prolonged hospital stay was also associated with older age, smoking, hypertension, cardiovascular, pulmonary, and neurologic disease.

History of influenza vaccination was associated with prolonged stay in univariable analysis, while actually having a positive influenza test was associated with shorter stay. Use of antibiotics, particularly broad-spectrum antibiotics, was strongly associated with prolonged hospital stay. Severity on admission (SATS score), positive blood cultures, infiltrate on chest X-ray, and use of steroids were associated with prolonged stay in univariable analysis only.

DISCUSSION

Accurate and rapid laboratory diagnosis of influenza is essential to guide treatment and infection control. Clinical studies of the diagnostic accuracy of physician diagnosis of influenza report low sensitivity [9, 36].

This prospective, controlled clinical study is unique in studying the clinical and logistical effects of implementing a rapid influenza POCT in one hospital ED during the influenza season 2018–2019 and comparing it to a different rapid test, incurring specimen transport time, in the neighboring control hospital.

We found that use of POCT was associated with shorter LOS in both univariable and multivariable analysis. The finding that a history of influenza vaccination was associated with prolonged hospital stay is likely to have been because admissions for diseases other than influenza may be more severe and require longer treatment. Indeed, a positive test for influenza on admission was associated with shorter hospital stay. As expected, hospital stay was longer in older patient and those with underlying diseases, particularly diabetes and cancer. While triage severity of illness (SATS score) was similar in the 2 hospitals, it was associated with prolonged hospital stay within each hospital. The association between prolonged hospital stay and SATS score, positive blood cultures, infiltrate on chest X-ray, and use of antibiotics and steroids is not surprising as these factors all indicate more severe disease. While the particularly strong association with broad-spectrum antibiotic use could be attributed to severity of disease, it may reflect on other challenges such as risk of antibiotic-associated diarrhea and a lack of good peroral alternatives for tapering courses, both of which would lead to unnecessarily prolonged hospital stay. The interpretation of our results is limited by its observational character and by comparing 2 different hospitals. Hence, we cannot rule out that factors other than using POCT could explain the shorter LOS in hospital 1, including physicians' management preferences, discharge practices, bed occupancy rates, organization of patient flow, and complexity of patients' illnesses. Interestingly, hospital 1 is a referral hospital, which would be expected to increase rather than diminish the duration of hospital stay, but it receives the majority of patients as direct admissions. Importantly, both study hospitals had significantly shorter LOS compared to LOS reported in the global literature, despite older patients.

This study is unique in comparing 2 rapid tests. Others have not reported equally efficient TATs in both control and intervention groups; however, short TATs have been linked to improved antibiotic usage and early discharge. In our study, the POCT in hospital 1 was extremely rapid (15 minutes). The elimination of time-consuming test ordering and transport procedures lowered the threshold for rapid testing upon admission. In our cohort, 4/10 patients were regarded as mildly ill after the initial triage evaluation (Supplementary Figure 1) requiring further medical attention within 60 minutes. Interestingly, the median TAT from triage to test result of 69 minutes in hospital 1 shows that many patients had a rapid influenza POCT performed as part of the short initial triage assessment, despite a low initial SATS score. Early testing upon admission allowed incorporation of the influenza results into the ED clinician`s assessment, possibly influencing clinical management, emphasizing the importance of the close proximity of the test. The suggested benefits of rapid TATs are supported by Brendish et al's randomized controlled trial post hoc analysis on the impact of TAT on outcome with POCT, where they found a TAT below 1.6 hours was associated with improved clinical outcomes [37].

Our analysis confirmed a superiority in targeted use of isolation for influenza patients in hospital 1 where the new POCT was implemented, in agreement with previous findings [27]. The overall experience of implementing the rapid POCT was positive amongst health care workers and patients, and in line with the findings of a recent Dutch study, which demonstrated improved hospital patient flow after implementing an influenza POCT in the ED [26]. The use of POCT led to improved priorities for isolation facilities, and importantly avoiding prolonged unnecessary isolation of influenza-negative patients, which may save cost.

Antibiotic overuse due to the difficulties in diagnosis is common in adults with viral respiratory tract infections [38]. Bacterial coinfection is common with influenza [39], but antibiotic treatment is not indicated for viral infection alone. Furthermore, studies found that influenza-positive patients were more likely to receive treatment with antibiotics than with NAIs [27, 30]. Frequent prescription of antibiotics in both influenza-positive and -negative patients, without detection of bacteria, indicates that primary bacterial infection or coinfection is of great concern for the clinician. In our study, the presence of a rapid influenza POCT was not associated with a reduction in initial antibiotic prescription in patients with acute respiratory illness. Additional RT-PCR findings did not significantly change ongoing prescriptions. As influenza-positive patients presented to the ED with a high symptom load

(high SATS score), we speculate that the POCT result alone was insufficient for ED clinicians to rule out bacterial coinfection initially. However, antibiotic stewardship initiatives focus on reevaluating the choice of antibiotics after 24–72 hours. Consequently, we further investigated the effect of POCT on duration of antibiotic treatment. Our results demonstrated an earlier termination of antibiotics in influenza-positive patients in hospital 2, despite using the laboratory-based influenza test (Figure 2). This could be explained by differences in antibiotic prescribing culture and overall adherence to guidelines between the 2 hospitals, with perhaps the smaller hospital being better at antibiotic stewardship control. Furthermore, the small difference in TATs of the influenza POCT and the laboratory-based influenza test probably does not influence treatment choices from day 2 onwards. Hence, POCT could have greater impact in hospitals with higher antibiotic usage or standard laboratory-based RT-PCR yielding results in 24–48 hours.

Both hospitals exhibited high performance in targeted antiviral therapy, as NAIs were given to >80% of influenza-positive patients, and only 3% of influenza-negative patients. The mean symptom duration upon hospitalization was 2 to 3 days, comparable to the 2009 pandemic [40]. According to updated national guidelines, NAIs are recommended for influenza patients with a symptom duration <48 hours or when severely ill and in need of hospital admission. In severely ill influenza patients, NAIs have been shown to reduce morbidity and mortality even with later treatment onset [15, 17, 41]. NAIs are administered on the wards, not in the ED. However, our findings of a mean NAI treatment initiation only 6 hours after initial triage in both hospitals is encouraging, as rapid treatment is beneficial [15, 40, 42]. Time to NAI treatment in hospital 1, with POCT, could possibly be further shortened if NAIs were given in the ED.

Our study highlights the positive effects of a rapid influenza POCT in the ED on initial TATs, treatment decisions such as isolation procedures, initiation of antiviral therapy, and reduced LOS. Our findings support the implementation of POCT in the hospital setting. In light of the ongoing severe acute respiratory syndrome coronavirus 2 pandemic, there is currently an even greater demand for rapid and accurate feedback of test results, both regarding influenza and other respiratory pathogens. Randomized studies are needed to ascertain the benefits of using POCT. Future studies should aim to investigate the overall impact and cost-benefits from targeted use of isolation, and also the benefits of implementing molecular influenza diagnostics in primary health care facilities and outpatient clinics.

Supplementary Data

Supplementary materials are available at *The Journal of Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

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Potential conflicts of interest. All authors: No reported conflicts of interest. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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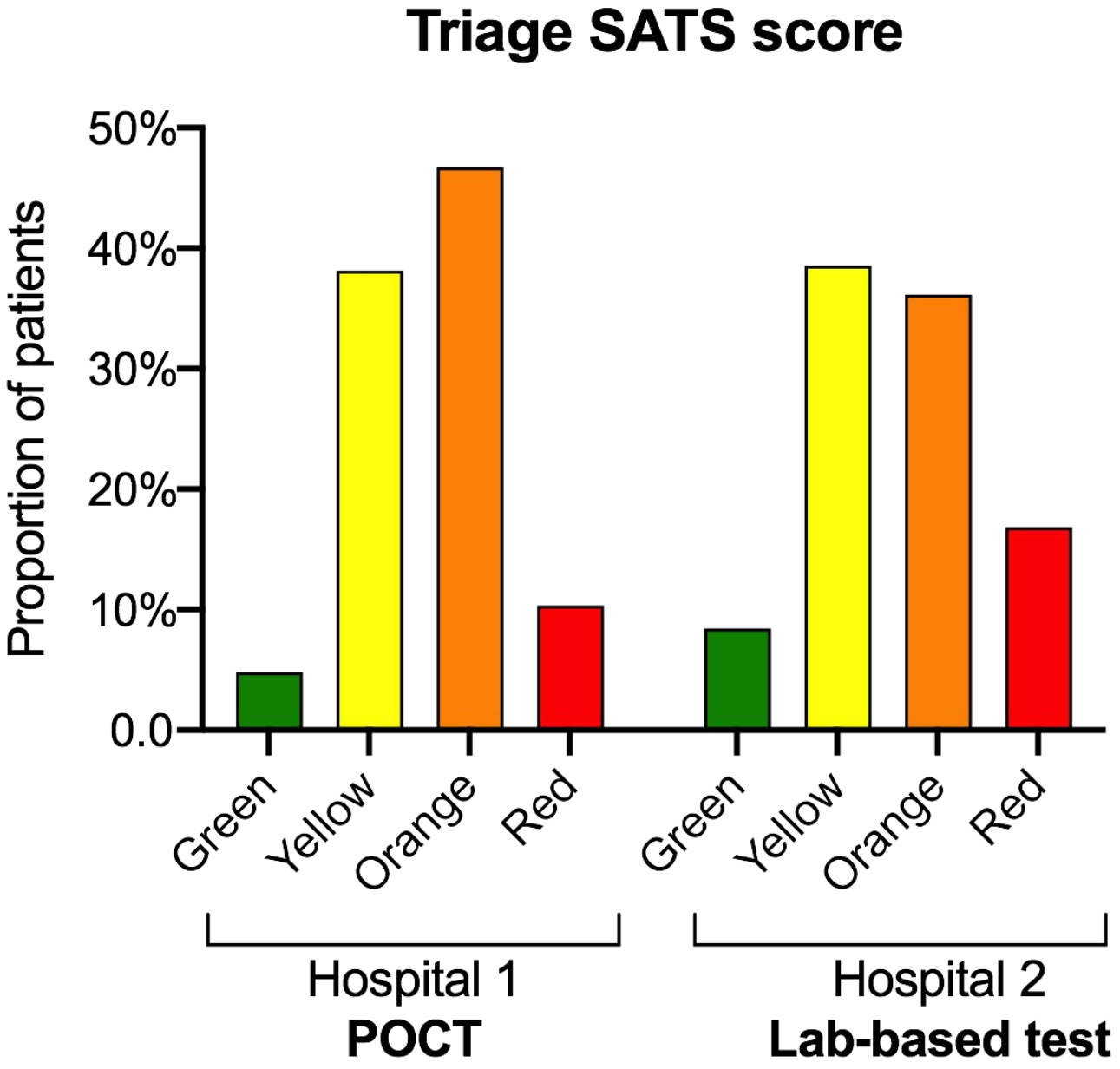

Triage SATS score
Proportion of patients
50%
40%
30%
20%
10%
0.0
Green
Yellow
Orange
Red
Green
Yellow
Orange
Red
Hospital 1
POCT
Hospital 2
Lab-based test

Supplementary table 1.

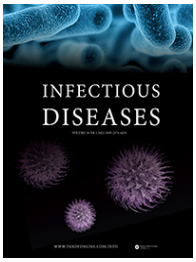
Symptoms upon admission	Hospital 1 **POCT N=400**	Hospital 2 **Lab-based test N=167**	**2-sided p-value**
Temperature >37.5°C	290 (73%)	141 (85%)	**0.002**
Influenza positive	130 (85%)	60 (95%)	**0.028**
Influenza negative	160 (65%)	81 (78%)	**0.018**
Malaise	382 (96%)	164 (98%)	0.120
Influenza positive	154 (100%)	63 (100%)	-
Influenza negative	228 (93%)	101 (97%)	0.111
Dyspnoe	308 (77%)	138 (84%)	0.058
Influenza positive	115 (75%)	49 (82%)	0.278
Influenza negative	193 (79%)	89 (86%)	0.124
Sore throat	178 (45%)	63 (39%)	0.215
Influenza positive	78 (51%)	26 (43%)	0.288
Influenza negative	100 (41%)	37 (37%)	0.470
Dry cough	163 (41%)	61 (37%)	0.341
Influenza positive	79 (52%)	32 (52%)	0.998
Influenza negative	84 (35%)	29 (28%)	0.233
Productive cough	242 (61%)	111(67%)	0.205
Influenza positive	108 (70%)	43 (68%)	0.785
Influenza negative	134 (55%)	68 (65%)	0.070
Myalgia	180 (45%)	71 (43%)	0.668
Influenza positive	81 (53%)	33 (53%)	0.933
Influenza negative	99 (40%)	38 (37%)	0.559
Arthralgia	161 (40%)	64 (39%)	0.747
Influenza positive	85 (55%)	32 (52%)	0.633
Influenza negative	76 (31%)	32 (31%)	0.974
Gastrointestinal symptoms	119 (30%)	57 (34%)	0.304
Influenza positive	54 (35%)	31 (49%)	0.053
Influenza negative	65 (26%)	26 (25%)	0.782
Neurological symptoms (headache, dizziness, syncope)	190 (49%)	82 (55%)	0.173
Influenza positive	85 (56%)	30 (60%)	0.646
Influenza negative	105 (44%)	52 (53%)	0.132

Supplementary table 2. RT-PCR Respiratory panel results.

	Hospital 1 n=325/400	Hospital 2 n=34/167
Influenza A virus	108	8
Influenza B Virus	2	0
Parainfluenza virus	9	0
Respiratory syncytial virus	23	6
Human Metapneumovirus	1	0
Adenovirus	0	1
Rhinovirus	11	1
Mycoplasma pneumonia	2	1
Chlamydophila pneumonia	4	0
Bordatella Pertussis	1	0
Other viruses (coronaviruses)	1	5

Papers I-III

II



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Lower antibiotic prescription rates in hospitalized COVID-19 patients than influenza patients, a prospective study

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ORIGINAL ARTICLE

Lower antibiotic prescription rates in hospitalized COVID-19 patients than influenza patients, a prospective study

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ABSTRACT

Background: COVID-19 patients are extensively treated with antibiotics despite few bacterial complications. We aimed to study antibiotic use in hospitalized COVID-19 patients compared to influenza patients in two consecutive years. Furthermore, we investigated changes in antibiotic use from the first to second pandemic wave.

Methods: This prospective study included both patients from two referral hospitals in Bergen, Norway, admitted with influenza ($n = 215$) during the 2018/2019 epidemic and with COVID-19 ($n = 82$) during spring/summer 2020, and national data on registered Norwegian COVID-19 hospital admissions from March 2020 to January 2021 ($n = 2300$). Patient characteristics were compared, and logistic regression analysis was used to identify risk factors for antibiotic use.

Results: National and local COVID-19 patients received significantly less antibiotics (53% and 49%) than influenza patients (69%, $p < .001$). Early antibiotics contributed to >90% of antibiotic prescriptions in the two local hospitals, and >70% of prescriptions nationally. When adjusted for age, comorbidities, symptom duration, chest X-ray infiltrates and oxygen treatment, local COVID-19 patients still had significantly lower odds of antibiotic prescription than influenza patients (aOR 0.21, 95%CI 0.09–0.50). At the national level, we observed a significant reduction in antibiotic prescription rates in the second pandemic wave compared to the first (aOR 0.35, 95% CI 0.29–0.43).

Conclusion: Fewer COVID-19 patients received antibiotics compared to influenza patients admitted to the two local hospitals one year earlier. The antibiotic prescription rate was lower during the second pandemic wave, possibly due to increased clinical experience and published evidence refuting the efficacy of antibiotics in treating COVID-19 pneumonia.

KEYWORDS
COVID-19
SARS-CoV-2
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antibiotic stewardship
respiratory infection

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Supplemental data for this article can be accessed here.

