Diagnosis, prevention and treatment of acute respiratory infections

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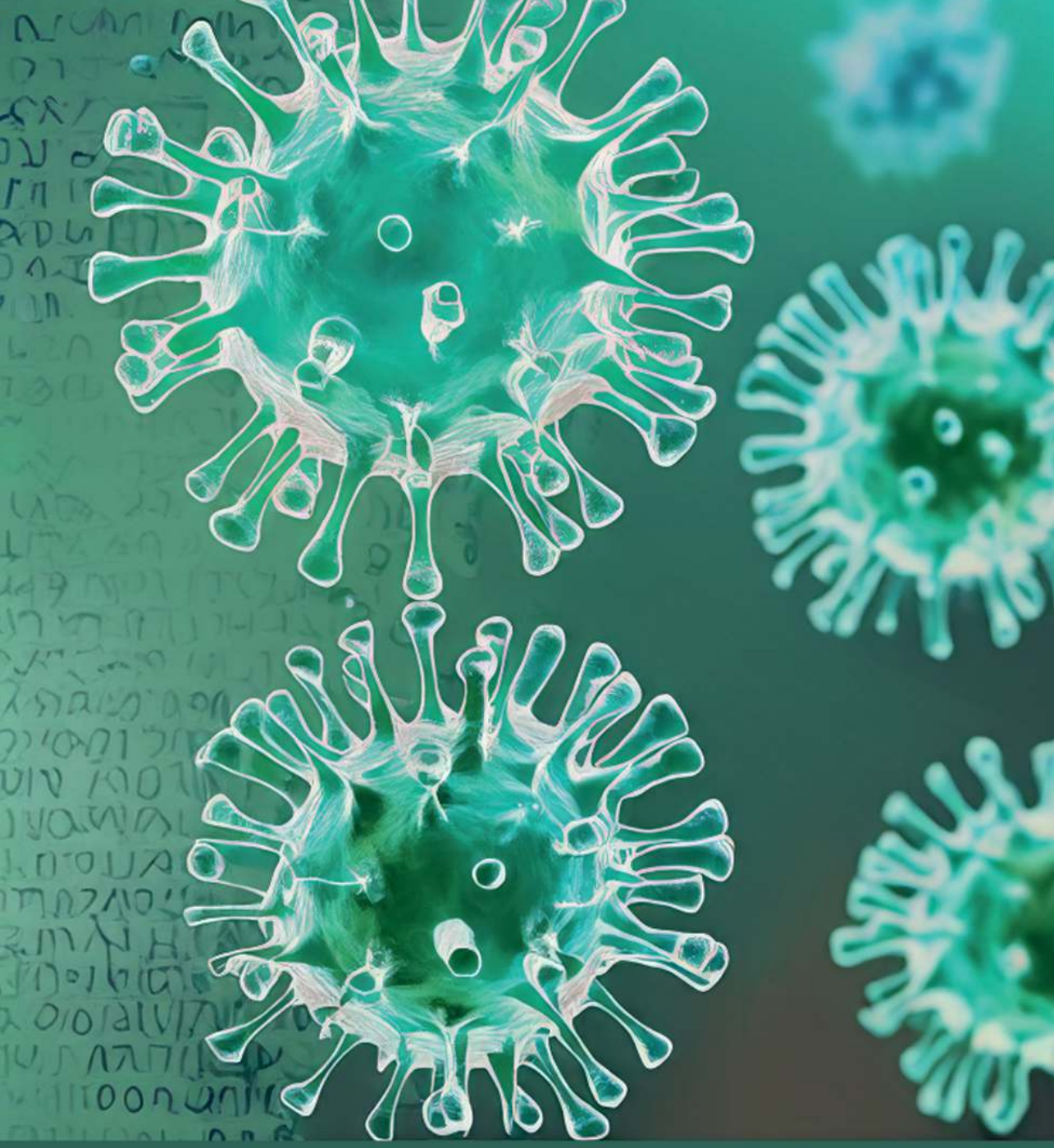


Chapter 1

General introduction and outline of this thesis

General introduction

Respiratory airway infections caused by viruses are an integral part of our lives and our society. Most of these infections are mild, leading to common colds, but some are leading to rapid deterioration which requires hospital admission or even mechanical ventilation. Many respiratory viruses are well known, such as influenza and respiratory syncytial virus (RSV), but in 2019 we were surprised by a new kid on the block: severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), causing COVID-19 disease. This virus will remain etched in our memory for the rest of our lives as it caused a pandemic of such magnitude that almost no one currently alive had experienced before. Nevertheless, the previous major pandemic which occurred one century ago, the influenza pandemic (1918-1920)[1], had not been forgotten, and parallels have been drawn between the two pandemics to understand and potentially predict the course of a global infectious disease threat.

While the influenza virus has existed for hundreds of years and influenza pandemics have occurred throughout the history, SARS-CoV-2 was identified at the end of 2019 in Wuhan, China. SARS-CoV-2 spread rapidly to other countries in the world, resulting in the global pandemic from February 2020 until May 2023.[2,3] Since the start until December 2024, the COVID-19 pandemic has reached over 776 million confirmed cases and many more unconfirmed cases, and over 7 million reported deaths.[4] However, the full impact of the pandemic has been much more significant than wat is reflected in the reported deaths from COVID-19 alone. Excess mortality helps capture not only the deaths directly caused by the virus, but also those indirectly related to the pandemic. Global studies on excess mortality during the COVID-19 pandemic have estimated a pooled global excess mortality rate of 104.84 deaths per 100.000 people.[5] In low- and lower-middle-income countries, the calculated excess mortality was 100.3 deaths per 100,000 people per year. However, these estimates remain uncertain due to relatively poor data.[6]

