Clinical and immunological aspects of COVID-19

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2024

Document Version:
Publisher's PDF, also known as Version of record

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DOCTORAL DISSERTATION

Doctoral dissertation for the degree of Doctor of Philosophy (PhD) at the Medical Faculty at Lund University to be publicly defended on 20th of September at 10.00 in Fullriggaren, Hallands hospital Halmstad

Faculty opponent
Anders Wallensten, Assistant professor, Assistant state epidemiologist, Public Health Agency Sweden

Organization: LUND UNIVERSITY

Document name: Doctoral dissertation **Date of issue:** 2024-09-20

Author(s): Emma Löfström **Sponsoring organization:**

Title and subtitle: Clinical and immunological aspects of COVID-19

Abstract:

The COVID-19 pandemic caused millions of deaths and had a huge impact on society. Initially little was known concerning long-term complications, the immune response and the risk of reinfections. The overall aim of this thesis was to explore the immune response, both after COVID-19 and after vaccination, focusing on the avidity (the “functional affinity” of antibodies) of anti-spike IgG, and to explore the symptoms of COVID-19 including the impact on health-related quality of life.

Patients with non-severe, PCR-confirmed COVID-19 were prospectively followed with health-questionnaires and repeated blood samples after infection and vaccination. A cohort with COVID-naïve patients were followed after vaccination. Symptoms and health-related quality of life were assessed by questionnaires including EQ5D-VAS and BIPQ. Qualitative content analysis was used for interviews with patients living with the post-COVID condition. An ELISA assay was used manually for SARS-CoV-2 antibody detection. For avidity index, the same assay with an extra step using a chaotropic agent was included. Neutralization titre was determined by a cytopathic effect-based microneutralization assay.

Most patients experienced unspecific symptoms such as fatigue, headache and fever, during 1-4 weeks, with 12% experiencing long-term symptoms. COVID-19 had a general negative impact on self-rated quality of life. BIPQ index had a negative correlation with EQ5D-VAS score after infection. Patients’ experience of living with post-COVID condition can be described as “moving between uncertainty and new insights”.

Even though antibody levels declined after infection, avidity increased, indicating a maturation of the immune system over time. However, infection failed to yield high-avidity antibodies. Vaccination induced a stronger immune response with higher levels of antibodies, with high avidity, and higher neutralization titres. COVID-19 prior to vaccination further enhanced the immune response. Already 3 months after vaccination, declining antibody-levels and neutralization titres were seen, emphasising the importance of vaccine boosters.

Key words: COVID-19, SARS-CoV-2, Avidity, Immune response, Quality of life, Post-COVID

Language English **Number of pages**: 79

ISSN and key title: 1652-8220

ISBN: 978-91-8021-608-1

Recipient’s notes Price Security classification

Signature Date 2024-07-06

Clinical and immunological aspects of COVID-19

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ISBN 978-91-8021-608-1
ISSN 1652-8220

Printed in Sweden by Media-Tryck, Lund University
Lund 2024

To Frans & Mårten

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II. **Health-related quality of life and long-term symptoms among patients with non-severe covid-19 - a prospective cohort study.** Löfström E, Kunkel S, Kötz A, Lingman M, Undén J, Nygren JM. Infectious Diseases (Lond). 2023 Apr; 55(4):272-281.

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V. **Avidity maturation of anti-spike IgG after vaccination in COVID-19 convalescent vs COVID-19 naïve patients.** Löfström E, Eringfält A, Kötz A, Tham J, Undén J. Submitted.

Abstract

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Even though antibody levels declined after infection, avidity increased, indicating a maturation of the immune system over time. However, infection failed to yield high-avidity antibodies. Vaccination induced a stronger immune response with higher levels of antibodies, with high avidity, and higher neutralization titres. COVID-19 prior to vaccination further enhanced the immune response. Already 3 months after vaccination, declining antibody-levels and neutralization titres were seen, emphasising the importance of vaccine boosters.