Introduction

The World Health Organization (WHO) has declared increasing antibiotic resistance a major threat to global health. Widespread use of broad-spectrum and long antibiotic treatment courses are important driving factors for development of resistance [1]. Community-acquired infections, particularly acute respiratory infections (ARI), are the main indicators for antibiotic prescription in hospitals [2]. Viral pathogens are detected in up to one-third of community-acquired cases of pneumonia (CAP) [3,4], but remains challenging to distinguish from ARI with bacterial or mixed aetiology in the clinic. Consequently, antibiotics are often given empirically to hospitalized patients with ARI, even after detection of a viral pathogen [4,5]. The emergence of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), causing coronavirus disease 2019 (COVID-19), actualizes the risk of antibiotic overuse. Initial published reports on treatment of COVID-19 included excessive antibiotic use, despite evidence of low rates of concurrent bacteraemia (3.8%) and other bacterial complications (6–15% of hospitalized cases) outside of intensive care units (ICU) [6–10]. However, the latest WHO interim guidance recommends antibiotic therapy only in severe COVID-19, or when signs of bacterial infection are present, and antibiotics should be adjusted to local microbiological epidemiology [11]. Prior to the COVID-19 pandemic, influenza accounted for the highest respiratory virus disease burden globally, with up to 650,000 deaths annually despite available vaccines and antiviral drugs [12]. Furthermore, influenza entails a significant risk of concurrent bacterial infections (co-infections), found in 10–35% of hospitalized patients, and secondary bacterial pneumonia (after onset or clearance of the initial viral infection), associated with fatality during the 1918 and 2009 influenza pandemics [13–16]. Co- and secondary bacterial infections require appropriate treatment, but despite awareness of antimicrobial resistance, antibiotic prescription rates increase annually during influenza season [17,18]. There is concern that the COVID-19 pandemic has halted progress in antibiotic stewardship and changed the antibiotic prescription patterns in hospitals. To address this, we initiated a prospective comparative cohort study and hypothesized that, after adjusting for clinical characteristics and severity of illness, hospitalized COVID-19 patients were prescribed more antibiotics, particularly broad-spectrum antibiotics, than influenza patients.

Methods

Study design

In this study, we compared clinical data from hospitalized patients $\geq$18 years old in Bergen, Norway, admitted with either influenza during the 2018/2019 influenza epidemic or with COVID-19 during March 2020–September 2020.

Patients were prospectively included from two academic referral hospitals in Bergen with emergency care services, Haukeland University Hospital (HUH) and Haraldsplass Deaconess Hospital (HDH). To investigate differences between local and national antibiotic prescription patterns, as well as changes in COVID-19 treatment during consecutive pandemic waves, we included national data on COVID-19 patients hospitalized between March 2020 and January 2021 from the Norwegian Intensive Care and Pandemic Registry (NIPaR) as a separate, national comparison. Similar surveillance on national influenza admissions do not exist. The NIPaR included the vast majority of hospital admissions due to COVID-19 since the first case on February 26, 2020. Registration became compulsory from March 30, 2020, and most admissions prior to this date were included retrospectively. We defined the second pandemic wave as the period from July 2020 to January 2021. According to viral aetiology and geographic location, we assigned patients to one of three cohorts; local influenza or COVID-19 cohorts – admitted to HUH or HDH – and the national COVID-19 cohort, the latter with data limited to age, gender, comorbidities, antibiotic use, in-hospital complications, length-of-stay (LOS) and 30-days mortality.

Data collection and patient consent statement

Patients recruited from HUS and HDH, or by next-of-kin when necessary, provided written informed consent (the KVIKKFLU study, #2018/1772; COVID-19 study #118664) [19]. NIPaR is based on the right for reservation, as a result active consent was waived for this group of patients.

The study was approved by the Western Norway Ethics committee (#118664) and conducted according to the principles of good clinical practice (GCP) and the Declaration of Helsinki.

Diagnostic assay

The diagnosis of influenza was confirmed by either a commercially available nucleic acid amplification test (Abbott™ ID NOW Influenza A and B 2 (Abbott Park, IL),

Cepheid GeneXpert® II (Sunnyvale, CA) with Xpert Xpress Flu/RSV and Xpert Flu test kit, Eplex Respiratory pathogen panel from GenMark Dx®) or an in-house reverse transcription-polymerase chain reaction (RT-PCR). Both hospitals used a common in-house RT-PCR test to confirm the diagnosis of COVID-19.

Statistical analysis

Patient characteristics were compared using chi-square statistics and Fisher's exact test. The significance of differences in median and interquartile range for continuous variables was assessed using the Mann–Whitney *U* test. As antibiotic stewardship aims to shift prescription practices from resistance driving broad-spectrum towards narrow-spectrum antibiotics, the frequency of broad- and narrow-spectrum antibiotic prescriptions in the two diagnostic groups were compared. We classified second- and third-generation cephalosporins, piperacillin-tazobactam, macrolides, quinolones and carbapenems as broad-spectrum, and phenoxy methyl- and benzyl-penicillins, aminopenicillins, and aminoglycosides as narrow-spectrum antibiotics. Odds ratios (ORs) between dichotomous categorical variables were calculated using binomial logistic regression. Factors with a significance level <0.05 in bivariable analysis were included as covariates in the multiple logistic regression analysis of factors associated with antibiotic prescription in local patients (age, diagnosis, symptom duration, comorbidities, oxygen treatment and chest X-ray infiltrates). Age was assessed as a continuous and categorical variable in the exploratory bivariable analysis, but as a continuous variable in the multiple logistic regression analysis. When adjusted analysis included national COVID-19 patients, covariates were limited to diagnosis, age, comorbidities and chest X-ray infiltrates, due to lack of data on symptom duration and oxygen treatment in this cohort. Microbiological data on co-infections were assessed but found insufficient for inclusion in statistical analysis.

Statistical analyses were performed in IBM SPSS statistics version 26 (SPSS, Inc., Chicago, IL) and Prism version 8.1.2 (GraphPad Software Inc., La Jolla, CA).

Results

Overall, 215 patients were included in the influenza cohort, and 82 patients in the local COVID-19 cohort. National data on COVID-19 patients from NIPaR was screened ($n = 2331$), and hospital admissions of adult patients (≥ 18 years old) were included in the subsequent data analysis ($n = 2300$), representing 2177 individual patients as shown in Figure 1. The distribution of gender, age- and comorbidities was comparable in local and national COVID-19 patients (Table 1). Among national COVID-19 patients, there was a significantly higher proportion of male patients than in the local influenza cohort (59% versus 51%, $p = .015$). Fewer COVID-19 patients than influenza patients had comorbidities, temperature above 37.5° and respiratory

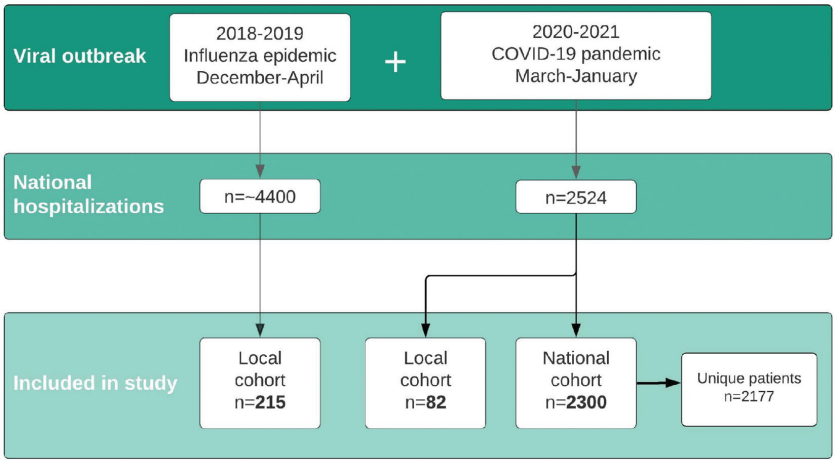

Figure 1. Study design. Local influenza and COVID-19 patients were included from Haukeland University Hospital and Haraldsplass Deaconess Hospital during the 2018/2019 influenza season and spring/summer of 2020. The national cohort included COVID-19 patient data from the Norwegian Intensive Care and Pandemic Registry. Inclusion criteria were age >= 18 years, and a diagnosis of either influenza in 2018/2019 or COVID-19 in 2020/2021.

Table 1. Clinical characteristics and outcomes of COVID-19 and influenza patients.

	Cohorts			*p*-values		
	Influenza *n* = 215	Local COVID-19 *n* = 82	National COVID-19 *n* = 2300	Influenza versus Local COVID-19	Influenza Versus National COVID-19	Local COVID-19 versus national COVID-19[d]
Demographics						
Age (median, IQR)[a]	65 (47–78)	57 (45–72)	61 (48–74)	.048*	.168	.083
Gender (male)	109/215 (51%)	44/82 (54%)	1362/2300 (59%)	.648	.015*	.282
BMI (median, IQR)[a]	24.8 (22.5–28.3)	27.4 (24.6–31.6)	27.4 (24.7–31.4)	<.001*	<.001*	.655
Smoker	34/212 (16%)	5/82 (6%)	72/2300 (3%)	.027*	<.001*	.139
Time from symptoms to admission (median days, IQR)[a]	3 (1–4)	7 (4.3–10)		<.001*		
Temperature > 37.5 °C	188/215 (87%)	61/82 (74%)	216/378 (57%)	.006*	<.001*	.005*
Pregnant	3/102 (3%)	0	13/938 (1%)		.053	
Comorbidities (any)	170/214 (79%)	58/82 (71%)	1516/2287 (66%)	.111	<.001*	.409
Diabetes	30/215 (14%)	8/82 (10%)	400/2300 (17%)	.333	.200	.065
Chronic lung disease (including asthma and COPD)	81/215 (38%)	23/82 (28%)	514/2300 (22%)	.120	<.001*	.209
Cardiovascular disease (including hypertension)	102/215 (47%)	26/82 (32%)	935/2300 (40%)	.014*	.053	.093
Chronic renal disease	28/215 (13%)	5/82 (6%)	134/2300 (6%)	.090	<.001*	.923
Chronic hepatic disease	1/215 (1%)	3/82 (4%)	30/2300 (1%)	.065[e]	.286	.064
Chronic neurological disease	52/215 (24%)	7/82 (9%)	101/2300 (4%)	.003*	<.001*	.076
Cancer	18/215 (8%)	5/82 (6%)	105/2300 (5%)	.512	.013*	.494
Immunosuppression	24/215 (11%)	8/82 (10%)	112/2300 (5%)	.727	<.001*	.031*
First antibiotic prescription before 24 h of admission						
Any antibiotics	142/215 (66%)	36/82 (44%)	824/2188 (38%)	<.001*	<.001*	.252
Penicillin	123/215 (57%)	27/42 (33%)	384/2188 (18%)	<.001*	<.001*	<.001*
Penicillin with ß-lactamase inhibitor	8/215 (4%)	3/82 (4%)	84/2188 (4%)	1.000[e]	.931	1.000[e]
Aminoglycosides	44/215 (20%)	11/82 (13%)	81/2188 (4%)	.162	<.001*	<.001*
Cephalosporins	31/215 (14%)	10/82 (12%)	309/2188 (13%)	.619	.905	.621
Fluoroquinolones	2/215 (1%)	0/82 (<1%)	57/2188 (3%)	1.000[e]	.165[e]	.268[e]
Carbapenems	1/215 (<1%)	0/82 (<1%)	5/2188 (<1%)	1.000[e]	.431[e]	1.000[e]
Macrolides	9/215 (4%)	2/82 (2%)	33/2188 (2%)	.733[e]	.004*	.363[e]
Other antibiotics in	21/215 (10%)	2/82 (5.6%)	47/2188 (2%)	.049*[e]	<.001*	.697[e]
First antibiotic prescription after 24 h of admission						
Any antibiotics	6/215 (3%)	4/82 (5%)	327/2188 (15%)	.471[e]	<.001*	.010*[e]
Narrow-spectrum[b]	3/215 (1%)	1/82 (1%)	148/2188 (7%)	1.000[e]	<.001*[e]	.040*[e]
Broad spectrum[c]	3/215 (1%)	3/82 (3%)	220/2188 (10%)	.352[e]	<.001*[e]	.058[e]
Clinical outcomes						
Chest X-ray infiltrates	63/181 (35%)	57/78 (73%)	1328/1983 (67%)	<.001*	<.001*	.260
Length-of-stay (median days, IQR)[a]	2.0 (1.0–5.0)	5.0 (2.0–8.0)	5.0 (2.4–9.5)	<.001*	<.001*	<.001*
30 day mortality	4/215 (2%)	3/82 (4%)	160/2300 (7%)	.399	.002*	.369

[a]Mann–Whitney *U* test.
[b]Penicillins without penicillinase-activity and aminoglycosides.
[c]Carbapenems, cephalosporins, macrolides, tetracyclines, quinolones, piperacillin/tazobactam and others.
[d]Overlapping patients ($n = 76$) were excluded from analysis.
[e]Fisher's exact test.
*p-value <.05. p-values $<= 0.05$ were considered significant.
Chi-square statistics were used unless otherwise noted.

symptoms upon admission (Table 1, Supplementary Table 1). Influenza patients were older than local COVID-19 patients (65 years versus 57 years, $p = .048$), but not significantly older than national patients (median age 61 years, $p = 0.083$, Table 1). COVID-19 patients were significantly more obese (body mass index >30) than influenza patients (33% versus 18%, x^2 $n = 1296$, $p < .001$). Smoking was significantly more prevalent in influenza patients (16%) than in local and national COVID-19 patients, 6% ($p = .027$) and 3% ($p < .001$), respectively. Local patients reported symptom duration upon admission. Influenza patients were symptomatic for 3 days before admission, compared to 7 days in local COVID-19 patients ($p < .001$, Table 1). Chest X-ray infiltrates were more common in COVID-19 patients (73% locally and 67% nationally) than in influenza patients (35%, both $p < .001$). COVID-19 patients had higher 30- day mortality rate than influenza patients (7% nationally and 4% locally versus 2%, $p = .002$ and $p = .399$), and longer hospital stays, with a median length-of-stay of 5 days compared to 2 days, $p < .001$ (Table 1).

Complete data on antibiotic prescription were available for all local patients and 95% nationally. Influenza patients received antibiotics (69%) significantly more often than both local and national COVID-19 patients (49% and 53% of patients respectively, $p = .001$ and $p < .001$). Antibiotics initiated within 24 h accounted for 90% of the prescriptions in local COVID-19 patients and 96% in influenza patients. In the national COVID-19 cohort, 72% of the antibiotics were given within the first 24 h of admission. Overall, COVID-19 patients nationally received broad-spectrum antibiotics more frequently than local influenza patients (36% versus 25%, $p = .002$) and less frequently narrow-spectrum antibiotics (28% versus 60%, $p < .001$). In local COVID-19 patients, the use of broad-spectrum antibiotics (23%) was similar to that of influenza patients ($p = .728$) and narrow-spectrum antibiotics (37%) similar to that of national COVID-19 patients ($p = .446$).

Among national COVID-19 patients receiving antibiotics, the most commonly prescribed were penicillins and second- and third-generation cephalosporins. Penicillins were prescribed to 51% of national COVID-19 patients who received antibiotics, compared to 75% of local COVID-19 and 86% of influenza patients. Cephalosporins were prescribed to 49% of national COVID-19 patients receiving antibiotics, but only to 33% of local COVID-19 and 22% of influenza patients. Internationally, azithromycin gained attention due to a possible effect on COVID-19, as it was shown to possess antiviral properties against multiple viral agents *in vitro* and anti-inflammatory effects *in vivo* [20,21]. In our study, only 3% of national patients received treatment with macrolides, mainly in the beginning of the pandemic. To study whether increased knowledge of the clinical picture of COVID-19 influenced the choice of antibiotic treatment, we divided patients into two groups, corresponding to the first (spring 2020) and the second wave (autumn 2020) of the pandemic in Norway (Figure 2 and Table 2). The adjusted ORs of antibiotic prescription in the two pandemic waves compared to influenza are presented in Figure 3(a) (crude OR in Supplementary Figure 1), demonstrating higher odds of broad-spectrum antibiotic prescription in the first pandemic wave than in influenza, but higher odds of overall and narrow-spectrum antibiotic prescriptions in influenza patients. In the second wave, use of broad-spectrum antibiotics was reduced by 20% (Table 2), and comparable to prescription rates in influenza patients (aOR 0.96, 95% CI 0.64–1.43). The adjusted ORs of antibiotic prescription in local versus national COVID-19 patients during the first pandemic wave are shown in Figure 3(b), demonstrating higher odds of the use of overall and broad-spectrum antibiotics nationally. Furthermore, national COVID-19 patients received significantly less antibiotics during the second pandemic wave than during the first (42% compared to 65% respectively, aOR 0.35, 95% CI 0.29–0.43, Figure 3(c)). The reduction was due to reduced rates of early antibiotic prescriptions (from 49% to 27%, $p < .001$, Table 2). Length-of-stay was significantly shorter during the second wave, with a median of 4.6 days versus 5.8 days in the first wave from admission to discharge ($p < .001$).