In contrast to SARS-CoV-2, the influenza virus has caused pandemics and epidemics every year (seasonal influenza) and is associated with 290.000 to 650.000 respiratory deaths annually worldwide.[7] The COVID-19 lockdown measures implemented during the pandemic led to a reduced circulation of influenza and other respiratory infections, by limiting opportunities for their reintroduction and local transmission. Furthermore, the implementation of COVID-19 vaccines in 2021 resulted in a decline in the administration of other vaccines, including those for influenza.[8-10]This lack of exposure to influenza and the reduced vaccination rates may have weakened population immunity, potentially resulting in more severe seasonal epidemics in the future.[11] Indeed, following the ease of pandemic restrictions, influenza showed pre-epidemic numbers of detection during the 2022/2023 and 2023/2024 winter season in the Netherlands.[12]

Among the many respiratory viruses causing RVIs (e.g. parainfluenza virus, rhinovirus, adenovirus, human metapneumovirus, other non-SARS-CoV-2 coronaviruses, bocavirus and RSV), SARS-CoV-2 and influenza are the most common infections worldwide in patients who seek healthcare due to respiratory illness.[13] Both the COVID-19 pandemic and the yearly influenza epidemics posed a threat of overwhelming the acute health care system. Overcrowding in the ED was an important

worldwide healthcare problem, as was the increasing number of hospitalizations and intensive care unit (ICU) admissions.[14] Similar pressure on healthcare is seen during peak respiratory virus seasons, which may affect the quality and access of health care.[15] Emergency services need to be ready to tackle the obstacles presented by future pandemics or epidemics or other infectious diseases. Prevention and adequate early treatment of these highly transmissible respiratory viruses could be very effective to lower the impact of these infections on the healthcare system. In this chapter, I will first outline and compare the structure and characteristics of both influenza virus and SARS-CoV-2. Subsequently, I will focus on the primary emphasis of my thesis: addressing gaps in scientific knowledge regarding SARS-CoV-2 and influenza, and how closing this gaps can contribute to enhancing future (acute) care.

Structure of influenza virus and SARS-CoV-2

Influenza virus

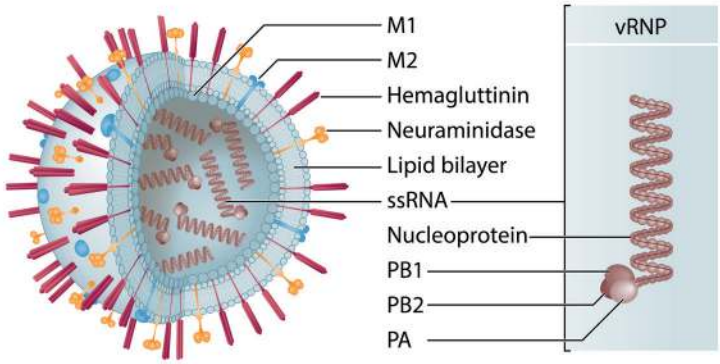
There are four types of influenza viruses: type A, B, C and D, of which only A and B are associated with human infections, causing seasonal epidemics of disease. Influenza C is infrequently detected and typically leads to mild infections, hence is of little medical concern when assessing healthcare impact and patient perspectives. In contrast, influenza D viruses predominantly impact cattle and are not known to affect humans.[13]

Influenza A, B and C are members of the Orthomyxoviridae, which is a family of negative single-stranded RNA (ssRNA) genomes. Although the structure of the influenza virus is somewhat variable, influenza A and B are virtually indistinguishable by electron microscopy. They are classified based on antigenic differences in their nucleoprotein (NP) and matrix protein (M1), both major structural components of their virions. Two different varieties of glycoprotein spikes are embedded in the envelope of the virus: the hemagglutinin (HA) and the neuraminidase (NA). Influenza A is further classified in subtypes based on specific combinations of these surface glycoproteins, A(H1N1) and A(H3N2).[16] The A(H1N1) is also denoted as A(H1N1)pdm09, the previous A(H1N1) virus leading to the 2009 pandemic.[13] Its noteworthy that only influenza type A viruses, with three HA (H1, H2, H3) and two NA (N1 and N2) subtypes, have been associated with causing pandemics.[16] Influenza B is not classified in subtypes, but is categorized by distinct lineages; type B viruses are grouped into either the B/Yamagata or B/Victoria lineage.

For a schematic presentation of influenza A, see figure 1. The influenza genome is organized in eight ssRNA segments. The RNA is bound to nucleoproteins. Together with three polymerase peptides (polymerase basis 1 (PB1), polymerase basic 2 (PB2) and polymerase acid (PA)) for each RNA segment, it forms the viral ribonucleoprotein (vRNP), that functions as a complex for replication of genomic RNA. The surface glycoprotein HA mediates the attachment of the virus to the host cell, whereas NA is predominantly involved in facilitating the release of newly produced virus particles from the host cell. M1 forms a coat inside the virus envelope. The membrane protein (M2) is a proton ion channel.[16]

The organization of influenza B is similar, with four envelope proteins: HA, NA, and instead of M2, NB protein and B matrix protein 2 (BM2).[16]

Figure 1. Schematic representation of the influenza A virus



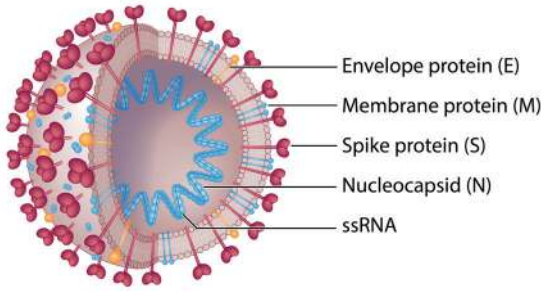
M1= matrix protein; M2= membrane protein; ssRNA= single-stranded RNA; PB1= polymerase basis 1; PB2= polymerase basis 2; PA= polymerase acid; vRNP= viral ribonucleoprotein