Populärvetenskaplig sammanfattning

I december 2019 kom de första rapporterna från Kina om ett okänt virus som orsakade svåra lunginflammationer. Inom kort kunde man konstatera att ett helt nytt coronavirus, SARS-CoV-2, orsakade sjukdomen COVID-19 och i mars 2020 deklarerade WHO att världen drabbats av en pandemi av historiska mått. Under åren som följde fick pandemin stora konsekvenser världen över och miljontals människor har dött till följd av COVID-19. I början av pandemin fanns ett enormt behov av kunskap om sjukdomen. Eftersom viruset och sjukdomen var helt nya fanns ingen tidigare kunskap om kliniskt förlopp, komplikationer, diagnostik och behandling eller hur immuniteten skulle utvecklas över tid. Tack vare en exceptionellt snabb utveckling av vaccin samt efter omfattande insatser för att hindra smittspridning kunde pandemin stoppas. I dagsläget orsakar COVID-19 fortfarande många sjukdomsfall men svår sjukdom är mer ovanligt.

Immunitet innebär att kroppens immunförsvar svarat på en infektion eller en vaccination, genom att utveckla både ett cellulärt försvar (T-celler) och ett antikropps-försvar som skyddar tillräckligt mycket mot ett smittämne, så att man inte blir sjuk när man stöter på smittämnet igen. Immunsvaret och immunitetsutvecklingen påverkar alltså både sjukdomsförlopp men framförallt avgör det huruvida vi kan få infektionen upprepade gånger eller om vi utvecklar immunitet, vilket har en avgörande betydelse i en pandemi.

Studien *COVID-19 Symtom och Immunitet*, ligger till grund för hela avhandlingen. Det är en studie som startades i juni 2020 i Region Halland, med fokus på just symtom och immunitets utveckling hos patienter med en PCR-verifierad, COVID-19 infektion som inte krävt sjukhusvård. Patienterna inkluderades under tidsperioden juni 2020 till januari 2021. Alla deltagarna följdes med digitala hälsoenkäter där de rapporterade sina symtom 1 gång/vecka under totalt två år efter den initiala infektionen. De lämnade blodprover 1, 3 och 6 månader efter den första infektionen och om en ny infektion konstaterades lämnade de ytterligare en omgång blodprover. Ungefär hälften av deltagarna bjöds in till del II av studien, vaccin-delen. Där följdes deltagarna med enkäter kring vaccinationen och med upprepade blodprover efter dos 2 och efter dos 3 av vaccinet. I samband med starten av vaccindelen av studien rekryterades ytterligare en grupp av patienter till studien. Det var patienter som inte haft COVID-19 men som skulle vaccineras. Även de följdes med digitala hälsoenkäter och upprepade blodprover efter vaccinationen.

Syftet med studien var att följa symtomutvecklingen över tid, identifiera komplikationer, mäta hur infektionen påverkade livskvalitén samt följa hur immunförsvaret utvecklade sig bland de patienter som haft en mildare COVID-19. Immunförsvaret är oerhört komplext och består av många olika delar och reaktioner som kan mätas på många olika sätt. I studien fokuserade vi på antikropps-

utvecklingen och aviditet, dvs en slags funktionstest av antikropparna där man mäter hur bra/hårt de kan binda till viruset.

I tre delarbeten (I, IV och V) har vi undersökt immunsvaret ur olika aspekter. I studie I analyserades blodprover tagna efter COVID-19 infektionen. Vi kunde visa att majoriteten av patienterna utvecklade ett immunsvar med påvisbara nivåer av IgG antikroppar riktade mot spike-proteinet på viruset (antispike IgG) efter infektionen. Antikropparna var fortfarande detekterbara 6 månader efter infektionen men nivåerna hade sjunkit rejält. Trots att nivåerna av antikropparna sjönk så visade det sig att aviditeten, dvs bindningsförmågan hos antikropparna, ökade signifikant under samma tidsperiod. Detta tyder på att immunförsvaret fortsätter att ”mogna” och utvecklas lång tid efter att själva infektionen är över och viruset är borta.