Local cohorts of influenza and COVID-19 patients were combined in the analysis of association between diagnosis and antibiotic use in the two referral hospitals. In the bivariable and multivariable analysis, a significant association between influenza and antibiotic prescription was found (Table 3). Other factors associated with antibiotic use in multivariable analysis of all local patients were chest X-ray infiltrates and oxygen treatment. In addition, the bivariable analysis showed significantly higher odds of antibiotic prescription with increasing age, shorter symptom duration, and underlying comorbidities (in particular cardiovascular disease, hypertension, and immunosuppression).

Discussion

We were surprised, that contrary to our hypothesis, when adjusted for important differences in patient

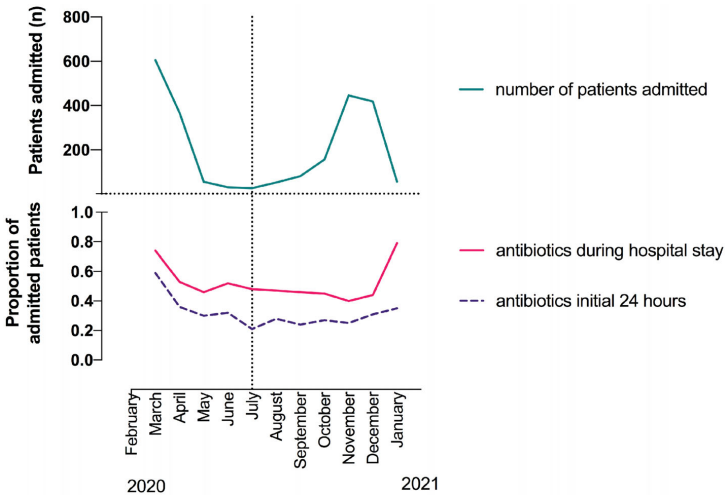

Figure 2. Monthly COVID-19 hospital admissions and antibiotic prescriptions from February 2020 to January 2021. Upper part: National COVID-19 hospital admissions per month (green line). Admissions peaked during spring and autumn of 2020 corresponding to the first and second pandemic wave (divided by the vertical dotted line). Lower part: Proportion of admitted patients receiving antibiotics any time during admission (pink line) and within 24 h of admission (purple line).

Table 2. Clinical characteristics of COVID-19 patients during the first and second pandemic wave.

Demographics	First wave $n = 1059$	Second wave $n = 1129$	Odds ratio (95% CI)	*p*-value
Age (median, IQR)[a]	60 (49–73)	60 (47–74)		.892
BMI (median, IQR)[a]	27 (24–30)	28 (25–32)		.002*
Length-of-stay (median days, IQR)[a]	5.8 (2.8–11.1)	4.6 (2.1–8.0)		<.001*
Gender (female)	432 (41%)	464 (41%)	0.99 (0.85–1.18)	.992
Known comorbidity	654 (62%)	781 (69%)	1.39 (1.16–1.66)	<.001*
Diabetes	146 (14%)	229 (20%)	1.61 (1.29–2.01)	<.001*
Chronic lung disease	216 (21%)	268 (24%)	1.22 (0.99–1.49)	.060
Chronic heart disease	384(36%)	491 (44%)	1.35 (1.14–1.60)	.001*
Chronic renal disease	56 (5%)	64 (6%)	1.08 (0.74–1.56)	.696
Chronic hepatic disease	13 (1%)	16 (1%)	1.16 (0.55–2.42)	.699
Chronic neurological disease	47 (5%)	45 (4%)	0.98 (0.66–1.46)	.928
Cancer	48 (5%)	50 (4%)	0.98 (0.65–1.46)	.907
Immunosuppression	60 (6%)	41 (4%)	0.63 (0.42–0.94)	.025*
Pregnancy	6 (1%)	7 (1%)	1.10 (0.37–3.27)	.871
Smoker	30 (3%)	37 (3%)	1.16 (0.71–1.90)	.547
Chest X-ray infiltrates	626 (69%)	635 (66%)	0.87 (0.72–1.06)	.162
First antibiotic prescription before 24 h of admission				
Any antibiotics	520 (49%)	304 (27%)	0.38 (0.32–0.46)	<.001
Narrow-spectrum[b]	240 (23%)	154 (14%)	0.54 (0.43–0.67)	<.001*
Broad-spectrum[c]	298 (28%)	157 (14%)	0.41 (0.33–0.51)	<.001*
First antibiotic prescription after 24 h of admission				
Any antibiotics	163 (15%)	164 (15%)	0.93 (0.73–1.18)	.570
Narrow-spectrum[b]	68(6%)	80 (7%)	1.11 (0.80–1.55)	.536
Broad-spectrum[c]	114 (11%)	106 (9%)	0.86 (0.65–1.14)	.285
Total antibiotics				
Any antibiotics	683 (65%)	468 (42%)	0.39 (0.33–0.46)	<.001*
Narrow-spectrum[b]	344 (33%)	260 (23%)	0.62 (0.52–0.75)	<.001*
Broad-spectrum[c]	490 (46%)	289 (26%)	0.86 (0.65–1.14)	<.001*

The first pandemic wave was defined as the time-period from March to June 2020, and the second pandemic wave as the time-period from July 2020 to January 2021.
[a]Mann–Whitney *U* test.
[b]Penicillins without penicillinase-activity and aminoglycosides.
[c]Carbapenems, cephalosporins, macrolides, tetracyclines, quinolones, piperacillin/tazobactam and others.
*p-value <.05. p-values <= .05 were considered significant. Chi-square statistics were used unless otherwise noted.

populations, antibiotic prescription rates in hospitalized COVID-19 patients were lower than in influenza patients in the same two referral hospitals.

Our study provides detailed findings and comparison of antibiotic prescription practices during the COVID-19 pandemic and 2018/2019 influenza epidemic,

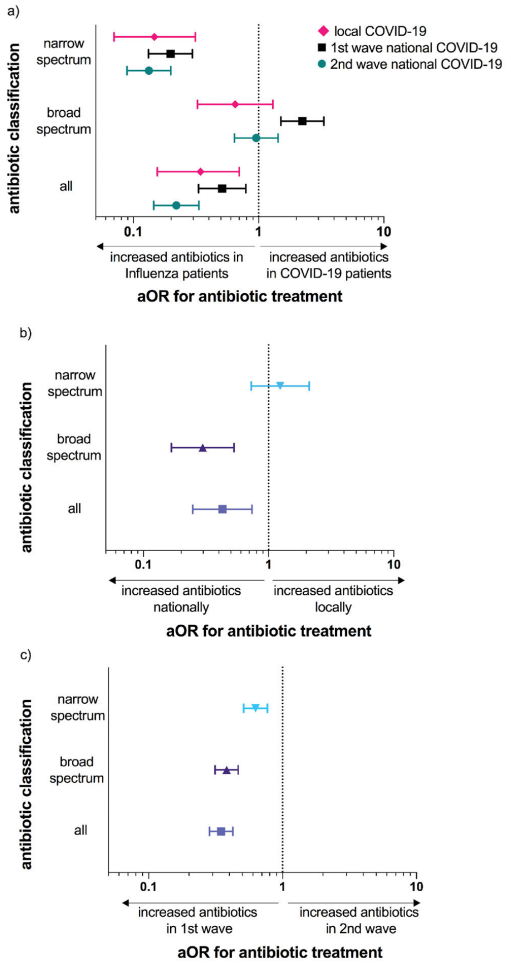

Figure 3. Adjusted odds ratios for antibiotic prescription. Adjusted odds ratios (aOR) for antibiotic prescription in (a) COVID-19 patients compared to influenza patients, (b) local COVID-19 patients compared to national COVID-19 patients in the first pandemic wave and (c) national COVID-19 patients in the second compared to first pandemic wave. Odds were adjusted for chest X-ray infiltrates, age and comorbidities.

contributing to the growing evidence of differences in clinical management, and patient outcomes of the two viral diseases [22,23].

In the spring of 2020, reports from European countries, such as Italy, depicted a healthcare system collapsing in the encounter with the pandemic virus SARS-CoV-2. The fear of the novel virus affected decision-making at many levels in society and may have impacted on antibiotic use. Since the previous influenza epidemic, national guidelines on antibiotic prescription remained unchanged through the first year of the pandemic, and did not include consideration of infection markers [24]. In influenza, co- and secondary bacterial infections require appropriate treatment, as they aggravate disease outcome [22,23]. COVID-19 has proved to be more lethal than seasonal influenza [22,25], possibly encouraging high initial antibiotic use.

Since the start of the COVID-19 pandemic, knowledge of the prevalence of bacterial coinfections, and experimental treatment options have rapidly advanced [6,26–28]. The development of co- and secondary infections appears to be rare in COVID-19 [29,30]. At time, reports on antibiotic prescription trends over time are scarce [31,32]. We observed a significant reduction in antibiotic prescriptions in clinically comparable patients from the first to second wave of the COVID-19 pandemic, indicating that the reduction in antibiotic prescriptions was due to fundamental changes in prescribing practices rather than changes in patient populations.

These findings are encouraging and show that important change in prescribing patterns is possible, especially with rapidly evolving knowledge during a pandemic.

We find it concerning that almost 70% of influenza patients received antibiotics, and that early antibiotics accounted for 96% of prescriptions, despite rapid influenza testing in the Emergency Department, short median symptom duration of 3 days and established knowledge of influenza pathology [33,34]. Prescription rates in our study were lower than or comparable to several international studies [35–38]. A recent study documented higher rates of 30-day respiratory disease readmission in influenza patients only treated with antivirals as compared to both antivirals and antibiotics, although the absolute differences in risk were low [39]. In COVID-19 patients early antibiotic prescriptions were significantly reduced from the first to second pandemic wave (from 49% to 27%, proportionally 76% and 65% of all prescriptions). In comparison, a study from the US reported a wide range (27–84%) of early empirical antibiotic use in COVID-19 patients in 32 hospitals [32]. High rates of empirical antibiotic treatment indicate the presence of unnecessary prescribing, potentially both resistance-driving and harmful at patient level, thus an important target for antimicrobial stewardship. In our experience, SARS-CoV-2 test turn-around-times has improved since the beginning of the pandemic outbreak, possibly affecting antibiotic prescribing patterns. Simultaneously, the superior local rapid influenza test

Table 3. Factors associated with antibiotic prescription.

	n	Antibiotic prescription (%)	OR (95%CI)	*p*-Value	aOR (95%CI)	*p*-Value
Diagnosis						
COVID-19	82	40 (49%)	0.43 (0.26–0.73)	.002*	0.21 (0.09–0.50)	<.001*
Influenza	215	148 (69%)				
DEMOGRAPHICS						
Age[a]	297	188 (63%)	1.03 (1.02–1.04)	.001*	1.01 (1.00–1.03)	.155*
Age groups						
Older (≥ 65 years)	141	105 (75%)	2.57 (1.57–4.20)	<.001*		
Younger(<65years)	156	83 (56%)				
10–19	2	1 (50%)	1.58 (0.90–27.78)	.753		
20–29	29	13 (45%)	1.29 (0.46–3.60)	.631		
30–39 (ref)	31	12 (39%)	ref			
40–49	28	12 (43%)	1.19 (0.42–3.36)	.746		
50–49	43	29 (67%)	3.28 (1.25–8.60)	.016*		
60–69	49	34 (69%)	3.59 (1.40–9.23)	.008*		
70–79	54	43 (80%)	6.19 (2.32–16.50)	<.001*		
80–89	46	31 (67%)	3.27 (1.27–8.46)	.014*		
90–99	15	13 (87%)	10.29 (1.97–53.85)	.006*		
Sex						
Female	144	90 (62%)	0.94 (0.58–1.50)	.781	–	
Male	153	98 (64%)				
Comorbidities						
Present	228	154 (68%)	2.21 (1.27–3.83)	.005*	1.16 (0.54–2.50)	.705
Absent	68	33 (49%)				
Cardiovascular disease						
Present	88	67 (76%)	2.32 (1.32–4.07)	.003*	–	
Absent	209	121 (58%)				
Hypertension						
Present	94	69 (73%)	1.95 (1.14–3.33)	.015*	–	
Absent	203	119 (59%)				
Chronic lung disease						
Present	104	72 (69%)	1.49 (0.90–2.48)	.120	–	
Absent	193	116 (60%)				
Smoking						
Current	39	30 (77%)	2.11 (0.96–4.63)	.063	–	
Previously or never	258	158 (61%)				
Obesity (BMI > 30)						
Present	52	32 (62%)	0.90 (0.48–1.69)	.747	–	
Absent	197	126 (64%)				
Diabetes Mellitus						
Present	38	28 (74%)	1.73 (0.81–3.72)	.159	–	
Absent	259	160 (62%)				
Chronic renal disease						
Present	33	24 (72%)	1.62 (0.73–3.64)	.237	–	
Absent	264	164 (62%)				
Chronic neurological disease						
Present	57	40 (70%)	1.46 (0.78–2.73)	.233	–	
Absent	240	148 (62%)				
Immunosuppression						
Present	32	26 (81%)	2.76 (1.10–6.92)	.031*		
Absent	265	162 (61%)				
Active cancer						
Present	23	17 (74%)	1.70 (0.65–4.47)	.276	–	
Absent	274	171 (64%)				
Clinical presentation						
Time from symptoms to admission[a]						
Days	293		0.91 (0.85–0.96)	.002*	0.93 (0.84–1.02)	.103
Temperature > 37.5 °C						
Present	249	162 (65%)	1.58 (0.84–2.94)	.154	–	
Absent	48	26 (54%)				
Diagnostics						
Chest X-ray						
Infiltrate	120	94 (78%)	2.51 (1.45–4.36)	.001*	4.39 (1.94–9.93)	<.001*
No infiltrate	139	82 (59%)				
Interventions						
Oxygen treatment						
Received	131	107 (82%)	4.74 (2.77–8.11)	<.001*	2.88 (1.49–5.57)	.002*
Not received	165	80 (49%)				
NIV treatment						
Received	36	36 (100%)	–	–	–	
Not received	260	151 (58%)				
Respirator treatment						
Received	13	13 (100%)	–	–	–	
Not received	282	174 (62%)				

Antibiotic prescription was defined as the dependent variable. Independent variables entered in multiple logistic regression analysis were 'diagnosis', 'age', 'comorbidities', 'duration of symptoms', 'chest X-ray infiltrates' and 'oxygen treatment'.

[a]Continuous variables. Approximate percentage of variance accounted for in multivariable analysis was 25% (Cox & Snell R^2=0.213 and Nagelkerke R^2=0.299).

turn-around-times is not reflected in lower empiric antibiotic prescriptions.

We found a higher prevalence of respiratory symptoms in local influenza patients than in local COVID-19 patients, in line with results of a recent meta-analysis [40]. The presence of respiratory symptoms and clinical findings has previously been associated with antibiotic prescribing in respiratory tract infections [41]. However, our study was not designed to examine such an association.

Broad-spectrum antibiotics was used more prevalently in COVID-19 patients than in influenza patients. The most common co-infecting pathogens in influenza are *Streptococcus pneumonia*, *Staphylococcus aureus*, and *Haemophilus influenzae* [34,42], most often treatable with narrow-spectrum antibiotics in Norway. Accumulated data demonstrates low prevalence of community-onset bacterial co-infection in COVID-19 patients, however, numerous different co-pathogens have been detected internationally [32,43]. These findings might encourage the use of broad-spectrum antibiotics in COVID-19 patients with suspected bacterial co-infection.