SARS-CoV-2

The SARS-CoV-2 virus belongs to the genus Betacoronavirus, a genus that includes several coronaviruses (SARS-CoV, MERS-CoV and others). Coronaviruses are enveloped positive sense ssRNA viruses. SARS-CoV-2 is genetically closely related to the SARS-CoV, which was the initial pandemic threat of a novel and lethal coronavirus that emerged in late 2002, leading to an outbreak of SARS. The structural proteins of SARS-CoV-2 are the spike (S), envelope (E), membrane (M), and nucleocapsid (N) proteins (figure 2).[17] The S protein binds to the angiotensin-converting enzyme 2 (ACE2) receptor, a membrane protein located at the outer surface of many human cells and facilitates virus entry into the host cell by inducing membrane fusion. The S protein is recognized as a focal point for mutations that substantially influence virus virulence, transmissibility, and the ability to evade the host immune responses.[18] The N protein is associated with the RNA genome inside virus particles. The M protein serves as a structural component influencing the morphology of the envelope and it binds to the N protein to strengthen the nucleocapsid. Additionally, the M protein contributes to the viral envelope composition by interacting with protein E, which has minor impact on the virus's envelope formation.[19]

A crucial difference between SARS-CoV-2 and influenza lies in the make-up of their genome.[20] The genome of positive sense ssRNA coronaviruses functions as messenger RNA (mRNA); the genome does not have to be transcribed as mRNA first.[21] In the case of influenza, the vRNPs are transported into the nucleus of the host cell after infection. A RNA-dependent RNA polymerase is essential for transcription of the negative sense ssRNA and replication of the viral RNA genome.[22]

Figure 2. Schematic representation of the SARS-CoV-2 viral particle



The structural proteins of SARS-CoV-2 are the spike (S), envelope (E), membrane (M), and nucleocapsid (N) proteins [17]

Viral genomic evolution leading to decreased immune cell recognition

Influenza virus

Influenza is a continuously evolving virus and is widely recognized for its susceptibility to undergo antigenic drift and antigenic shift. Mutations in the antigenic structure of the influenza virus have resulted in a number of different influenza subtypes and strains.

Antigenic drift is a process where relatively minor point mutations in the viral genome accumulates, thereby giving rise to new strains of the influenza virus due to the expression of different HA or NA glycoproteins. Consequently, surface antigens differ from those in previous antigens and are therefore not recognized by the human's immune system. The virus strain is no longer effectively neutralized by host antibodies. Through this mechanism, the virus can effectively evade the body's immune system. Thus, immunity acquired from either earlier infection or vaccination does not necessarily protect against symptomatic infections with the newly developed strains. Influenza A and B both frequently undergo antigenic drift, yet the yearly reformulation of influenza vaccines often allows scientists to consider and incorporate any newly emerged strains.[16]

Antigenic shift occurs when there is an exchange of RNA segments between genetically distinct influenza viruses, enabling the creation of new antigens and leading to the emergence of a new strain or subtype. This virus may encode entirely new antigenic proteins to which the human population lacks any pre-existing immunity. As a result, it has the potential to rapidly cause a global pandemic and scientist are unable to prepare effective vaccines in advance, as these new strain may develop abruptly and unpredictably.[16]

SARS-CoV-2

Like the influenza virus, SARS-CoV-2 has changed over time due to antigenic drift. In addition, studies have demonstrated that, in comparison to previous variants, the Omicron variant exhibited an unprecedented degree of antigenic novelty, which can be compared to an influenza-like antigenic shift event.[23,24] It has contributed to the need for booster vaccinations and required the update of vaccines to align with evolving variants.[23,25] Newly emerged, noteworthy variants, with important effects on the properties of the virus (such as an increase in transmissibility or virulence, a change in clinical presentation or decrease in effectiveness of public health and social measures or available diagnostics, vaccines and therapeutics), are classified as variants of concern (VOC), based on working definitions used by the World Health Organization (WHO).[26] Each variant has several designations, based on the nomenclature by distinct phylogenetic classification system. In addition, from May 2021 onwards, the WHO has also assigned labels to key variants, using the Greek alphabet (table 1).[27]

Table 1. Previously circulating VOCs[26]

WHO label	Pango lineage*	GISAID clade	Nextstrain clade	Country first detected	Date of dominance in the Netherlands
Alpha	B.1.1.7	GRY	20I (V1)	United Kingdom, Sep-2020	Dec-2020
Beta	B.1.3.51	GH/501Y.V2	20H (V2)	South Africa, May-2020	Jan-2021
Gamma	P.1	GR/5-1Y.V3	20J (V3)	Brazil, Nov-2020	NA
Delta	B.1.617.2	G/478G.V1	21A, 21I, 21J	India, Oct-2020	Summer of 2021
Omicron	B.1.1.529	GR/484A	21K	Multiple countries, Nov-2021	Early 2022

The different names associated with different variants of concern (VOC) are determined according to various nomenclature systems (e.g. those assigned by the WHO, Pango, GISAID and Nextstrain).
GISAID= Global Initiative on Sharing all Influenza Data
**includes all descendent lineages*

Clinical presentation

Although RVIs can be classified according to the causative pathogen, they are generally classified clinically according to the affected system as upper respiratory tract infections (rhinitis [common cold], sinusitis, ear infections, acute pharyngitis, epiglottitis, laryngitis) or lower respiratory tract infection (pneumonia, bronchiolitis).[28] Although characteristic clinical features such as olfactory and gustatory dysfunction appear to be common in the early stages of a SARS-CoV-2 infection[29] (with a lower prevalence of olfactory dysfunction caused by the omicron variant[30]), the clinical presentation of both viruses varies widely and symptoms could be similar. Both viruses have the

potential to induce various respiratory and systemic symptoms, such as sneezing, coughing, rhinitis, sore throat, nasal congestion, fever, malaise, myalgia, headache and other non-specific symptoms. Therefore, it is not possible to discriminate the causative virus, based on clinical presentation alone. The severity of viral respiratory illness due to both influenza and SARS-CoV-2 could range from asymptomatic and mild, self-limiting upper respiratory tract infection to severe disease requiring mechanical ventilation.