I studie IV ville vi jämföra hur immunitetsutvecklingen skiljer sig åt efter en naturlig infektion med COVID-19 jämfört med vaccinationen. I den studien undersökte vi inte bara nivån och aviditeten av antikropparna utan också dess neutraliserande förmåga. Neutralisations förmåga mäts i en analys där man använder sig av levande virus. Serumet (blodprovet) blandas med levande virus och tillsätts sedan till en cellkultur (alltså levande celler). Efter några dagar mäter man hur stor andel av cellerna som inte förstörts av viruset, dvs i hur stor omfattning som antikropparna i serumet har skyddat cellerna mot viruset genom att hindra, dvs neutralisera viruset. Det vi kunde visa var att immunsvaret efter två doser vaccin var betydligt kraftigare och bättre än efter infektionen. Både nivåerna av antispike IgG, aviditets index och neutralisations-förmågan var betydligt högre i vaccin-gruppen än i infektions-gruppen. Troligen beror det på att när virus-delar/protein (antigen) kommer ut i blodet, som vid vaccination, så triggas immunförsvaret mer än vad det gör vid en mild infektion, där viruset sannolikt inte hinner ta sig ut i blodet innan det stoppas av immunförsvaret. Sammanfattningsvis visar detta att en mild infektion med COVID-19 inte ger upphov till någon kraftig immunreaktion och antikropparna som bildas vid infektion har sämre förmåga att binda till och neutralisera viruset än vad som ses efter vaccination, dvs det är sannolikt att man kan drabbas av COVID-19 upprepade gånger. Således är vaccination även till de som haft COVID-19 av värde.

I studie V jämförde vi immun-svaret efter vaccination mellan en grupp som haft COVID-19 innan de vaccinerades respektive en grupp som inte haft COVID-19 innan vaccinationen. Där visade resultatet att immunsvaret, mätt som nivåer av antispike IgG och aviditet, var kraftigare om man haft COVID-19 innan man blev vaccinerad. Dock sjönk nivåerna av antispike IgG relativt snabbt efter vaccinationen. Resultatet visar att upprepad exponering för virus-antigen ger kraftigare immunsvar, men då nivåerna sjunker snabbt är det sannolikt nödvändigt med upprepade vaccinationer för att bibehålla skyddande immunitet.

I studie II undersökte vi symtomen vid COVID-19 och hur den hälso-relaterade livskvalitén påverkades. Resultatet visade att de vanligaste symtomen som rapporterades var sjukdomskänsla, trötthet, huvudvärk, feber och hosta samt att

majoriteten hade symtom under 1-4 veckor. Intressant var att 12% av patienterna fortsatte att rapportera symtom mer än 8 veckor efter infektionen och efter 6 månader angav 7% att de fortfarande hade symtom. De vanligaste långtids-symtomen var nedsatt fysisk kondition, svår trötthet, luktbortfall och huvudvärk. När pandemin började var det inte känt att COVID-19 skulle kunna resultera i persisterande symtom men ganska snart kom rapporter och studier om kvarvarande symtom, sk lång-tids COVID eller post COVID. Mekanismen bakom tillståndet är inte känd och det finns inga biomarkörer för diagnos och heller ingen specifik behandling. I vår studie kunde vi inte se att de initiala symtomen skiljde sig för de som senare utvecklade post COVID och i delarbete I fann vi ingen skillnad i antikropps-svaret för de med post COVID.

Hälso-relaterad livskvalité mättes med ett formulär, EQ5D-VAS, som är ett validerat frågeformulär kring fem dimensioner av hälsa (rörlighet, personlig vård, vardaglig aktiviteter, smärta/besvär, oro/nedstämdhet) som kan användas oavsett sjukdom och svårighetsgrad. Vi fann att medelvärdet för EQ5D-VAS index sjönk från hur det var innan COVID-19 till 1 månad efter infektionen. Även om vi inte kan utesluta att pandemi situationen i sig också påverkat resultatet så visar det att infektionen med COVID-19 påverkade den hälso-relaterade livskvalitén negativt.