Currently, Norway has no national registry of antibiotic treatment of hospitalized influenza patients, and, to our knowledge, our current cohorts are the most comprehensive in the country, including both regional influenza patients and all COVID-19 hospitalizations in Norway until January 2021. In a national survey of antibiotic stewardship, one of the two participating hospitals, -HDH-, ranked top in the country in adhering to narrow-spectrum antibiotic use when appropriate, while HUS was among those using most broad-spectrum antibiotics. Both hospitals are more restrictive than the country as a whole concerning antibiotic treatment of COVID-19. In Norway, there is low prevalence of multiresistant bacteria compared to most other countries. Hence, some of our findings on selection and prescription of antibiotics may only be generalizable to countries with similar microbial resistance patterns. Another limitation is that we lacked data on microbiological findings in most patients and therefore could not evaluate the appropriateness of the antibiotic prescription in each case. Furthermore, our study focussed solely on the proportionate use of antibiotics, and not on treatment duration. The core elements of antibiotic stewardship, particularly in patients with COVID-19, such as reassessment, de-escalation and early termination, should be investigated in future studies.

The 30-day mortality reported in our study was exceptionally low compared to other studies [30,44,45]. This could be influenced by a tendency to treat elderly and frail nursing home residents with COVID-19 outside hospital, where the majority of deaths during the early phase of the pandemic occurred [46].

We believe it is important to analyze present antibiotic prescribing patterns in the context of previous practices. Our study forms a valuable backdrop for reflection on decisive factors for antibiotic prescription in viral lung infections. A preprinted study of hospitalized influenza patients in Norway between 2014-2018 reported of unchanged antibiotic use in the study period [47], whereas in hospitalized COVID-19 patients, we observed rapid changes in antibiotic prescription rates during 2020. Improved rapid diagnostic tools, and targeted stewardship measures to reduce discrepancies between the true prevalence of bacterial co-infection and antibiotic use in viral respiratory infections is urgently needed, as antibiotic resistance may well be our next pandemic threat.

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Disclosure statement

The authors declare no conflicts of interest.

Short summary

Hospitalized COVID-19 patients received less antibiotics than influenza patients. The majority of antibiotic prescriptions were early and empirical in both COVID-19 (>70%) and influenza (>90%). Significant reduction of COVID-19 antibiotic prescriptions was observed from the first to second pandemic wave.

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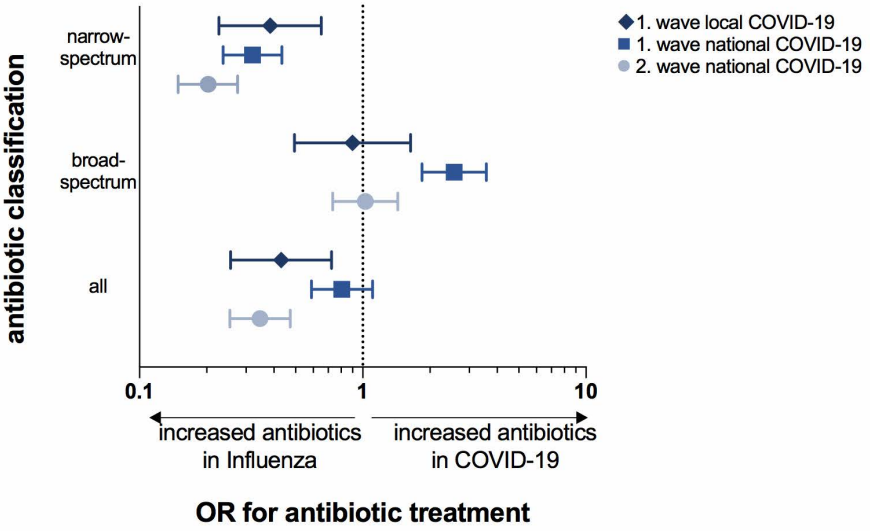
Supplementary figure 1: Crude odds ratios for antibiotic prescription

Odds ratios (OR) for antibiotic prescription with 95% confidence intervals in a) COVID-19 patients compared to influenza patients, b) local COVID-19 patients compared to national COVID-19 patients in the first pandemic wave and c) national COVID-19 patients in the second compared to first pandemic wave.

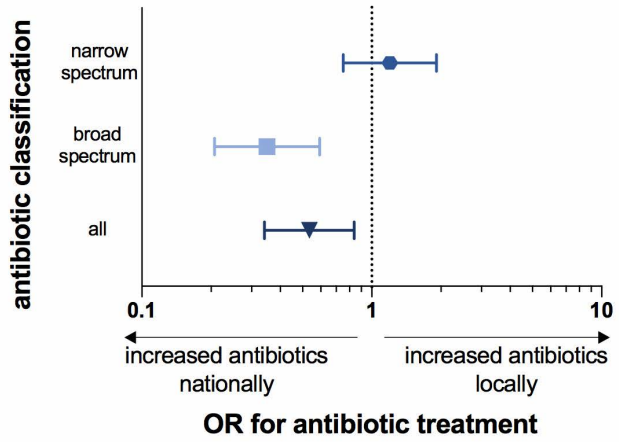
Supplementary table 1: Presenting symptoms in local influenza- and COVID-19 patients

Upon admission, COVID-19 patients reported fewer respiratory symptoms than influenza patients did. P-values were calculated using chi-square distribution. P-values <= 0.05 were considered significant.

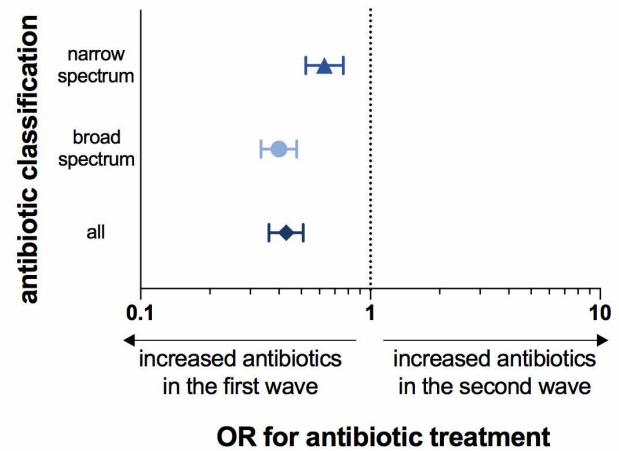
a)


1. wave local COVID-19
1. wave national COVID-19
2. wave national COVID-19
antibiotic classification
narrow-spectrum
broad-spectrum
all
0.1
1
10
increased antibiotics in Influenza
increased antibiotics in COVID-19
OR for antibiotic treatment

b)


antibiotic classification
narrow spectrum
broad spectrum
all
0.1
1
10
increased antibiotics nationally
increased antibiotics locally
OR for antibiotic treatment

c)


antibiotic classification
narrow spectrum
broad spectrum
all
0.1
1
10
increased antibiotics in the first wave
increased antibiotics in the second wave
OR for antibiotic treatment

Supplementary table 1: Presenting symptoms in local patients with influenza and COVID-19

Clinical presentation	**Influenza**	**COVID-19**	**p-value**
	n=215	**n=82**	
Temperature >37,5 °C	188/215 (87%)	61/82 (74%)	0.006*
Cough (with or without expectorate)	204/213 (96%)	67/82 (82%)	<0.001*
Dyspnoea	162/212 (76%)	52/81 (64%)	0.035
General fatigue	215/215 (100%)	64/82 (78%)	<0.001
Muscle- and joint-pain	123/214 (58%)	35/81 (43%)	0.028
Acute respiratory failure	72/215 (34%)	28/82 (34%)	0.915

Papers I-III

III

Symptom burden and immune dynamics 6 to 18 months following mild SARS-CoV-2 infection: A case-control study.

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Running title: Long COVID up to 18 months

40-word summary: A significant burden of long COVID symptoms were observed 6-18 months post-infection in SARS-CoV-2 positive cases, compared to SARS-CoV-2 naive controls. Associations between SARS-CoV-2 specific humoral and cellular immune responses and long COVID symptoms were identified.

Abstract

Background: The burden and duration of persistent symptoms after non-severe COVID-19 remains uncertain. This study aimed to assess post-infection symptom trajectories in home-isolated COVID-19 cases compared to age- and time-period matched seronegative controls, and investigate immunological correlates of long COVID.

Methods: A prospective case-control study conducted between February 28th and April 4th 2020 included home-isolated COVID-19 cases followed for 12 (n=233) to 18 (n=149) months, and 189 age-matched SARS-CoV-2 naive controls. We collected clinical data at baseline, 6, 12 and 18 months post-infection, and blood samples at 2, 4, 6 and 12 months for analysis of SARS-CoV-2 specific humoral and cellular responses.

Results: Overall, 46% (108/233) had persisting symptoms 12 months after COVID-19. Compared to controls, adult cases had a high risk of fatigue (27% excess risk, gender and comorbidity adjusted odds ratio [aOR] 5.86, 95% confidence interval [CI]3.27-10.5), memory problems (21% excess risk, aOR 7.42, CI 3.51-15.67), concentration problems (20% excess risk, aOR 8.88, CI 3.88-20.35), and dyspnea (10% excess risk, aOR 2.66, CI 1.22-5.79). The prevalence of memory problems increased overall from 6 to 18 months (excess risk 11.5%, CI 1.5, 21.5, p=0.024) and among women (excess risk 18.7%, CI 4.4, 32.9, p=0.010). Longitudinal spike IgG was significantly associated with dyspnea at 12 months. The spike-specific clonal $CD4^{+}TCR\beta$ depth was significantly associated with both dyspnea and number of symptoms at 12 months.

Conclusions: This study documents a high burden of persisting symptoms after mild COVID-19, and suggest that infection induced SARS-CoV-2 specific immune responses may influence long-term symptoms.

Keywords: long COVID, PASC, SARS CoV-2, antibodies, T-cells.

Introduction

Prolonged complications after Coronavirus disease 2019 (COVID-19) are a major health concern in the ongoing pandemic. New and persisting symptoms beyond 3 months after acute COVID-19, without other medical explanations[1-4], are referred to as long COVID. Long COVID significantly overlaps with the post-intensive care syndrome (PICS) observed in survivors of severe COVID-19[5, 6]. Although the burden of long COVID is greater after severe disease, long COVID can also develop after mild illness, with 39-77% of hospitalized and-non-hospitalized patients reporting persisting symptoms 12 months after COVID-19[7-13]. In two year longitudinal follow-up studies, symptom burden decreased with time, but residual symptoms persisted in 55% of hospitalized patients[14] and 38% of non-hospitalized patients[15]. Frequent persisting symptoms are fatigue, dyspnea, neurocognitive problems and mental health problems[16], but due to methodological heterogeneity, uncertainty remains about the true burden. Symptoms of long COVID may be wrongly attributed to infection as only a few studies included controls[14, 17, 18], making it difficult to identify any confounders[10, 15]. Online surveys where participants are included on their own initiative likely overestimate the symptom burden of long COVID[19]. In contrast, registry data may fail to pick up on symptoms that do not result in contact with health service, and may consequently underestimate symptom prevalence[20, 21]. Previously, we reported higher SARS-CoV-2 spike-specific antibodies associated with long COVID in a prospective cohort of home-isolated patients at 6 months[22]. Others have found potent antibody responses, aberrant T-cellular responses and pre-existing illness are associated with symptom sequelae[22-26]. Knowledge of the pathophysiology of long COVID is still evolving. In this study, we aimed to investigate symptom trajectories up to 18 months post-infection, assess the excess risk of symptoms in COVID-19 cases compared to age- and time-matched SARS-CoV-2 naïve controls, and explore the immunological and clinical correlates of long COVID.

Methods

Study population

Cases included home-isolated patients with Reverse transcription-polymerase chain reaction (RT-PCR) confirmed SARS-CoV-2 infection, tested at the city´s centralized testing facility (Bergen Municipality Emergency Clinic, BMEC) between February 28th, 2020, and April 4th, 2020. Household contacts of confirmed cases were invited to participate in a study of household attack rates during the same period[27], and those testing positive for SARS-CoV-2 spike antibodies within 2 months after recruitment were included as cases in the current study. One patient who was hospitalized in the weeks after acute infection was excluded from this cohort. All cases were assessed by clinical follow-up for 12 months (n=233), and a subgroup of adult cases agreeing to further follow-up (n=149) were followed for 18 months.

A control group was assessed at the clinic and recruited in two ways. Firstly, household contacts without symptoms, who did not seroconvert, and had no history of RT-PCR positivity, were included, and considered socioeconomically matched to the cases. Secondly, age-matched controls were recruited between January and March 2021 from the population of individuals who were prioritized for vaccination due to either age, comorbidity or occupation. All controls were seronegative at the time of symptom assessment. Hence, the seasonal timing of assessment, and the degree of national and local restrictions, were similar for cases at the 12-month follow-up and controls. The matching was therefore primarily chosen for comparison to the 12-month patient data.

Ethical considerations

The study was approved by the Regional Ethics Committee of Western Norway (#118664 and # 218629). All eligible individuals received both oral and written information about the study protocol and provided written informed consent upon inclusion. For children <16years old, parents provided consent.

Clinical data collection

Participant data were entered in electronic case report forms (eCRFs) using the Research Electronic Data Capture database (REDCap®, Vanderbilt University, Nashville, Tennessee) software, and subsequently stored on a secure research server.

All cases recruited at Bergen Municipality Emergency Clinic were followed up for 12 months (Interquartile Range [IQR] 11.5-12.4 months) with systematic interviews at baseline, 2, 6, and 12 months (supplementary methods), and blood samples at 2, 4, 6 and 12 months. 149 cases had an additional follow-up at 18 months (Figure 1). All subjects provided information on demographics and comorbidities, prescription drug use, and COVID-19 related symptoms at baseline and follow-up visits. Comorbidities recorded were asthma, chronic obstructive pulmonary disease (COPD), hypertension, chronic heart disease, rheumatic disease, diabetes, cancer, neurological disease, immunosuppressive conditions, or other severe or chronic disorders.

The baseline symptom questionnaire was limited to fatigue, headache, fever, myalgia and dyspnea. At 6- 12- and 18-month follow-up of cases, a dichotomized yes/no questionnaire was conducted for the following persistent symptoms: dyspnea, sleep problems, headache, dizziness, tingling, palpitations, gastrointestinal problems or low-grade fever. A general

questionnaire with dichotomized answers was used to assess fatigue, concentration, and memory problems in children ≤15 years old. For adult cases, the validated 11-item Chalder Fatigue Scale (CFS) was used. This CFS questionnaire identifies symptoms associated with both physical and mental fatigue, with graded responses that can be reported according to a Likert scale (0,1,2,3) or as a bimodal score (0,0,1,1)[28]. The prevalence of fatigue, impaired concentration, and memory problems was derived from the corresponding bimodal score of the CFS item 1, 8, and 11, respectively (supplementary table 1). We used a definition of long COVID as persistent or new onset symptoms at 3 months after COVID-19 [4].

Controls provided blood samples and replied to a survey including demographic and clinical information on comorbidities, assessment of dyspnea, and the 11-item CFS concomitantly with the 12-month follow-up of cases.

Blood sampling

Sera were stored at -80°C and heat-inactivated for 1 hour at 56°C after thawing before use.

Enzyme-linked Immunosorbent Assay (ELISA)

A two-step ELISA for detection of IgG was used, first by antibody screening for the Wuhan receptor-binding domain (RBD), followed by endpoint Wuhan spike ELISA, as previously described[27, 29] (supplementary methods).

Microneutralization assay

The microneutralization (MN) assay was performed using a local SARS-CoV-2 isolate from March 2020, as previously described [27, 29] (supplementary methods).

Identification of SARS-CoV-2 associated T-cell receptor β (TCRβ) sequences

Genomic DNA was extracted from EDTA blood using the Qiagen DNeasy Blood Extraction Kit (QIAGEN, Germantown, MD) and amplified in a bias-controlled multiplex PCR, followed by high-throughput sequencing. SARS-CoV-2 associated CDR3 regions of TCRβ chains were sequenced using the ImmunoSEQ Assay T-MAP™ COVID platform (Adaptive Biotechnologies, Seattle, WA) as previously described[30]. The relative number of SARS-CoV-2-associated TCRs was defined as the *clonal breadth*, and the relative proportion of SARS-CoV-2-associated TCRs as the *clonal depth*.