A difference between the two viruses is the risk of developing thrombotic complications. Respiratory viruses, including influenza, were already known to lead to a procoagulant state. Studies during early COVID-19 breakout suggested that SARS-CoV-2 infection could induce a hypercoagulable state, resulting in thromboembolism, and elevated D-dimer levels has been reported as a prognostic factor for severe COVID-19.[31,32] Studies examining thrombotic complications have demonstrated that the risk of venous thromboembolism was higher among hospitalized patients with COVID-19, compared with hospitalized patients with influenza.[33,34]

During the COVID-19 pandemic, the clinical course and clinical outcomes were affected by the evolution of the virus and the emergence of new VOCs, in contrast to influenza, where the clinical course and endpoints varies much less. Infection with a SARS-CoV-2 VOC increased the risk of disease severity and death, compared to other non-VOC variants. In addition, individuals infected and hospitalized with the Omicron variant have experienced a lower clinical severity of infection with different presenting symptoms (less cough, fever, shortness of breath, fatigue and anosmia) compared to earlier waves, with a notably reduced likelihood of hospitalization, severe disease, ICU admission or mortality.[35-38] This could be due to a lower pathogenicity of this variant or more likely pre-existing immunity. The impact of influenza fluctuates from one season to another, partly because of variations in the type or subtype of circulating influenza, as well as differences in influenza vaccine coverage and effectiveness. Typically, lower vaccine effectiveness and consequently higher hospitalization rates are linked to seasons where influenza A H3N2 is predominant.[39] However, also individuals hospitalized with influenza A H1N1pdm09 and B virus infections could have severe outcomes.[40] Thus, in contrast to COVID-19, the clinical course of influenza infection does not become milder over the years, despite achieving (herd) immunity.

Mortality for COVID-19 seems to be higher compared to influenza, particularly seasonal influenza.[41] This difference in mortality rate was more pronounced in the first year(s) of the COVID-19 pandemic. However, also late in the pandemic (in 2022/2023), hospitalization for COVID-19 was associated with a higher risk of death, compared to hospitalization for influenza (hazard ratio, 1.61 [95% confidence interval, 1.29-2.02]).[42] The decline in mortality rates among hospitalized COVID-19 patients later in the COVID-19 pandemic could be attributed to alterations in SARS-CoV-2 variants and to increased levels of immunity resulting from both vaccination and previous infection. Risk factors contributing to severe disease for both viruses include among other older age, diabetes mellitus and multiple (chronic) comorbidities and immunocompromised status[43-45], such as patients with a solid organ transplant (SOT). In SOT patients, disruption in critical components of both innate and adaptive immune responses may result in uncontrolled viral replication, leading to progression of the infection to the lower respiratory tract, thereby giving rise to consequential complications including bacterial or fungal co-infection, respiratory failure and even death.[44]

Transmission

Both the influenza virus and SARS-CoV-2 spread easily between people by infectious droplets, containing virus particles, through close-range contact.[46,47] These droplets are released into the air when an infected individual coughs, sneezes, talks or sighs, allowing other individuals to become infected through inhalation or direct contact with mucous membranes. SARS-CoV-2 virus uses the ACE2 as cell-entry receptor.[48] The binding of the S protein of the SARS-CoV-2 to the ACE2 receptor is a critical step in establishing an infection.[46] The influenza virus can also be transmitted through hand contact if the hands are contaminated with the virus.[13] There is currently no conclusive evidence for indirect contact transmission of SARS-CoV-2 in humans.[46,49]

An important difference between the two viruses is the speed of transmission. The transmission rate of SARS-CoV-2 is higher than that of seasonal influenza.[50] The incubation period of influenza is about two days (range 1-4 days)[13], while the incubation period of SARS-CoV-2 is longer, ranging from 1 to 14 days.[51] The serial interval (the duration between symptom onset in the primary case and symptom onset in its secondary case) was reported to be 2.3-5.8 days for the Delta and 2.1-4.8 days for the Omicron variant[52], while the serial interval for influenza virus is shorter: 2.2 days for influenza A(H3N2) and 2.8 for influenza A(H1N1)pdm09.[53] The reproductive number (the average number of secondary transmissions from one infected individual) seemed to be higher for SARS-CoV-2 than for influenza. The contribution of asymptomatic infection to onward transmission is in both viruses recognised.[54,55] However, while there is evidence of transmission from asymptomatic people with COVID-19[56], the proportion of transmission attributable to asymptomatic infections remain largely unanswered for the influenza virus.[57]

Diagnosis

As diagnosing these different respiratory infections is impossible purely on clinical symptoms, molecular diagnostics through reverse transcriptase polymerase chain reaction (RT-PCR) has become the primary method to identify which respiratory virus is causing the infection. The PCR can be performed on nasopharyngeal (NP) swabs, oropharyngeal (OP) swabs, anterior/mid-turbinate nasal swabs, bronchoalveolar lavage (BAL), sputum and saliva.[58] It amplifies viral RNA through a series of repetitive cycles. The test is considered positive when the amount of fluorescence signal generated during the reaction crosses a pre-established threshold, and the number of reaction cycles required to achieve this specified threshold is referred to as the cycle threshold (Ct) value. Ct values are indicators of the pathogen load, with lower numbers of RT-PCR amplification cycles and thus a lower Ct value indicating higher viral titres or viral load (a greater amount of viral RNA present in the original sample). In addition, higher viral loads are correlated with increased probability of isolating infectious virus. Thus, the Ct value is a proxy marker to assess whether a patient has an active, contagious infection and thus is considerable infectious. However, this is affected by various factors, including the assay itself (e.g. sample volume, chemical conditions and whether a single copy or multi-copy region design is targeted) as