Då patienter med post COVID var en helt ny patientgrupp bestämde vi oss för att gå vidare med en kvalitativ studie för att undersöka hur det är att leva med post COVID, delstudie III. De patienter som rapporterat kvarvarande symtom mer än 8 veckor bjöds in till delstudien och data samlades in genom djupintervjuer med tre öppna frågor, ”Hur skulle du beskriva din upplevelse av att leva med post COVID?”, ”Vad betyder hälsa för dig?” och ”Hur upplever du din hälsa nu efter sjukdomen?”. Data bearbetades sedan med sk innehållsanalys (Qualitative content analysis). Patienternas upplevelse av att leva med post COVID kan beskrivas som att ”röra sig mellan osäkerhet och nya insikter”. De beskrev tydligt förlorade förmågor (förlorad smak/lukt, förlorad energi), förlorad kontroll (inte känna igen sig själv, försöka hitta svar och förstå sjukdomen) men också att de omvärderat livet och prioriterade sin hälsa mer. Symtomen av post COVID, framförallt den svåra trötttheten (fatigue), påverkade sociala aktiviteter och både den fysiska och mentala hälsan. Många drog sig för att kontakta vården då de var rädda för att inte bli tagna på allvar och då det oftast inte fanns någon hjälp att få. Studien har gett viktiga perspektiv på hur det är att leva med post COVID. Framöver behövs fler studier för att identifiera mekanismerna bakom och för att hitta effektiva behandlingar.

I dagsläget vet vi inte exakt hur COVID-19 kommer utvecklas. Utifrån nuvarande kunskapsläge är det sannolikt att vi inte kommer kunna få bort viruset utan det är en sjukdom vi kommer få leva med. Fokus bör vara att skydda de patienter som har risk för svår sjukdom med vaccination och, i utvalda fall, tidig antiviral behandling.

Abbreviations

COVID-19	Coronavirus disease 2019
2019-nCoV	2019 novel Corona virus
SARS-CoV-2	Severe Acute Respiratory Syndrome Coronavirus 2
WHO	World health organisation
SARS-CoV	Severe Acute Respiratory Syndrome Coronavirus
MERS-CoV	Middle East Respiratory Syndrome Coronavirus
Orf	Open reading frame
RNA	Ribonucleic acid
RBD	Receptor binding area
ACE2	Angiotensin converting enzyme 2
VOC	Variants of concern
ARDS	Acute respiratory distress syndrome
CRP	C-reactive protein
BMI	Body mass index (kg/m^2)
PCR	Polymerase chain reaction
mRNA	Messenger ribonucleic acid
NK-cell	Natural killer cell
IFNγ	Interferon gamma
CD4+	Cluster of differentiation 4
APC	Antigen presenting cell
Ig	Immunoglobulin
CDRs	Complementarity determining regions
IL-7	Interleukin 7
HR-QoL	Health related quality of life
BIPQ	Brief illness perception questionnaire
CPE	Cytopathic effect
EBV	Epstein-Barr virus

ME/CSF	Myalgic encephalomyelitis/ Chronic fatigue syndrome
Ct value	Threshold cycles
RSV	Respiratory syncytial virus
NAATs	Nucleic acid amplification tests

Introduction

The COVID-19 pandemic

In December 2019 the first patients in Wuhan, China, were diagnosed with pneumonia from an unknown virus and in the beginning of 2020 it was confirmed that a novel coronavirus was the cause (1). Initially the virus was named 2019-nCOV, but was later changed to SARS-Coronavirus 2 (SARS-CoV-2), and the disease it causes was called COVID-19. The spread of the new virus increased rapidly all over the world and in March 2020, WHO officially declared the COVID-19 to be a pandemic.