Statistical analysis

Data analysis and visualization were performed in R version 4.2.1 (R Foundation for Statistical Computing, Vienna, Austria) (Figure 2,3 & 4) and IBM SPSS Statistics version 26 (New York, US) (Table 1 & Supplementary Table 1-4). Age-stratified analysis was performed using 15-year intervals to provide sufficient group sizes. Pearson's chi-square test and Fisher's exact test were used to compare proportions. The Mann-Whitney U-test was used to compare continuous variables between two groups. Confidence intervals (CI) and p-values for risk differences were calculated using the fmsb-package in R. Correlations between antibody titers and T-cell breadth and depth were assessed by Spearman´s rho. Multivariate binomial logistic regression was used for analyses of binary outcome variables and negative

binomial regression was used for the count outcome “number of symptoms”. Regression models are presented with adjusted odds ratios (aOR) and 95% CI or risk ratio (RR) with 95% CI or standard error (SE) and p-values. Scaling of TCR breadth was applied due to significant difference in the range between the depth and the breadth of the TCR variables. Microneutralization and IgG antibody titers were log(10) -transformed to adjust for non-normality. Generalized estimating equations (GEE) were used to compare longitudinal spike IgG antibody measurements between two groups (geepack package (v 1.3.3 in R).

Results.

Study population

A population of 233 home-isolated COVID-19 cases were followed for 12 months, and 189 controls were assessed at the time when cases had their 12 months follow-up. Cases and controls had similar median age (44 vs 41 years, p=0.576), 16/233 cases and 7/189 controls were ≤18 years. There were fewer females among cases (53% vs 66%, p=0.010). Overall, more cases reported comorbidities than controls (53% vs 42%, p=.026), most frequently chronic lung disease (12% vs 8%, p=0.168), hypertension (11% vs 7%, p=0.241), rheumatic disease (7% vs 3% p=0.047), and chronic heart disease (6% vs 6%, p=0.915) (Supplementary table 1).

Symptom burden in cases at 12-month follow-up compared to controls

Compared to controls, adult cases had excess risk, and higher gender and comorbidity adjusted odds of fatigue (37% vs 9%, aOR 5.86, CI 3.27-10.5, p<0.001), impaired

concentration (24% vs 4%, aOR 8.88, CI 3.88-20.35, p<0.001), memory problems (26% vs 5%, aOR 7.42, CI 3.51-15.67, p<0.001), and dyspnea (15% vs 5%, aOR 2.66, CI 1.22-5.79, p=0.014). Children 0-15 years old reported no symptoms at 12 months follow-up in either cases or controls. Cases aged 16-30, 31-45, and 46-60 had the highest risk of memory problems and impaired concentration (p<0.05) (Table 1). Fatigue, on the other hand, was more frequently reported by cases aged 46-60 (41% vs 2% in controls, p<0.001) and 61-81 (42% vs 13% in controls, p=0.033). Age-stratified prevalence of 11 symptoms is presented in Figure 2.

Longitudinal symptom development

We assessed the trajectories of 11 symptoms in a subgroup of 149 cases followed for 18 months (Figure 3a-c). The prevalence of reported memory difficulties increased overall from 6 to 18 months follow-up, with an excess risk of 11.5% (CI 1.5, 21.5, p=0.024), the excess risk was significant among women (excess risk 18.7%, CI 4.4, 32.9, p=0.010), but not among males (9.6%, CI -3.6, 22.8, p=0.154). The risk difference from 6 to 18 months for other specific symptoms and symptoms overall was not statistically significant (Figure 3a).

Compared to males, women had excess risk of having symptoms overall at 18 months (17.5%, CI: 1.6, 33.3, p=0.030, Fig 4b) and at 12 months follow-up (20.2%, CI: 4.5, 36.0, p=0.012), but not at 6 months (6.8%, CI: -9.3, 22.8, p=0.41). There was no statistically significant risk difference between the sexes for each specific symptom at 18 months follow-up (Figure 4b), although women had more memory problem at 12 month and scored higher on Chalder fatigue score at 6 and 12 months (Supplementary Table 2 and 3).

Assessing different intensities according to the Likert-scale ("more than usual" versus "much more than usual") we found that cases had excess risk of fatigue, memory problems, impaired concentration and dyspnea compared to controls at all three time points (Table 2). However, the proportion with severe symptoms was low, and there was no significantly increased risk of severe cognitive symptoms at 12 and 18 months.

Association between acute-phase symptoms and long COVID

The majority of cases were symptomatic in the acute phase (226/233 cases). When adjusted for age, gender and comorbidities, acute-phase dyspnea was associated with an increased risk of fatigue, (OR 2.14, CI 1.16-3.95, p=0.010) and dyspnea (OR 8.55, CI 2.77-26.32, p=0.002) at 12 months follow-up, and acute-phase headache was associated with impaired concentration (OR 2.34, CI 1.03-5.29, p=0.040) (Table 3).

Association of antibody titers and long COVID

We measured SARS-COV-2 spike-specific IgG antibody titers at 2, 4, 6, and 12 months after infection. Antibodies waned over time (supplementary table 4), and antibody titers measured at 2 months were considered to reflect the peak of humoral response[31]. Peak spike-binding IgG (geometric mean titer 6128, range 50-98924) and longitudinal antibody titers from 2-12 months, were associated with dyspnea at 12 months and persistent dyspnea from 6 to 12 months, in adjusted analysis (p=0.02 and p=0.05)(Table 3, Figure 4a). Longitudinal antibody responses were not significantly higher in cases with ≥3 symptoms at 12 months compared to those with no symptoms, or in cases with persistent fatigue at 6 and 12 months compared to cases without fatigue (Figure 4b-c).

Association of persisting symptoms and T-cell responses

We measured the correlations between SARS-CoV-2 associated class I restricted ($CD8^+$) or class II restricted ($CD4^+$) TCRs and spike IgG titers from the same time points. Spike IgG antibodies correlated more strongly with $CD4^+$ than $CD8^+$ spike-specific TCRs. Significant correlations between spike IgG and $CD4^+$ clonal breadth and depth were observed at 2 months ($r = 0.371$, $p<0.0001$ and $r=0.315$, $p<0.001$), respectively, and at 6 months ($r=0.276$, $p<0.001$ and $r=0.251$, $p<0.001$). Whereas only the spike IgG and $CD8^+$ clonal depth correlation at 2 months was significant ($r=0.139$, $p=0.039$). SARS-CoV-2 specific clonal depth, (Total, $CD4^+$, and spike-specific $CD4^+$) at 6 months was associated with increased symptom burden at 12 months, when adjusted for age, gender, and the reciprocal TCR breadth (Table 4). Total $CD4^+$ spike-specific clonal depth was also associated with dyspnea at 12 months.

Discussion

In this longitudinal observational case-control study, we found that half of the home-isolated cases still had at least one residual symptom 12 and 18 months post-infection. Compared to controls, cases had significant excess risk of the dominant long COVID symptoms; fatigue, memory- and concentration problems, and dyspnea.

A key strength of our study is the inclusion of age-matched, seronegative controls recruited from the same geographical location and during the same time-period as the cases. Both cases and controls, therefore, had similar exposures to pandemic-related public infection control measures, disrupted social services, and psychosocial stress. We show that the excess fatigue, cognitive symptoms, and dyspnea reported by cases are likely sequelae of mild SARS-CoV-2

infection. Other case-control studies find excess burden of main long COVID symptoms in cases compared to influenza controls[32], healthy adults[14], and children, but the quality-of-life scores were lower in pediatric controls[17], suggesting that pandemic circumstances have affected the health of young people considerably.

Investigating longitudinal symptoms trajectories is important to predict the long COVID burden. In our study, specific symptoms evolved differently over time in individual cases, supporting the fluctuating nature of long COVID previously described[33] . Symptom debut later than 6 months post-infection could also reflect a coincidental overlap with emerging symptoms attributable to other causes or personal circumstances.

In non-controlled studies, the proportion of patients with residual symptoms at 12 months varies considerably (39%-77%)[7, 9-13], and we found a prevalence of 46% in our cases. The prevalence of fatigue, a dominating long COVID sequelae, ranges from 27%[12] in non-hospitalized, 16%-53% in mixed populations[11, 13] to 10% -33%[7, 8, 10] in hospitalized patients, partly reflecting differences in patient selection and symptom assessment[9]. In our subgroup of cases followed for 18 months, the prevalence of most symptoms remained at similar levels throughout, while memory difficulties increased, particularly among women. Although a body of research essentially describe improvement of long COVID over time, studies have described durable symptoms concerning mental health and cognition[14, 15] . Our finding of a lack of improvement in memory difficulties over time is of concern. Although sometimes perceived as vague symptoms, not always being recognized by the health care systems, cognitive symptoms may have significant impact on daily activity and work performance. Our study provides some reassurance for patients with persistent cognitive symptoms in that most cases reported *moderate* symptoms, and that there was no significant excess risk of *severe* cognitive symptoms at 12 and 18 months.

SARS-CoV-2 infection leads to sustained alteration of immune responses and spike-specific IgG titers appears to be associated with long COVID in both hospitalized and home-isolated patients[22, 25, 34]. Our study found that higher peak and longitudinal spike-specific IgG was associated with persistent dyspnea at 12 months. Interestingly, neutralizing antibodies levels were not associated with long-term symptoms, suggesting that other antibody effector mechanisms such as complement activation, Fc receptor binding or cross-reactivity to autoantigens, could be involved in long COVID[35-37]. No association was observed between spike-specific IgG and cognitive symptoms. The role of antibodies in this pathology remains unclear, although SARS-CoV-2 specific antibodies have been discovered in the cerebrospinal fluid (CSF) of COVID-19 patients[38], with abnormal oligoclonal banding patterns found in mild COVID-19 with cognitive sequelae[39]. Furthermore, cerebral elevated cytokine levels and brain abnormalities found in long COVID patients are compatible with inflammatory damage[40].

Dysregulation of T-cell activation and their associated cytokine mediators suggest an aberrant systemic immune response in long COVID patients[26]. Here, we found that the spike specific $CD4^+$ TCR clonal depth at 6 months was associated with increased number of long COVID symptoms and dyspnea at 12 months, suggesting a role for $CD4^+$ T-cells in long COVID. This may indicate an extensive immune stimulation driving T-cell proliferation, resulting in an increased magnitude and duration of circulating spike-specific T-cells and their associated antibodies. T-cell mediated tissue damage, disruption of cytokines and cell signalling homeostasis, may thus be involved in the pathogenesis of long COVID. Further studies should investigate the role of antigen-driven dysregulation of T-cells in long COVID including functional and phenotypic characteristics of T-cell subsets.

Our study is limited by the small size hampering subgroup analysis, potential bias in self-reported symptoms, suboptimal gender- and comorbidity-matching for controls, and lack of information for controls on certain variables of interest for long COVID, such as smoking and BMI. Strengths of our study are the inclusion of a near-complete geographical cohort from the first pandemic wave and the personalized follow up to detect long COVID symptoms, which may be missed in healthcare-based registry studies. All cases were infected with the ancestral Wuhan-like strain, and the prevalence of long COVID may differ after infection with subsequent variants of concern, which have increased infectivity and cause a different range of organ-specific symptoms.

Overall, our findings should be considered as intermediate, as longer follow-up will be required to understand the nature and chronicity of long COVID. Nonetheless, it is worrisome that fatigue, dyspnea, and cognitive problems post-infection have affected an important portion of the working-age population over this extensive period.

Conclusion

The positive association between spike IgG antibodies and $CD4^+$ associated SARS-CoV-2 specific TCR sequences with long-term symptoms, supports previous published results linking immune responses to long COVID pathogenesis. Hallmark long COVID symptoms occurred far more frequently in cases than in time- and age-matched confirmed seronegative controls, suggesting a causal relationship between COVID-19 and sequelae. The high proportion of symptomatic patients at 18 months, particularly those with cognitive symptoms is concerning. It is somewhat reassuring that few patients perceived their cognitive symptoms as severe at 18 months.

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Potential conflict of interest

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Author contributions

N.L. and R.J.C. conceived and designed the study. K.K. and E.B.F. performed literature search. K.K., E.B.F., C.T. and K.G.I.-M., recruited the participants and followed them up. F.Z. developed and ran the neutralization assays. T.B.O conducted the ELISA assays. K.A.B. organized sample collection and the TCR analyses. R.E. and I.M.K. conducted the TCR analyses. T.Ö. and E.B.F. analyzed the data. B.B., R.J.C., N.L. and E.B.F. interpreted the data. N.L., R.J.C., B.B and E.B.F. wrote the manuscript. Members of the COVID-19 research group contributed to the study follow-up, data collection and laboratory assays. All authors reviewed, edited and approved the final version of the manuscript.

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Tables

Table 1 Risk of frequently reported symptoms at 12-months in age-stratified COVID-19 cases aged ≥16 compared to non-infected controls.

	All (16-81 years)				16-30 years			31-45 years			46-60 years			61-81 years		
	Case % (n) n=220	Control % (n) n=182	aOR* (95% CI) p-value	Crude risk difference 95% CI p-value	Case % (n) n=55	Control % (n) n=38	aOR* (95% CI) p-value	Case % (n) n=56	Control % (n) n=78	aOR* (95% CI) p-value	Case % (n) n=68	Control % (n) n=43	aOR* (95% CI) p-value	Case % (n) n=41	Control % (n) n=23	aOR* (95% CI) p-value
Fatigue	37% (81)	9% (17)	5.86 (3.3-10.5) <0.001	27% (20- 35) <0.001	26% (14)	13% (5)	2.95 (0.9-9.5) 0.070	39% (22)	10% (8)	5.67 (2.3-14.2) <0.001	41% (28)	2% (1)	32.98 (4.2-260.8) 0.001	42% (17)	13% (3)	4.64 (1.1-19.1) 0.033
Memory problems	26% (57)	5% (9)	7.42 (3.5-15.7) <0.001	21% (14, 28) <0.001	18% (10)	3% (1)	12.97 (1.5-110.0) 0.019	25% (14)	8% (6)	4.62 (1.6-13.2) 0.005	32% (22)	0% (0)	NA**	27% (11)	9% (2)	3.42 (0.7-17.3) 0.137
Impaired concentration	24% (53)	4% (7)	8.88 (3.9-20.4) <0.001	20% (14, 27) <0.001	26% (14)	5% (2)	7.03 (1.4-34.6) 0.016	23% (13)	3% (2)	12.66 (2.7-59.8) 0.001	29% (20)	0% (0)	NA**	15% (6)	14% (3)	1.15 (0.3-5.3) 0.860
Dyspnea	15% (34)	5% (9)	2.66 (1.2-5.8) 0.014	11% (5,16) <0.001	13% (7)	0% (0)	NA**	14% (8)	5% (4)	2.61 (0.72-9.5) 0.144	18% (12)	4% (2)	3.62 (0.7-18.0) 0.115	17% (7)	14% (3)	1.19 (0.3-5.4) 0.827

*aOR=Gender and comorbidity adjusted odds ratios with 95% confidence intervals. Comorbidity=includes the presence of any comorbidity. Corresponding 2-sided p-values <0.05 are shown in bold.

**NA=not applicable

Table 2 Longitudinal data on crude risk difference of long COVID symptoms† in 149 cases aged ≥16 years who came for 6-, 12- and 18-months follow-up compared to non-infected controls

					Excess risk compared to controls, % (CI) p		
	Controls	Cases 6m	Cases 12m	Cases 18m	6m	12m	18m
	N=182	N=148	N=149	N=149			
Fatigue	9% (17)	39% (58)	41% (61)	36% (53)	30% (21, 39) <0.001	32% (23, 41) <0.001	26% (17, 35) <0.001
more than usual	9% (17)	32% (47)	36% (53)	32% (48)	22% (14, 31) <0.001	26% (17, 35) <0.001	23% (14, 31) <0.001
much more than usual	0% (0)	7% (11)	5% (8))	3% (5)	7% (3, 12) <0.001	5% (2, 9) 0.004	3% (0, 6) 0.023
Concentration problems	4% (7)	22% (33)	29% (43)	26% (38)	18% (11, 26) <0.001	25% (17, 33) <0.001	22% (14, 29) <0.001
more than usual	4% (7)	18% (27)	27% (40)	23% (35)	14% (8, 21) <0.001	23% (15, 31) <0.001	20% (12, 27) <0.001
much more than usual	0% (0)	4% (6)	2% (3)	2% (3)	4% (0, 7) 0.012	2% (0, 4) 0.080	2% (0, 4) 0.080
Memory problems	5% (9)	21% (31)	29% (43)	33% (49)	16% (9, 23) <0.001	24% (16, 32) <0.001	28% (20, 36) <0.001
more than usual	4% (8)	20% (29)	27% (40)	30% (45)	15% (8, 22) <0.001	22% (15, 30) <0.001	26% (18, 34) <0.001
much more than usual	1% (1)	1% (2)	2% (3)	3% (4)	1% (-1, 3) 0.227	1% (-1, 4) 0.251	2% (-1, 5) 0.136
Dyspnea†	5% (9)	16% (23)	17% (25)	16% (24)	11% (4,17) 0.002	12% (5,19) <0.001	11% (4, 18) 0.001

† Severity of dyspnea was not recorded at 6 and 12 months.