well as factors inherent to the sample (e.g. sample quality, and inhibitors) that can influence the efficiency of amplification.[59] For SARS-CoV-2, many studies have found correlations between SARS-CoV-2 RT-PCR Ct values and the ability to culture live virus.[60] The Ct value of viral RNA level above which infectiousness is unlikely may vary from >24 to ≥32 for SARS-CoV-2.[61,62] In contrast, for influenza, the threshold Ct value above which infectivity is unlikely, has not been thoroughly investigated. Influenza-positive specimens are identified by RT-PCR Ct values below ≤40, with lower Ct values associated with more illness severity.[63]

Traditionally, respiratory specimens collected from the nasopharynx have been regarded as having the highest sensitivity for detecting viruses, including SARS-CoV-2.[64, 65] For influenza B and A(H3N2)pdm09, NP swabs had a better combined calculated diagnostic sensitivity than OP samples.[66,67] However, another study reported that OP swabs were significantly more sensitive than NP swabs for the 2009 pandemic influenza A(H1N1) virus, with no difference between the two methods for influenza A(H3N2) virus.[68] Collecting combined NP and OP specimens would be the most effective approach.[68,69] In addition, a combination of two less-invasive swabbing methods, such as nasal and OP swabs, had about the same sensitivity as NP swabs for both influenza and SARS-CoV-2.[67,70] For patients who produce sputum, sputum samples might be more appropriate than NP for the detection of respiratory viruses, for both influenza and SARS-CoV-2[71-73], and for the assessment of bacterial superinfections.

Vulnerable populations

Certain groups of individuals are at an increased risk of severe illness due to RVIs, making them particularly vulnerable. High-risk groups are people older than 60 years, those with underlying health conditions, and those with immune-compromising conditions that severely impair their immune response. Specifically, high-risk groups include patients with haematological malignancies, renal failure, solid tumours, those undergoing treatment with immunosuppressants, and patients who have received an solid organ, stem cell or bone marrow transplant.[74] In this thesis I have investigated the burden of RVIs in solid organ transplant (SOT) recipients, as there has not been much research on this in non-lung transplant recipients. They are at increased risk due to their daily use of immunosuppressive medication, affecting the functionality of B- and T- cells and leading to a diminished serological immune response to vaccinations.[75-78] Preventing (severe) influenza and COVID-19 is crucial for this vulnerable population.

Prevention of transmission

Effective influenza and COVID-19 control relies both on vaccinations to protect persons before they getting infected and on the use of control measures and transmission prevention strategies in infectious individuals.[79,80]

Influenza virus

The first inactivated influenza vaccine was licensed for wide use in 1945.[1] Influenza vaccines are updated yearly and designed to provide protection against the four

main groups of type A and B influenza viruses anticipated to be most prevalent in the upcoming season. The WHO makes a recommendation on the composition of flu vaccines in February and September each year, for the Northern Hemisphere's and the Southern Hemisphere's, respectively. These recommendations are based on review of a variety of data, including epidemiologic data (which influenza viruses are circulating in different parts of the world), genetic data, antigenic data, human serology studies, evolutionary analyses and vaccine effectiveness studies. Subsequently, each country independently determines which viruses should be incorporated into influenza vaccines licensed in their country.[81] As of 2019, the quadrivalent influenza vaccine (designed to provide active immunisation against four influenza virus strains [two A subtypes and two B types]) has replaced the trivalent influenza vaccine (which protects against three different influenza virus strains) in the nation immunization program in the Netherlands. The majority of influenza vaccines are administered through injection (inactivated and recombinant influenza vaccines), although live attenuated influenza vaccines are also available as a nasal spray for children.[13] Yearly influenza vaccination is recommended for people who are at higher risk of developing serious influenza-related complications, such as older patients (≥60 years old), patients with underlying (chronic) diseases, and immunocompromised patients (such as patients receiving a SOT).[7,44]

SARS-CoV-2

In contrast to influenza vaccines, COVID-19 vaccines have been developed more recently and in a short(er) time frame due to the urgent need. Several different vaccine platforms were used: live-attenuated virus vaccines, inactivated virus vaccines, nucleid acid vaccines (DNA vaccines, RNA vaccines), viral vector vaccines and protein subunit vaccines. All but the nucleid acid platform were already used for licensed vaccines prior to the COVID-19 pandemic.[17,18] Among the four major structural proteins, the S protein is the major antigenic target for COVID-19 vaccines and one of the main immunogens widely used in developing serological assays.[18,82,83] Antibodies binding to the receptor binding domain (RBD) of the SARS-CoV-2 S protein prevent attachment to the host cell and neutralize the virus.[48] The first SARS-CoV-2 vaccines became available approximately one year after the start of the COVID-19 pandemic. In the Netherlands, the first mRNA vaccines were approved in January 2021 (Pfizer/BioNTech and Moderna). All were given intramuscularly, as this is the standard inoculation route of most vaccines.

Effectiveness of a vaccine

In the literature, several methods are commonly used to measure the effectiveness of a vaccine. Measuring an immunological response is one of these methods. This can include both antibody levels measured through serological tests, as well as cellular immunity, specifically the evaluation of T-cell responses, using assays such as ELISPOT or flow cytometry. However, a stronger immune response does not always indicate the clinical effectiveness of a vaccine, since there can be discrepancies between the virus strains included in the vaccine and those circulating in the population. Therefore,

assessing the vaccine effectiveness (VE) is regarded as a key outcome measure for evaluating clinical effectiveness. Assessing VE is also imported for several other reasons, such as public health decisions, guiding recommendations for specific subgroups, monitoring the real-world vaccine impact, boosting public health confidence in vaccines, and resource allocation.