CI = 95% confidence interval, p = p-values

Table 3 Associations between acute symptoms, early immune responses and long COVID symptoms at 12 months in adult cases†.

	Fatigue	**Memory problems**	**Impaired concentration**	**Dyspnea**
	aOR (CI)	**aOR (CI)**	**aOR (CI)**	**aOR (CI)**
	p-value	**p-value**	**p-value**	**p-value**
Acute phase headache (n=196)	1.37(0.69-2.74)	1.38 (0.65-2.93)	**2.34 (1.03-5.29)**	1.40 (0.55-3.53)
	0.3700	0.4100	**0.0400**	0.4800
Acute phase dyspnea (n=198)	**2.14 (1.16-3.95)**	1.85 (0.95-3.59)	1.11(0.58-2.12)	**8.55 (2.77-26.32)**
	0.0100	0.0700	0.7600	**0.0002**
Acute phase fever (n=198)	1.40 (0.72-2.70)	1.17 (0.58-2.39)	1.50 (0.73-3.08)	0.90 (0.39-2.09)
	0.3200	0.6600	0.2700	0.8100
Acute phase myalgia (n=198)	1.51 (0.80-2.86)	1.48 (0.73-2.98)	1.46 (0.73-2.93)	**3.73 (1.34-10.35)**
	0.2100	0.2800	0.2900	**0.0100**
Spike IgG titer at 2 months** (n=209)	1.16 (0.64-2.1)	1.14 (0.58-2.22)	1.39 (0.71-2.73)	**3.06 (1.23-7.61)**
	0.6300	0.7100	0.3300	**0.0200**
Microneutralizing antibody titer	0.95 (0.55-1.64)	0.80 (0.43-1.49)	0.98 (0.54-1.8)	1.21 (0.59-2.48)
at 2 months** (n=195)	0.8500	0.4800	0.9600	0.6000

† Presented as age, gender, and comorbidity adjusted odds ratios, with corresponding 2-sided p-values <0.05 shown in bold.
CI= 95% confidence interval
** IgG titer range: 50-98924. Samples with undetectable spike IgG titers were given a value of 50. Titers were log(10) transformed for calculation purposes.
** MN titers range: 10-16096. Samples with undetectable microneutralizing (MN) antibodies were given a value of 50. Titers were log(10) transformed for calculation purposes.

Table 4 Associations between SARS-CoV-2 associated T-cell clonal depth† and fatigue, memory/concentration, dyspnea and number of symptoms at 12 months.

SARS-CoV-2 associated T-cell receptor sequences	Fatigue aOR (SE)* p-value	Memory problems + impaired concentration aOR (SE)* p-value	Dyspnea aOR (SE)* p-value	Number of symptoms at 12 months¤ aRR (SE)** p-value
Total T-cell clonal depth	1.55 (0.332)	1.86 (0.378)	1.49 (0.497)	**1.71 (0.274)**
	0.1880	0.102	0.4250	**0.0499**
Total $CD4^+$ T-cell clonal depth	1.80 (0.362)	1.98 (0.395)	1.85 (0.568)	**1.94 (0.294)**
	0.1060	0.0827	0.2780	**0.0242**
Total spike specific $CD4^+$ T-cell clonal depth	2.70 (0.583)	2.77 (0.608)	**7.12 (0.969)**	**3.15 (0.483)**
	0.0889	0.0943	**0.0427**	**0.0176**

†One-tailed Fisher's exact test identified 8630 SARS-CoV-2-associated TCRβ sequences, and potential false positive TCRβ sequences associated with cytomegalovirus (CMV) or human leukocyte antigen (HLA) alleles were removed. SARS CoV-2 associated TCRβ sequences subsets were classified as Class I associated ($CD8^+$ T-cells) or Class II associated ($CD4^+$ T-cells), and spike or non-spike-associated. T-cell depth corresponded to the relative expansion of SARS-CoV-2 associated T-cell clonal subtypes

¤Number of symptoms at 12 months were encoded as integers from 0 to 11 including the total of 11 symptoms assessed.

*Odds ratio (aOR), adjusted for age, gender and scaled reciprocal SARS-CoV-2 associated T-cell breadth, with standard error (SE), corresponding 2-sided p-value <0.05 are shown in bold.

** aRate ratio (aRR), adjusted for age, gender and scaled reciprocal SARS-CoV-2 associated T-cell breadth, with standard error (SE), corresponding 2-sided p-value <0.05 are shown in bold.

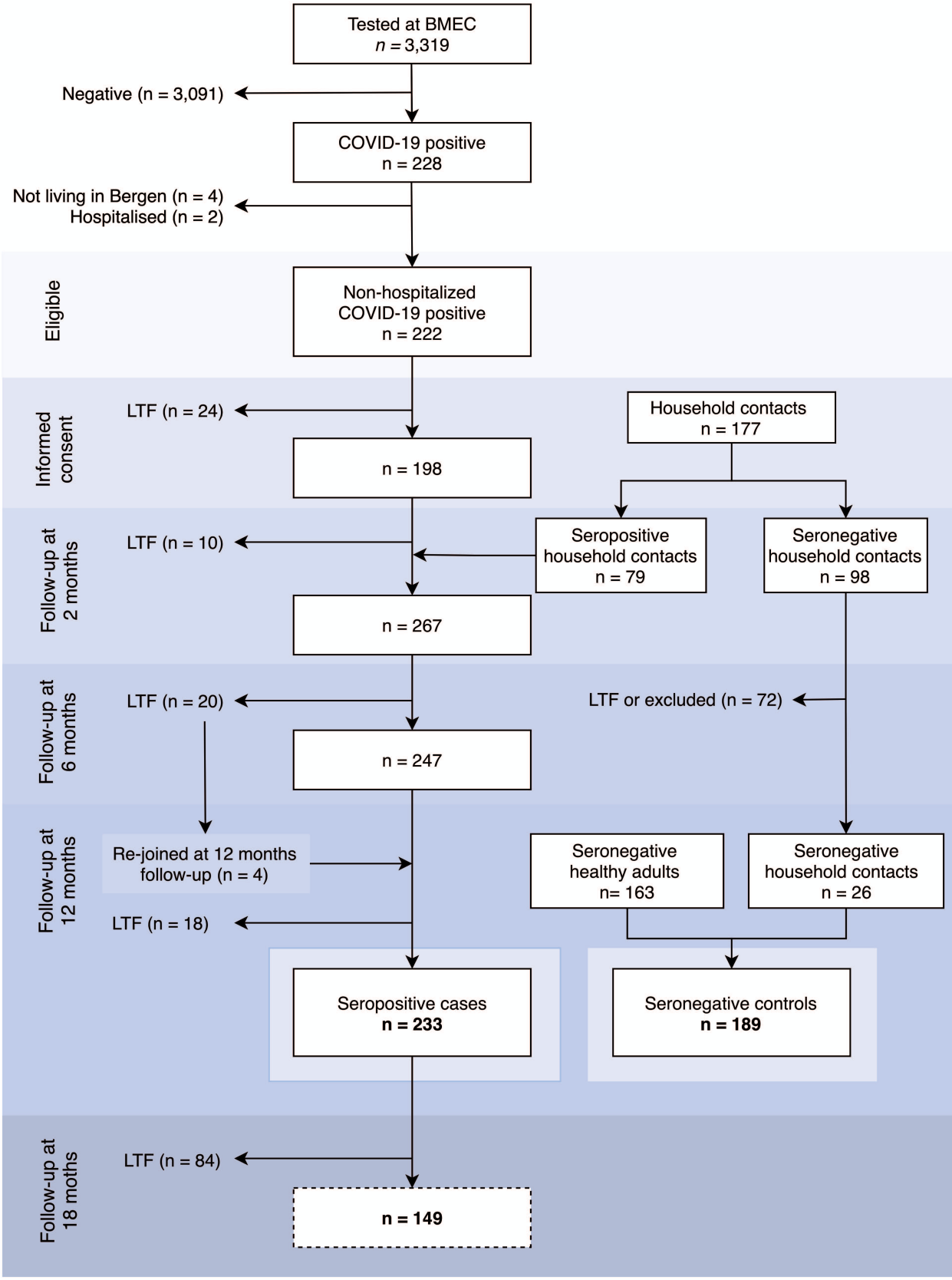
Figure legends

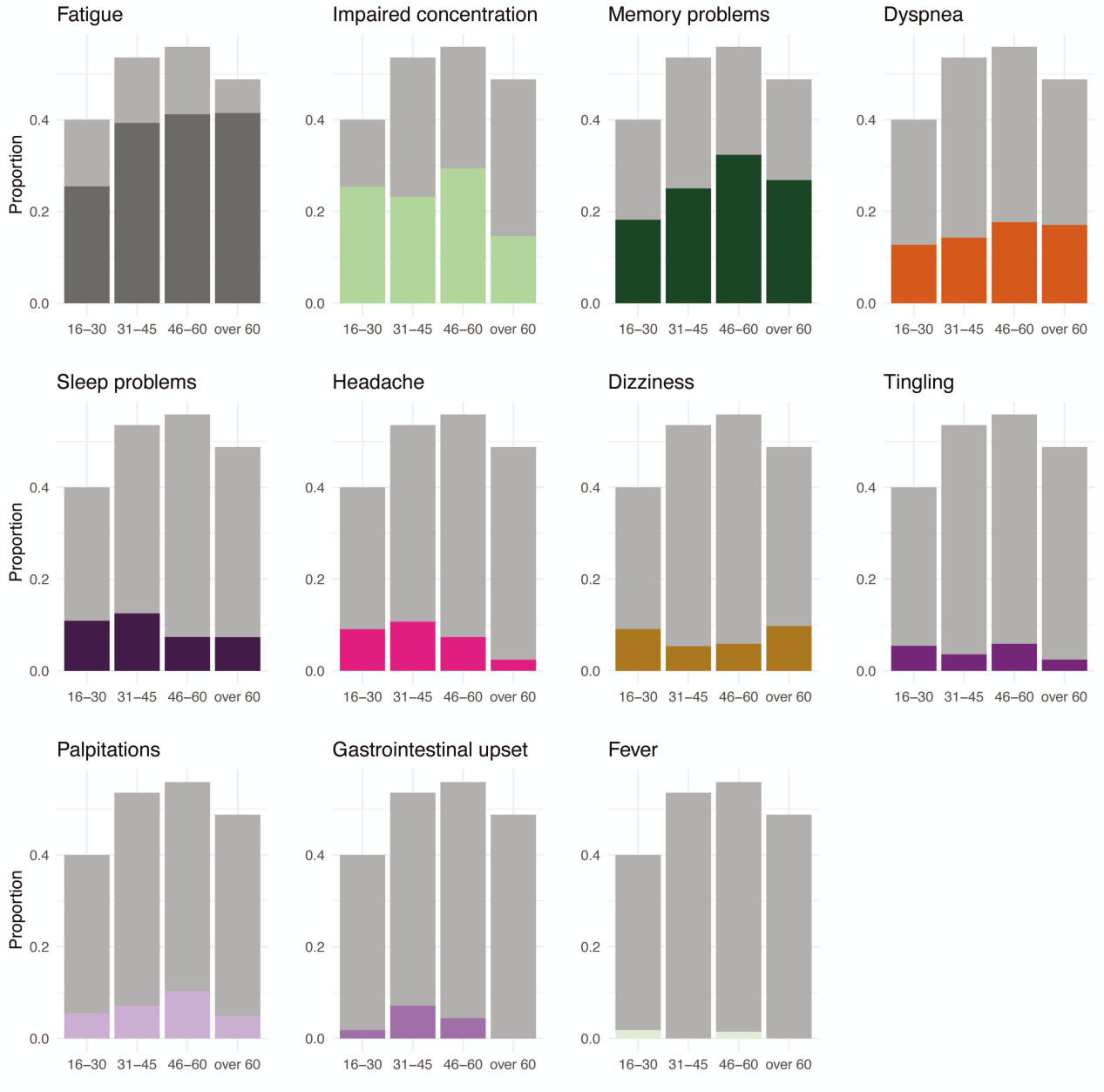
Figure 1 Study population.
Inclusion of SARS-CoV-2 cases (left) and control group (right). Eligible participants tested for SARS-CoV-2 infection by RT-PCR at Bergen Municipality Emergency Clinic (BMEC) were recruited between February 28th and April 4th, 2020. Only one case (the first most symptomatic) from each household was tested due to limited testing capacity, thus individuals living with COVID-19 positive study participants were included as household contacts. If household contact had positive SARS-CoV-2 serology (RBD and spike-IgG ELISA) within 2 months after recruitment, they were registered as cases. Seronegative household contact without a history of COVID-19 symptoms were included as controls. Additional controls were recruited amongst individuals who were prioritized for vaccination, either because of their age, comorbidity or occupation. At the time of symptom recording, all controls were confirmed seronegative. LTF = Lost to follow-up

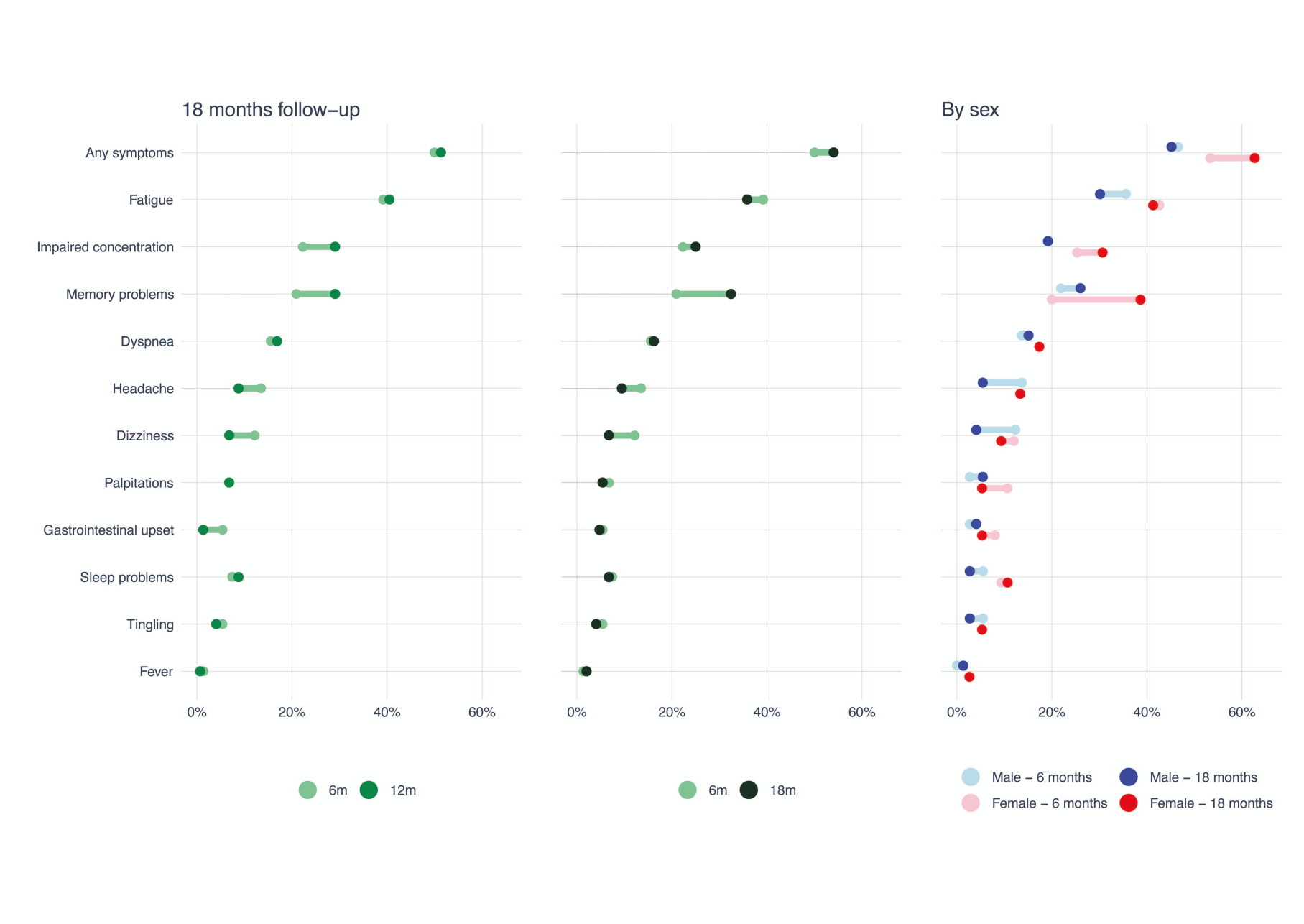
Figure 2 Age-stratified symptom prevalence at 12 months post infection.
Bar plot representing the proportion of cases reporting 11 key symptoms at 12 months follow-up. The cases reported a mean of 1.4 symptoms overall. The age group 0-15 years old (n=13) is not shown due to absence of symptoms. The light gray area in the bar charts represent the overall proportion with any of the 11 symptom in the current age group. The colored areas represent the proportion with the specified symptoms.