During the development of a vaccine, it is essential to establish efficacy thresholds. This helps to identify the best candidates for different roles (e.g. vaccines that can prevent or eradicate an epidemic versus those that can reduce its impact). Vaccine efficacy refers to the performance of a vaccine under controlled conditions, such as a clinical trial, while VE refers to how well a vaccine performs in real-world conditions, assessing the impact of a vaccine on disease incidence in the general population. It reflects data from observational studies and real-world use. The effectiveness in the general population can be lower than the efficacy in a trial due to variations in healthcare access, population health and timing of vaccine administration. The preferred efficacy rate according to the WHO is 70%, but its minimum is 50%.[84] A vaccine with lower efficacy can still be effective for targeted vaccination, such as the protection of specific groups. Even if a vaccine doesn't fully protect or eliminate an epidemic, it can still save a significant number of lives, reduce hospitalizations, and lower costs.[85]

Effectiveness of the influenza vaccine

The effectiveness of the seasonal influenza vaccine is examined each year in the general population, utilizing both Dutch and European data, and is published annually by the National Institute for Public Health and the Environment (RIVM). Over the past decade, the estimated effectiveness of the (inactivated) influenza vaccine in the Dutch population has ranged from 31% to 57% in the general population, regardless of age.[86-90] However, the VE for the seasonal influenza vaccine in SOT recipients remain unknown, despite their increased risk of complications. Research indicates that these patients experience a reduced serological immune response to vaccinations compared to the general population due to the inhibition of the immune system.[75-78] Consequently, the VE in SOT patients may also be lower than is observed in the general population; however the available literature on this topic is limited. Benefits of influenza vaccination in SOT recipients have only been reported in relation to disease severity, occurrence of complications and outcomes, like graft outcomes, disease severity and length of hospital stay.[91-94] Further research is needed to determine the VE in specific vulnerable population. In this thesis, we determined the influenza VE in immunocompromised adults with a SOT.

Effectiveness of SARS-CoV-2 vaccines

Since the introduction of COVID-19 vaccines, many studies have been conducted (in the general population and across various subgroups) to evaluate the clinical VE of different vaccines. All approved Western vaccines have shown effectiveness exceeding 50%, with the original mRNA-based vaccines demonstrating the highest total efficacy (Pfizer 95%[95], Moderna 94%[96]). VE was subsequently confirmed in real-world settings beyond clinical trials.[97,98] Despite the ongoing evolution of SARS-CoV-2, individuals who have been previously infected or vaccinated maintained significant protection

against COVID-19 due to either natural or vaccine-induced immunity.[99]

The risk of SARS-CoV-2 infection among SOT recipients has drawn significant attention, with various studies indicating an increased risk of severe disease in this group. Similar to influenza, many studies have concentrated on vaccine immunogenicity by examining antibody production following immunization. These studies have found that SOT recipients exhibit a markedly lower humoral and cellular immune response to two doses of SARS-CoV-2 vaccines compared to immunocompetent individuals, although this response can vary across target organs and is different for different age groups. Booster vaccinations could elicit a more robust immune response, and the immunogenicity of mRNA based vaccines was greater than that of inactivated vaccines in SOT recipients.[100]

In contrast to influenza, more data is available regarding the clinical effectiveness of SARS-CoV-2 vaccines in SOT recipient. The real-world VE of SARS-CoV-2 vaccines against infection in this population was reported to be 31%, 46% and 72% after one, two and three doses, respectively. When examining clinically important outcomes (including hospitalization or death), the VE was 38%, 54% and 67%, respectively. Therefore, while the VE for SARS-CoV-2 infection in SOT recipients is lower compared to the general population (approximately 90% against infection and clinically import outcomes after two doses[97,101]), effectiveness notably improves after three doses.[102] This finding aligns with another study, that reported a significant protective effect of the SARS-CoV-2 vaccines against severe disease, with the third dose providing additional protection beyond the initial two doses (overall effectiveness was 47% after two doses and 64% after three or more doses).[103]

Vaccine inequity

During the COVID-19 pandemic, vaccine inequity, referring to the disparities in access to vaccines among different populations, became prominently visible worldwide. Despite the rapid development and production of COVID-19 vaccines, world-wide immunization had been hampered by limited vaccine supplies, especially in low- and middle-income countries.[4,104] Consequently, the vaccine coverage in low-income countries was low, with high morbidity and mortality. A large unvaccinated population continues to pose a threat of emerging new SARS-CoV-2 variants that may be more infectious, more virulent and more resistant to vaccines than current strains.[105] Therefore, increasing global immunity was crucial in combatting the pandemic. Intradermal (ID) vaccination is a dose-sparing strategy with the benefit that more people can be vaccinated with the same stockpile, and the potential additional advantage of fewer side effects.[06,107] In ID administration, vaccine is introduced directly into the papillary dermis (figure 3 & 4), which contains a higher density of antigen presenting dendritic cells (APC) than muscle tissue, which makes it an obvious compartment to administer vaccine antigens into.[108] In addition, the extensive dermal lymphatic system aids efficient transport of vaccine antigen and APCs to the draining regional lymph nodes allowing an optimal presentation to B- and T-lymphocytes (figure 5).[109] ID vaccination through a 1/10th or 1/5th fractional vaccine dose introduced directly into the papillary dermis can be as effective as the IM administration of the standard dose. This has already been demonstrated for the seasonal influenza vaccine and several

other vaccines, such as rabies, yellow fever, and inactivated polio.[110,111] However, ID vaccination was not evaluated during the development of the COVID-19 vaccines. In this thesis, we discuss the ID route of administration of the mRNA COVID-19 vaccine as a dose sparing technique. This has also been the first time that an mRNA vaccine was administered directly into the skin.