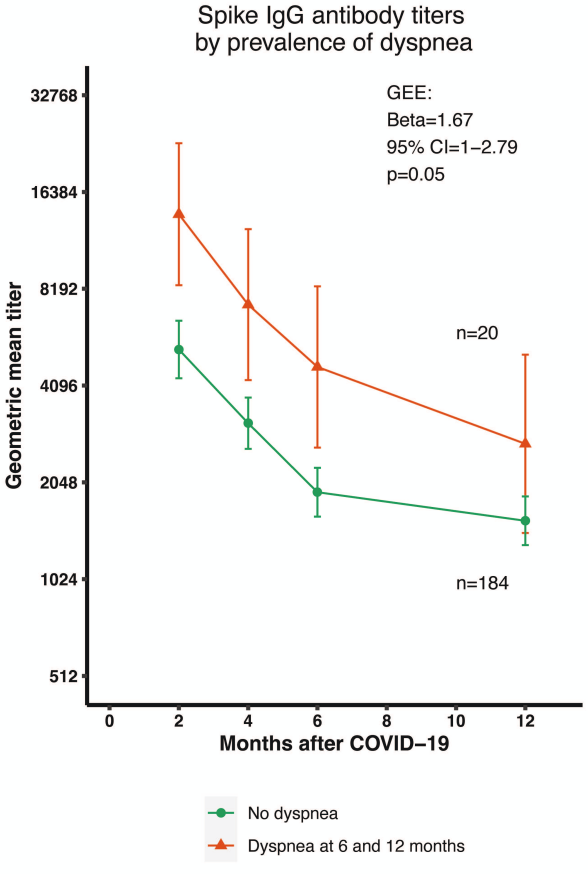
Figure 3 Longitudinal symptom changes up to 18 months post-infection.
Dumbbell charts present longitudinal data on development of 11 specified symptoms in a subcohort of patients followed for 18 months (n=148, one patient was excluded due to missing data on all symptoms at 6 months). 3a) presents the overall symptom change from 6 to 12 months, 3b) presents the overall symptom change from 6 to 18 months and 3c) presents the symptom change in men (n=73) and women (n=75) from 6 to 18 months.

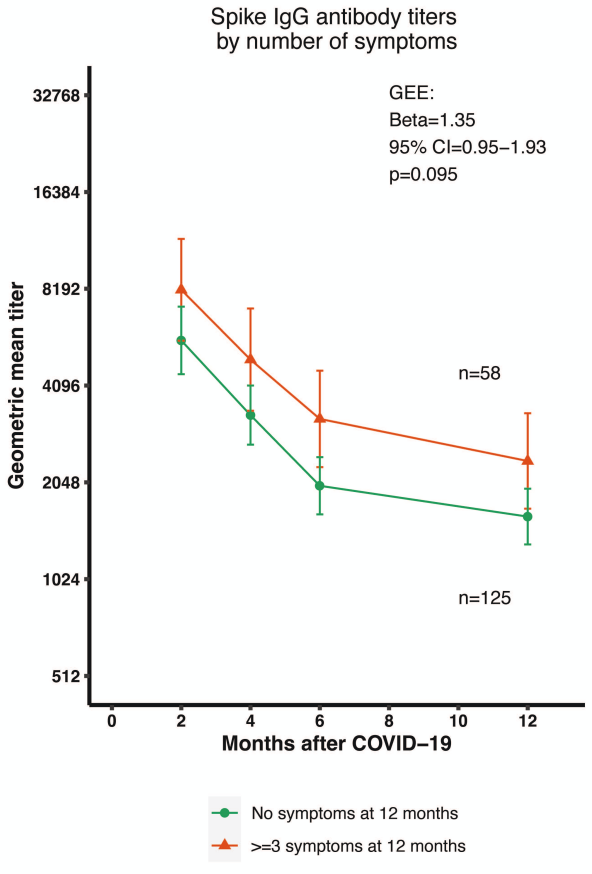
Figure 4 Kinetics of the spike IgG antibody response in relation to symptoms at 6 and 12 months.
The relationship between longitudinal antibody titers and a) persistent dyspnea versus no dyspnea, b) 3 or more symptoms at 12 months versus no symptoms and c) persistent fatigue versus no fatigue. The generalized estimating equation (GEE) coefficients with 95% Confidence intervals (CI) are adjusted for age, gender, comorbidity and time of measurement. All cases who had been vaccinated against SARS-CoV-2 during the follow-up period (n=20) were excluded from the analysis of immunological parameters at 12 months.

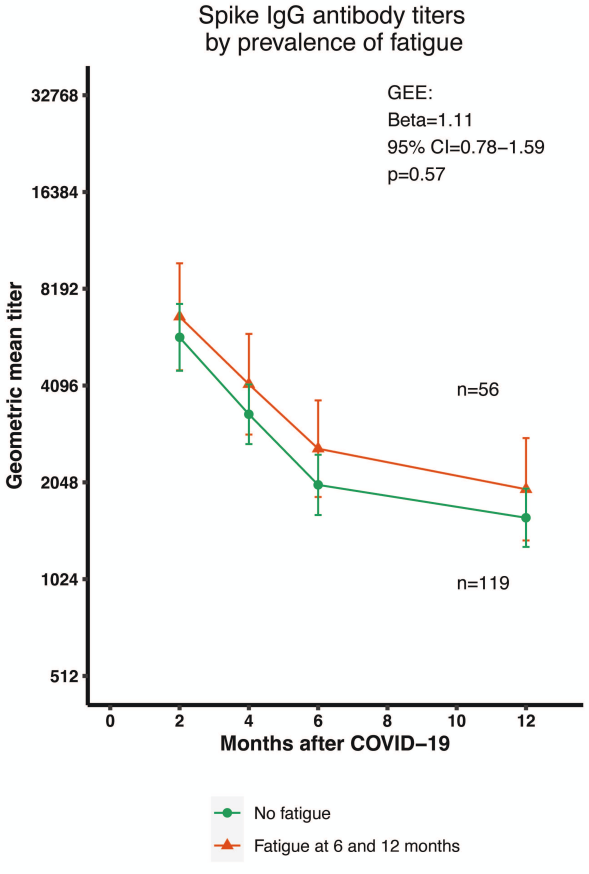

Tested at BMEC
n = 3,319
Negative (n = 3,091)
COVID-19 positive
n = 228
Not living in Bergen (n = 4)
Hospitalised (n = 2)
Eligible
Non-hospitalized
COVID-19 positive
n = 222
Informed consent
LTF (n = 24)
n = 198
Household contacts
n = 177
Follow-up at 2 months
LTF (n = 10)
Seropositive household contacts
n = 79
Seronegative household contacts
n = 98
n = 267
Follow-up at 6 months
LTF (n = 20)
LTF or excluded (n = 72)
n = 247
Follow-up at 12 months
Re-joined at 12 months follow-up (n = 4)
Seronegative healthy adults
n= 163
Seronegative household contacts
n = 26
LTF (n = 18)
Seropositive cases
n = 233
Seronegative controls
n = 189
Follow-up at 18 moths
LTF (n = 84)
n = 149


Fatigue
Impaired concentration
Memory problems
Dyspnea
Sleep problems
Headache
Dizziness
Tingling
Palpitations
Gastrointestinal upset
Fever
Proportion
0.0
0.2
0.4
16–30
31–45
46–60
over 60


18 months follow–up
By sex
Any symptoms
Fatigue
Impaired concentration
Memory problems
Dyspnea
Headache
Dizziness
Palpitations
Gastrointestinal upset
Sleep problems
Tingling
Fever
0%
20%
40%
60%
6m
12m
18m
Male – 6 months
Male – 18 months
Female – 6 months
Female – 18 months


Spike IgG antibody titers
by prevalence of dyspnea
GEE:
Beta=1.67
95% CI=1–2.79
p=0.05
n=20
n=184
Geometric mean titer
32768
16384
8192
4096
2048
1024
512
0
2
4
6
8
10
12
Months after COVID–19
No dyspnea
Dyspnea at 6 and 12 months


Spike IgG antibody titers
by number of symptoms
GEE:
Beta=1.35
95% CI=0.95–1.93
p=0.095
n=58
n=125
Geometric mean titer
32768
16384
8192
4096
2048
1024
512
0
2
4
6
8
10
12
Months after COVID–19
No symptoms at 12 months
>=3 symptoms at 12 months


Spike IgG antibody titers
by prevalence of fatigue
GEE:
Beta=1.11
95% CI=0.78–1.59
p=0.57
n=56
n=119
Geometric mean titer
32768
16384
8192
4096
2048
1024
512
0
2
4
6
8
10
12
Months after COVID–19
No fatigue
Fatigue at 6 and 12 months

Supplementary table 1. Demographic and clinical profile of COVID-19 cases and age-matched SARS-CoV-2 naive controls.

	All cases (0-81 years)			0-15 years			16-30 years			31-45 years			46-60 years			61-81 years		
	Case	Control	p-value	Case	Control	p-value	Case	Control	p-value	Case	Control	p-value	Case	Control	p-value	Case	Control	p-value
	233	189		13	5		55	38		56	78		68	45		41	23	
Age, median (IQR)	44 (27)	41 (21)	0.576	7 (9)	8 (4)	0.289	24 (5)	26 (4)	0.056	37 (8)	38 (8)	0.887	53 (6)	52 (8)	0.897	68 (9)	73 (17)	0.377
Female	53% (124)	66% (124)	0.010	62% (8)	40% (5)	0.608	55% (30)	79% (30)	0.016	52% (29)	58% (45)	0.498	54% (37)	73% (33)	0.043	50% (20)	61% (14)	0.352
Any comorbidity*	53% (124)	42% (80)	0.026	15% (2)	0% (0)	1.000	47% (26)	47% (18)	0.993	52% (29)	41% (32)	0.217	52% (35)	31% (14)	0.033	78% (32)	70% (16)	0.452
Chronic lung disease	12% (28)	8% (15)	0.168	15% (2)	0% (0)	1.000	9% (5)	11% (4)	1.000	11% (6)	5% (4)	0.319	13% (9)	4% (2)	0.195	15% (6)	22% (5)	0.470
Hypertension	11% (25)	7% (14)	0.241	0% (0)	0% (0)	-	0% (0)	3% (1)	0.409	4% (2)	3% (2)	1.000	16% (11)	2% (1)	0.026	29% (12)	44% (10)	0.251
Rheumatic disease	7% (16)	3% (5)	0.047	0% (0)	0% (0)	-	6% (3)	0% (0)	0.267	4% (2)	0% (0)	0.173	9% (6)	0% (0)	0.079	12% (5)	22% (5)	0.313
Chronic heart disease	6% (11)	6% (11)	0.915	0% (0)	0% (0)	-	0% (0)	0% (0)	-	0% (0)	4% (3)	0.265	4% (3)	2% (1)	1.000	24% (10)	30% (7)	0.599
Diabetes	4% (9)	4% (7)	0.932	0% (0)	0% (0)	-	2% (1)	3% (1)	1.000	2% (1)	3% (2)	1.000	3% (2)	2% (1)	1.000	12% (5)	13% (3)	1.000
Neurological disease	3% (8)	2% (3)	0.359	0% (0)	0% (0)	-	4% (2)	3% (1)	1.000	2% (1)	0% (0)	0.418	2% (1)	2% (1)	1.000	5% (2)	13% (3)	0.341

Immuno-suppression	2% (5)	4% (7)	0.387	0% (0)	0% (0)	-	2% (1)	0% (0)	1.000	2% (1)	4% (3)	0.640	2% (1)	2% (1)	1.000	10% (4)	4% (1)	0.646
Malignancy	2% (4)	1% (1)	0.386	0% (0)	0% (0)	-	0% (0)	0% (0)	-	0% (0)	0% (0)	-	2% (1)	0% (0)	1.000	7% (3)	4% (1)	1.000
Smoking Currently	3% (8)	-	-	0% (0)	-	-	2% (1)	-	-	2% (1)	-	-	6% (4)	-	-	5% (2)	-	-
Smoking previously	26% 61	-	-	0% (0)	-	-	9% (5)	-	-	29% (16)	-	-	31% (21)	-	-	46% (19)	-	-
BMI (median, IQR)	24.4 (22.4)	-	-	17.8 (5.0)	-	-	23.4 (15.6)	-	-	24.9 (17.3)	-	-	24.7 (4.7)	-	-	25.4 (15.0)	-	-

Data are presented as overall and age-stratified cases and controls. Continuous data are presented as median (interquartile range) and categorical data as percentages. 2-sided p-values with a significance level <0.05 are marked in bold.

*Any comorbidity is defined as one or more of the following: chronic lung disease, hypertension, rheumatic disease, chronic heart disease, diabetes, neurological disease, immuno-suppression, malignancy and other chronic diseases.

Supplementary table 2. Chalder Fatigue Scale (CFS) by gender at 6 and 12 months in COVID-19 cases and in controls.

		Men Control	Women Control	p-value	Men Case 6m	Women Case 6m	p-value 6m	Men Case 12m	Women Case 12m	p-value
	CFS-item*	n=61	n=121		n=104	n=115		n=104	n=116	
1	Do you have problems with tiredness?									
	More than usual	7% (4)	11% (13)	0.429	27% (28)	31% (35)	0.566	28% (29)	35% (41)	0.275
	Much more than usual	0% (0)	0% (0)	NA	4% (4)	10% (12)	0.072	3% (3)	7% (8)	0.225
2	Do you need to rest more?									
	More than usual	7% (4)	8% (10)	0.776	22% (23)	33% (38)	0.072	23% (24)	32% (37)	0.170
	Much more than usual	0% (0)	0% (0)	NA	2% (2)	6% (7)	0.176	1% (1)	5% (6)	0.124
3	Do you feel sleepy or drowsy?									
	More than usual	5% (3)	7% (8)	0.753	21% (22)	28% (32)	0.253	21% (22)	30% (35)	0.149
	Much more than usual	0% (0)	1% (1)	1.000	0% (0)	4% (5)	0.061	1% (1)	2% (2)	0.638
4	Do you have problems starting things?									
	More than usual	3% (2)	5% (6)	0.719	15% (16)	22% (25)	0.229	21% (22)	28% (33)	0.243
	Much more than usual	0% (0)	0% (0)	NA	2% (2)	4% (4)	0.686	1% (1)	4% (5)	0.218
5	Do you lack energy?									
	More than usual	12% (7)	14% (17)	0.817	21% (22)	29% (33)	0.199	30% (31)	35% (40)	0.520
	Much more than usual	0% (0)	1% (1)	1.000	4% (4)	7% (8)	0.382	1% (1)	7% (8)	**0.039**
6	Do you have less strength in your muscles?									
	Less than usual	7% (4)	6% (7)	1.000	16% (17)	14% (16)	0.615	19% (20)	16% (18)	0.427
	Much less than usual	0% (0)	0% (0)	NA	1% (1)	4% (5)	0.216	1% (1)	3% (3)	0.625
7	Do you feel weak?									
	More than usual	0% (0)	4% (5)	0.169	7% (8)	18% (21)	**0.021**	16% (17)	19% (22)	0.659
	Much more than usual	0% (0)	0% (0)	NA	1% (1)	4% (5)	0.216	0% (0)	1% (1)	1.000
8	Do you have difficulties concentrating?									
	More than usual	2% (1)	5% (6)	0.426	14% (15)	17% (19)	0.668	20% (21)	25% (29)	0.439
	Much more than usual	0% (0)	0% (0)	NA	0% (0)	8% (9)	**0.004**	0% (0)	3% (3)	0.250
9	Do you make slips of the tongue when speaking?									
	More than usual	2% (1)	1% (1)	1.000	5% (5)	10% (11)	0.177	8% (8)	10% (11)	0.668
	Much more than usual	0% (0)	0% (0)	NA	0% (0)	1% (1)	1.000	0% (0)	3% (3)	0.250
10	Do you find it more difficult to find the right word?									
	More than usual	2% (1)	3% (4)	0.664	21% (22)	24% (27)	0.680	24% (25)	26% (30)	0.819
	Much more than usual	0% (0)	0% (0)	NA	1% (1)	3% (3)	0.623	1% (1)	8% (9)	**0.021**
11	How is your memory?									
	Worse than usual	3% (2)	5% (6)	0.719	16% (17)	18% (21)	0.709	17% (18)	30% (35)	**0.031**
	Much worse than usual	2% (1)	0% (0)	0.337	1% (1)	3% (3)	0.623	1% (1)	3% (3)	0.625
	Total Chalder score** (mean, SD)	10.98 (1.93)	11.07 (2.39)	0.891	12.99 (3.47)	14.24 (4.87)	**0.033**	13.26 (3.71)	14.44 (4.40)	**0.024**

Table present the frequencies of the two highest scores according to the CFS by Likert scale (0-3). 2-sided p-values <0.05 are marked in bold

Supplementary table 3 Symptom prevalence by gender at 6 and 12months

	6 months			12 months		
	Men N=104, (100%)	Women N=116 (100%)	p-value	Men n(%) N=104, (100%)	Women n(%) N=116 (100%)	p-value
Any symptom*	45 (43%)	65 (67%)	0.050	40 (39%)	68 (59%)	**0.003**
Fatigue*	32 (31%)	47 (41%)	0.120	32 (31%)	49 (42%)	0.078
Impaired concentration*	15 (14%)	28 (24%)	0.065	21 (20%)	32 (28%)	0.200
Memory problems*	18 (17%)	24 (21%)	0.504	19 (18%)	38 (33%)	**0.014**
Dyspnea	14 (14%)	21 (18%)	0.347	15 (14%)	19 (16%)	0.689
Headache	11 (11%)	17 (15%)	0.365	4 (4%)	11 (11%)	**0.046**
Dizziness	11 (11%)	13 (11%)	0.881	2 (2%)	4 (12%)	**0.004**
Gastrointestinal upset	5 (5%)	10 (9%)	**0.263**	2 (2%)	6 (6%)	0.286
Palpitations	3 (3%)	12 (10%)	0.033	1 (1%)	15 (13%)	**<0.001**
Sleep problems	4 (4%)	9 (8%)	0.262	6 (6%)	15 (13%)	0.071
Tingling	5 (5%)	5 (4%)	1.000	1 (1%)	9 (8%)	**0.021**
Fever	0 (0%)	3 (3%)	0.249	0 (0%)	2 (2%)	0.499

*n=219 at 6 months.
P-values are 2-sided, p-values <0.05 are marked in bold.

Supplementary table 4. Overview of antibody titers over time, measured by Enzyme-linked Immunosorbent Assay (ELISA) and Microneutralization (MN) assay

	Spike IgG titers (ELISA)*			Microneutralization titers**		
Time point	Subjects (N)	Geometric mean titer	Titer range	Subjects (N	Geometric mean titer	Titer range
2 months	231	6128	50-98924	217	161.42	10-16096
4 months	212	3604	50-38632	211	95.09	10-3307
6 months	227	2225	50-36094	208	72,89	10-1640
12 months (- vac. cases)	199	1724	101-24955	186	69.72	10-2304
12 months (+vac. cases)	217	2201	101-315160	203	94.99	10-38776

*In ELISA, samples with no detectable antibodies were assigned a titer of 50. Samples with no detectable antibodies at 2m=2%, 4m=2%, 6m=2%, and 12m=0%.

** In MN assay, samples with no detectable antibodies were assigned a value of 10. Samples with no detectable antibodies at 2m=5%, 4m=6%, 6m=13% and 12m=12%.

Supplementary methods

Overview of study follow-up visits.

	Baseline/ enrolment	2 months	4 months	6 months	12 months	18 months
Demographics						
Age	x					
Gender	x					
Comorbidities*	x			x	x	x
BMI	x			x		
Smoking habits	x			x		
Pregnancy (females)	x	x		x	x	x
Household contacts	x					
Medication						
Immunosuppressive medication	x	x		x	x	x
Other	x	x		x	x	x
Original Infection	x					
Date of symptom onset	x					
Tested for RT-PCR	x					
Date of positive RT-PCR	x					
Contact with SARS CoV-2 infected persons	x					
Duration of acute illness		x				
Acute symptoms						
Fever	x	x				

Cough	x	x				
Fatigue	x					
Headache	x					
Dyspnea	x	x				
Myalgia	x					
Other symptoms	x					
Hospitalization	x	x		x	x	x
Persistent symptoms						
Yes/no		x		x	x	x
Cough		x				
Fever		x		x	x	x
Headache				x	x	x
Dyspnoea		x		x	x	x
Dizziness				x	x	x
Tingling/ Numbness				x	x	x
Palpitations				x	x	x
Sleep problems				x	x	x
Stomach problems/change in bowel movements	□			x	x	x
Chalder fatigue scale				x	x	x
Depression						x
Concentration/memory difficulties (Children only)					x	x
More tired than normal (Children only)					x	x

Re-infection (if relevant)						
Date of symptom onset		x		x	x	x
Tested for RT-PCR		x		x	x	x
Date of positive RT-PCR		x		x	x	x
Contact with SARS CoV-2 infected persons		x		x	x	x
COVID vaccination				x	x	x
Vaccine type				x	x	x
Number of doses				x	x	x
Date of doses				x	x	x
Adverse events after vaccination				x	x	x

*Chronic lung disease, hypertension, rheumatic disease, chronic heart disease, diabetes, neurological disease, immuno-suppression, malignancy and other chronic illnesses.

The case report forms, except for the Chalder Fatigue Questionnaire, were developed in-house.

Enzyme-linked Immunosorbent Assay (ELISA)

Serum samples were analyzed in a two-step ELISA, firstly by antibody screening for the Wuhan receptor-binding domain (RBD), followed by endpoint Wuhan spike ELISA using the Wuhan spike protein, 100ng/well as previously described [1, 2]. Briefly, duplicates of sera were serially diluted in a 5-fold manner from 1:100. The chromogenic substrate 3,3′,5,5′-tetramethylbenzidine (TMB; BD Biosciences, San Jose, CA, USA) was used to detect horseradish peroxidase (HRP)-labelled secondary antibodies directed against IgG (SouthernBiotech, Birmingham, AL, USA). Optical density (OD) was measured at 450/620 nm using the Synergy H1 Hybrid Multi-Mode Reader with the Gen5 2.00 (version 2.00.18) software (BioTek Instruments Inc., Winooski, VT, USA). Pooled pre-pandemic sera (n=128) were used as a negative control. IgG endpoint titers were reciprocal to the serum dilution with an OD value of 3 standard deviations above the mean of negative controls. For calculation purposes, samples with no detectable antibodies were appointed a titer of 50.

Microneutralization assay

The microneutralization (MN) assay was performed in a certified Biosafety Level-3 Laboratory using a local SARS-CoV-2 isolate from March 2020, hCoV-19/Norway/Bergen-01/2020 (GISAID accession ID EPI_ISL_541970) as previously described [1, 2]. Briefly, serially diluted paired sera (from 1:20) and 100 tissue culture infectious dose 50% ($TCID_{50}$) virus were incubated for 1 hour at 37°C and subsequently transferred to 96-well plates pre-seeded with Vero cells for 24-hour incubation at 37°C, before anti-nucleocapsid protein (NP) immunostaining. The MN titer was determined as the reciprocal of the serum dilution giving 50% inhibition of virus infectivity. For calculation purposes, samples without detectable antibodies were assigned a value of 10.

Identification of SARS-CoV-2 associated T cell receptor (TCRβ) sequences

Genomic DNA was extracted from EDTA blood using the Qiagen DNeasy Blood Extraction Kit (QIAGEN, Germantown, MD) and amplified in a bias-controlled multiplex PCR, followed by high-throughput sequencing. SARS-CoV-2 associated CDR3 regions of TCRβ chains were sequenced using the ImmunoSEQ Assay T-MAP™ COVID platform (Adaptive Biotechnologies, Seattle, WA) as previously described [3].
One-tailed Fisher's exact test identified 8630 SARS-CoV-2-associated TCRβ sequences, and potential false positive TCRβ sequences associated with cytomegalovirus (CMV) or human leukocyte antigen (HLA) alleles were removed. Subsets of SARS-CoV-2 associated TCRβ sequences were categorized as Class I or Class II clonal subtypes and further divided into spike and non-spike-associated sequences. The outcome is presented as the *clonal breadth*,

defined as the fraction of the overall T cell receptor repertoire that represented SARS-CoV-2 specific T-cell clonal lineages, and the *clonal depth*, defined as the relative frequency of SARS-CoV-2 specific T-cell clones in a repertoire.

References:

1. Kuwelker K, Zhou F, Blomberg B, et al. Attack rates amongst household members of outpatients with confirmed COVID-19 in Bergen, Norway: A case-ascertained study. Lancet Reg Health Eur **2021**; 3: 100014.
2. Trieu MC, Bansal A, Madsen A, et al. SARS-CoV-2-Specific Neutralizing Antibody Responses in Norwegian Health Care Workers After the First Wave of COVID-19 Pandemic: A Prospective Cohort Study. The Journal of infectious diseases **2021**; 223(4): 589-99.
3. Snyder TM, Gittelman RM, Klinger M, et al. Magnitude and Dynamics of the T-Cell Response to SARS-CoV-2 Infection at Both Individual and Population Levels. medRxiv **2020**.

Errata for

Clinical studies of epidemic influenza and pandemic COVID-19 to improve the chain of patient care: from bedside diagnostics to long-term complications

Elisabeth Berg Fjelltveit

Thesis for the degree philosophiae doctor (PhD)
at the University of Bergen

07.11.22 EBFjelltveit

(date and sign. of candidate)

08.11.22

(date and sign. of faculty)

Errata

Page IX,72,80,81 Missing hyphen: "Sars CoV-2" – corrected to "Sars-CoV-2"
Page X Rephrasing: "som illustrerer at antibiotikastyrings-verktøy utover akutt pasientmottak» – corrected to "som antyder at anitbiotikastyrings-verktøy i forløpet etter akuttmottak"
Page XIII Missing hyphen: "Point of care" – corrected to "Point-of-care"
Page XIII Added abbreviation: "WWI World War I"
Page 3,9,11,13,23,67,77,88 Misspelling: "Influenza" – corrected to "influenza"
Page 6 Misspelling: "modelling" – corrected to "modeling"
Page 6 Missing words: "ARDS" – corrected to "acute respiratory distress syndrome (ARDS)"
Page 7 Missing word: "replication errors" – corrected to "RNA replication errors"
Page 8 Rephrasing: "vaccine roll-out" – corrected to "roll-out of vaccines"
Page 9 Wrong number: "five last influenza" – corrected to "four last influenza"
Page 11 Added referral to figure 2
Page 12 Rephrasing: "enable to infect a cell" – corrected to "capable of infecting a cell"
Page 12 Missing words: "RNA" – corrected to "ribonucleic acid (RNA)"
Page 12 Misspelling: "proteins" – corrected to "protein"
Page 13 Repetition: "of new viral RNA (vRNA)" – corrected to "of new vRNA"
Page 13 Added referral to figure 3: "The influenza replication cycle is illustrated in **figure 3**"
Page 15 Repetition: "acute respiratory distress syndrome (ARDS)" – corrected to "ARDS"
Page 16 Misspelling: "were" – corrected to "where"
Page 16 Repetition: "Norwegian Institute of Public Health (NIPH)" – corrected to "NIPH"
Page 17 Rephrasing "Intensive care influenza patients" – corrected to "Influenza patients in intensive care units (ICU) "
Page 17 Added referral to figure 4 in text
Page 19 Added referral to figure 5: "The SARS-CoV-2 replication cycle is illustrated in **figure 5**"
Page 19 Missing word: "The RBD" – corrected to "The receptor binding domain (RBD)"
Page 21 Misspelling: "VOCs" – corrected to "VOC"
Page 22 Missing full stop: "*Haemophilus influenzae*[128]" – corrected to "*Haemophilus influenzae*[128]."
Page 23 Misspelling: "paitents" – corrected to "patients"
Page 23 Misspelling: "correlates" – corrected to "correlate"
Page 23 Misspelling: "resent" – corrected to "recent"
Page 25 Repetition: "interferon response" – corrected to "IFN response"
Page 26 Misspelling: "homes" – corrected to "home".
Page 27 Missing word: "influenza and SARS-CoV-2" – corrected to "influenza and SARS-CoV-2 infection".
Page 27 Improve clarity: "hemagglutinin inhibiting effect" – corrected to "hemagglutination inhibition effect"
Page 28 Remove excess words: "and importantly."
Page 29 Spacing: " Depending" – corrected to "Depending"
Page 30 Misspelling: "co-stimuation" – corrected to "co-stimulation"
Page 30,33,57 Misspelling: "SARS-COV-2" – corrected to "SARS-CoV-2"
Page 31 Misspelling: "has" – corrected to "have"

Page 31 Missing words: "risk factors such as pregnancy," – corrected to "risk factors such as older age, pregnancy,"
Page 32 Misspelling: "to-hit model" – corrected to "two-hit model"
Page 32 Misspelling: "vaccine hesitation" – corrected to "vaccine hesitancy"
Page 33 Misspelling: "Pfizer/BioNTec" – corrected to "Pfizer/BioNTech"
Page 33 Misspelling: "aims" – corrected to "aim"
Page 34 Missing word and misspelling: "LAIV can elicit both systemic HI responses" – corrected to "Live attenuated influenza vaccines (LAIV) can elicit both systemic HA responses"
Page 34 Missing word "Studying infection" – corrected to "By studying infection"
Page 37 Missing hyphen: "antigen based" – corrected to "antigen-based"
Page 37 Misspelling: "result" – corrected to "results"
Page 37 Rephrasing: "better targeted" – corrected to "better suited"
Page 38 Rephrasing: "the hospital setting[200,201], but test sensitivity" – corrected to "the hospital setting[200,201]. However, test sensitivity".
Page 46 Missing word: "A subgroup of cases was also followed for 18 months." – corrected to "A subgroup of patient was additionally followed for 18 months (n=149)."
Page 46 Rephrasing: "with the purpose of following immunological studies, still ongoing." - corrected to "with the purpose of still ongoing immunological studies."
Page 48 Missing word: "electronic CRFs" -corrected to "electronic case report forms (eCRFs)"
Page 52 Replace word: "the *clonal depth,* defined as the relative proportion" – corrected to "the *clonal depth,* defined as the relative expansion"
Page 52 Corrected order of figure 11 and 12, referenced in text
Page 53 Misspelling. "gen" – corrected to "gene"
Page 55 Misspelling "was" – corrected to "were"
Page 56 Missing word "CI" – corrected to "confidence interval (CI)"
Page 59 Misspelling: "limits" – corrected to "limited"
Page 62 Rephrasing: COVID-19 infected – corrected to "SARS-CoV-2 infected"
Page 64 Missing word: "are present or beyond" – corrected to "are present at or persist beyond"
Page 64 Misspelling: "confounding" – corrected to "confounders"
Page 66 Corrected title: "one year" corrected to 6 to 18 months"
Page 66 Misspelling: "ads" – corrected to "adds", "health-care" – corrected to "health care", "12" – corrected to "18"
Page 68,71,72,75,78 Remove spacing before reference
Page 70 Repeated word: "sensitivity" – corrected to "specificity"
Page 72 Word removal: "length-of-stay" (LOS)" – corrected to "LOS"
Page 82 Word removal: "COVID-19 infection" – corrected to "COVID-19"

All text "aligned left" – corrected to "justified"

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