Figure 3. Skin anatomy

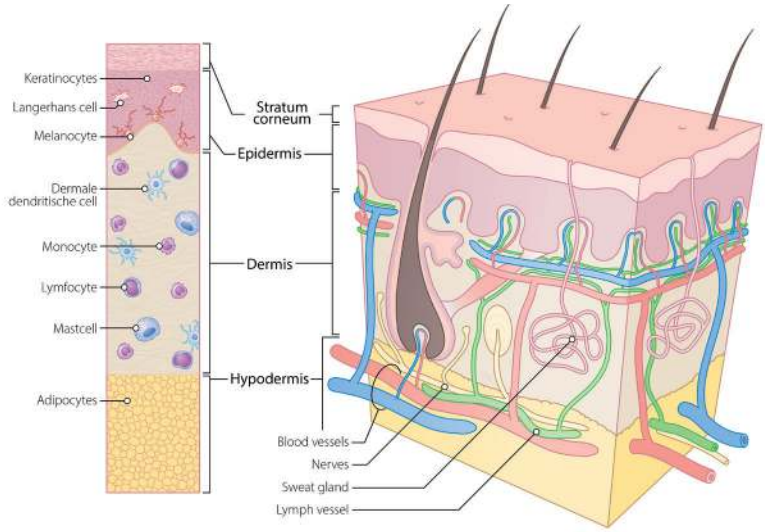
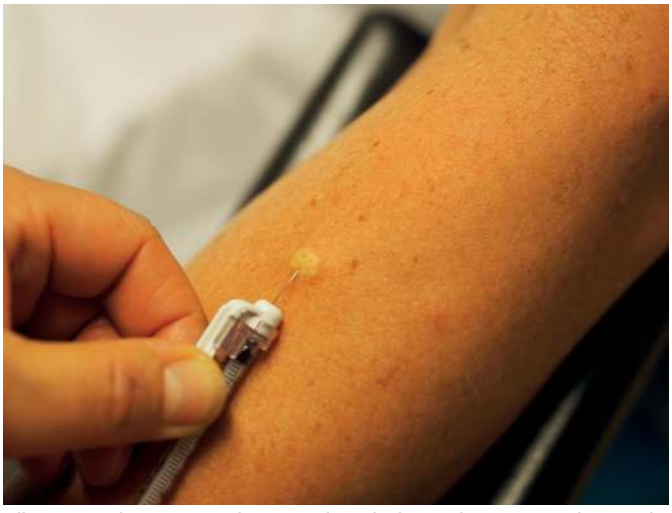
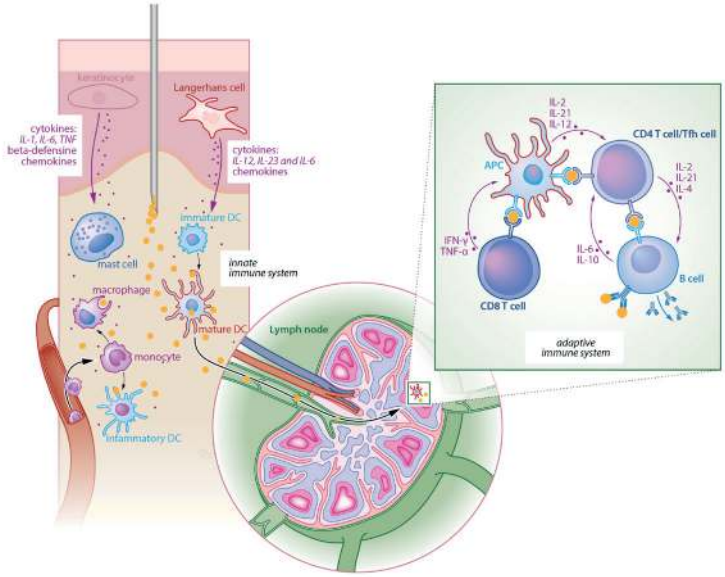


Figure 4. Intradermal vaccination with standard needle, using the Mantoux technique



A standard needle is inserted at a 5-to-15-degree angle and advanced approximately 3mm through the epidermis to ensure that the entire bevel was covered the skin, using the Mantoux technique.

Figure 5. Skin immune system



Immune-stimulating components of the vaccine trigger the activation of dendritic cells (DCs). Keratinocytes respond to stimuli by producing pro-inflammatory cytokines (including interleukin [IL]-1, IL-6, IL-18 and tumour necrosis factor [TNF]) and chemokines. These cytokines lead to activation of dermal DCs. Langerhans cells produce, among others, IL-2, IL-23, and IL-6 and can migrate to the lymph nodes to present antigens to T-lymphocytes. Additionally, it leads to extravasation of monocytes from capillaries into the dermis. Monocytes differentiate into inflammatory DCs and macrophages, creating a large pool of innate immune cells. After DCs have taken up antigens from the dermis, activated DCs migrate through the lymphatic vessels to the draining lymph nodes, where they present antigens to T-lymphocytes. IL-12 is the key cytokine produced by DCs after interaction with the naïve CD4 cell, which is crucial for the differentiation of the T-follicular helper cell (Thf cell). The Thf cell is important for the regulation of B-cell proliferation. Soluble antigens also migrate to the lymph nodes, where they activate the B-lymphocytes.[113]
APC=antigen-presenting cell; DC= dendritic cell; IFN=interferon, IL=interleukin, Thf=T-follicular helper cell, TNF= tumour necrosis factor.

Treatment

Neuraminidase inhibitors such as oseltamivir are the principal antiviral drugs for the treatment of influenza and are prescribed to accelerate recovery and prevent complications. However, evidence regarding the clinical benefits is inconsistent. It has been shown to be effective in uncomplicated influenza in the non-immunocompromised (non-)hospitalized population, in terms of mortality, reduction in severity of symptoms, symptom duration, viral load and the prevention of influenza.[113-116] Sharma et al found a significant reduction in 30-day readmissions and in hospital length of stay among hospitalized influenza patients who received oseltamivir <48h of hospital admission.[117] However, oseltamivir was not associated with a reduced risk of hospitalization in a recent meta-analysis. Regarding adverse events, oseltamivir is associated with increased rates of nausea and vomiting, but not with serious adverse events.[118,119]

In the fight against SARS-CoV-2, therapeutic options were also very important.

Currently, a large number of studies are registered for COVID-19 (clinicaltrials.gov) and several antiviral and anti-inflammatory medications have been approved/ advised by the European Medical Agency (EMA), including remdesivir, nirmatrelvir/ritonavir (Paxlovid), dexamethasone, monoclonal antibodies, tocilizumab and anakinra.[120] However, in the beginning of the pandemic, treatment options against COVID-19 were scarce and studies evaluating the effect of drug therapies were highly needed. In this thesis, we evaluated ensovibep as a therapeutic option for COVID-19.

Outline of this thesis

The overall aim of this thesis is to address gaps in the scientific knowledge regarding SARS-CoV-2 and influenza, the two major respiratory viruses. Enhanced knowledge in this area is crucial for the prevention and management of potential future pandemics, for healthy and immunocompromised persons.

This thesis comprises two parts. Part one focuses on patients with a SOT. Due to their lifelong use of immunosuppressive medication, RVIs may present differently in SOT patients compared to the general population, and outcomes may be worsen. **Chapter 2** provides an overview of the epidemiology and impact of both SARS-CoV-2, influenza and other respiratory viruses in these patients. A summary of clinical characteristics is provided, next to clinical outcomes and factors associate with a poor outcome.

Vaccination against influenza is recommended in the Netherlands for SOT patients. The benefits of influenza vaccination in SOT recipients is reported in several studies related to disease progression and the occurrence of complications.[121-123] However, studies regarding the clinical effectiveness of influenza vaccination are lacking. In **chapter 3**, the influenza VE among immunocompromised adults with a SOT is explored.

The second part of the thesis focuses on SARS-CoV-2 in the general population. **Chapter 4** reports on the results of a proof-of-concept, dose-escalation, randomized-controlled study in healthy volunteers in which we reduced the standard IM dose of the mRNA-1273 COVID-19 vaccine to an equivalent of 10% and 20%, which was given by the ID route. Based on published data on dose sparing of rabies and yellow fever vaccines[110], we hypothesized that a dose reduction of 80-90% was possible for a vaccination strategy in the COVID-19 pandemic. From these data we hypothesized that two fractional SARS-CoV-2 vaccine doses may be sufficient to provide a strong antibody response. We chose an mRNA-1273 vaccine for ID delivery because at the beginning of the COVID-19 pandemic, this was the only vaccine available from the National Institute for Public Health and the Environment for study purposes. Additionally, no previous research had been conducted on ID studies using mRNA vaccines. The results of this proof-of-concept study justified a larger randomized-controlled non-inferiority study in which we investigated whether the virus binding antibody response elicited by two fractional doses of mRNA-1273 vaccine by ID delivery was non-inferior to that of a control group receiving the doses intramuscularly (**chapter 5**). In addition, we measured SARS-CoV-2-specific memory B- and T-cell responses. Finally, we evaluated the performance of an easy-to-use ultra-short ID microneedle (Bella-mu® 1.4mm)

in this prospective study, as one drawback of ID vaccination is that it is technically more demanding to perform than IM injection, requiring a more trained staff. We also evaluated the alterations in the coagulation cascade in this cohort of participants, as thrombotic events following SARS-CoV-2 vaccination are reported during the first year of the vaccination period (**chapter 6**).

In **chapter 7** we compared the ID fractional-dose vaccination strategy for SARS-CoV-2 booster vaccination to the standard IM route. We administered a booster dose 6 months after the primary series of vaccination and determined the immune response after vaccination.

Needle-based immunisation has several other limitations such as pain, needle stick injuries and poor patient compliance due to needle-phobia. The development of needle-free delivery systems, like microneedle arrays (MNA)/ microneedle patches (MNP), jet-injectors and ID adapters, has been identified by the WHO as an important goal in global health care.[124] **Chapter 8** evaluates the safety and immunogenicity of a nanoporous microneedle array (npMNA) for SARS-CoV-2 vaccination with the mRNA-1273 vaccine in healthy participants as a booster vaccination compared to an IM booster dose. Microneedles are a platform for transdermal drug delivery: it is easy to self-administer, and it exhibits a high drug bioavailability.[125] The drug is typically deposit in or on the microneedle tip, which is fixed to the underlying base substrate to form an array, subsequently attached to the patch backing. The microneedles placed on the array or patch are minute, needle-like structures, with a length ranging from 0.10 to 3 mm, but typically 0.10 to 1 mm[126,127], that allows to overcome the skin's main barrier, the stratum corneum (figure 3).[128-130] Vaccine delivery through microneedle technology potentially combines several important advantages: dose-sparing, options for dry product forms distributed at ambient temperatures and better acceptability by the public as it does not involve (injection with) needles.

Chapter 9 evaluates ensovibep as a therapeutic option for COVID-19. Ensovibep is a recombinant multispecific DARPin® (designed ankyrin repeat proteins) molecule, engineered to neutralize SARS-CoV-2 with high potency. This study was designed to explore the viral clearance, pharmacokinetics and tolerability of ensovibep in non-hospitalized patients with mild to moderate COVID-19 in a prospective study, early in the COVID-19 pandemic. Next, it served as a feasibility study in the clinical development trajectory of ensovibep.

Chapter 10 summarizes the findings of this thesis and discusses future perspectives.

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