The pandemic affected people all over the world. By the end of March 2024 over 7 million COVID-19 deaths was reported to WHO and more than 770 million cases were confirmed (2). The impact of the pandemic on society in general, as well as on individuals worldwide, is hard to grasp. In addition to the burden of the disease, health-care systems were overrun, there were lock-downs with economic consequences and there were restrictions in daily life.

The first reported cases of COVID-19 were connected to the market in Wuhan, China. After an increasing spread in China, big outbreaks in Iran and northern Italy were reported in the beginning of 2020. By that time, many countries started to test travellers from areas with outbreaks for COVID-19. People were also instructed to stay home in case of symptoms such as fever or cough after a travel. In Sweden at that point, all suspected cases in travellers were tested and infection tracking, including testing of all contacts, was done for each confirmed case of COVID-19. However, the spread of the virus was extensive, despite actions for preventing the spread. Even though the testing capacity throughout Sweden had increased until March 2020, it did not meet the needs. It was not possible to catch all possible cases, instead patients in need of hospital care and health care workers were prioritized for testing. An intense work at all microbiological laboratories for setting up large-scale testing started. In June 2020, testing for the general population in Sweden could start and large scale testing was kept until late February in 2022. During this period, about 750 000 PCR-tests have been performed in the county of Halland (numbers of inhabitants 330 000).

The first case of COVID-19 in Sweden was diagnosed already in the end of January 2020 but not until March 2020 a rapid increase of the number of cases were seen.

Since then more than 2.5 million cases of confirmed COVID-19 have occurred in Sweden and more than 27 000 COVID-19 deaths have been reported in Sweden (WHO dashboard). The numbers of reported COVID-19 cases are influenced by testing strategies and the accessibility to testing, nevertheless the different phases, or waves of the pandemic, is well illustrated in figure 1.

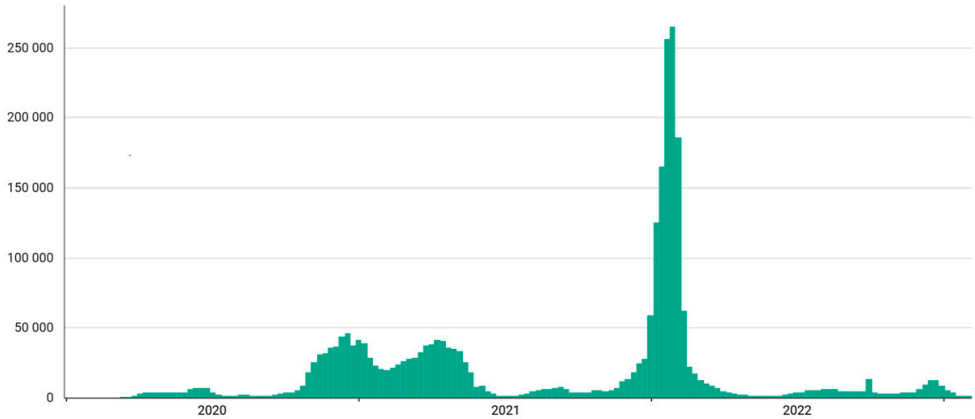

Figure 1. Numbers of confirmed COVID-19 cases in Sweden. Data from Public health agency Sweden. https://www.folkhalsomyndigheten.se/faktablad/fall-covid-19/

Thankfully, vaccines could be developed and produced faster than expected and contributed to end of the pandemic and the saving of millions of lives (3, 4).

SARS-CoV-2

The virus that causes COVID-19 is an enveloped, positive stranded RNA-virus belonging to the family Coronavirinae. The family can be divided into four genera, with SARS-CoV-2 belonging to the genera Betacoronavirus together with SARS-CoV and MERS-CoV. The name Coronavirus originates from the crown-like spikes on the surface that can be seen with electron microscope, reminiscent of the solar corona. Generally, Coronavirus can be found in both animals and humans. Many of the coronavirus are endemic in humans and cause predominately non-severe respiratory infections, such as the common cold.

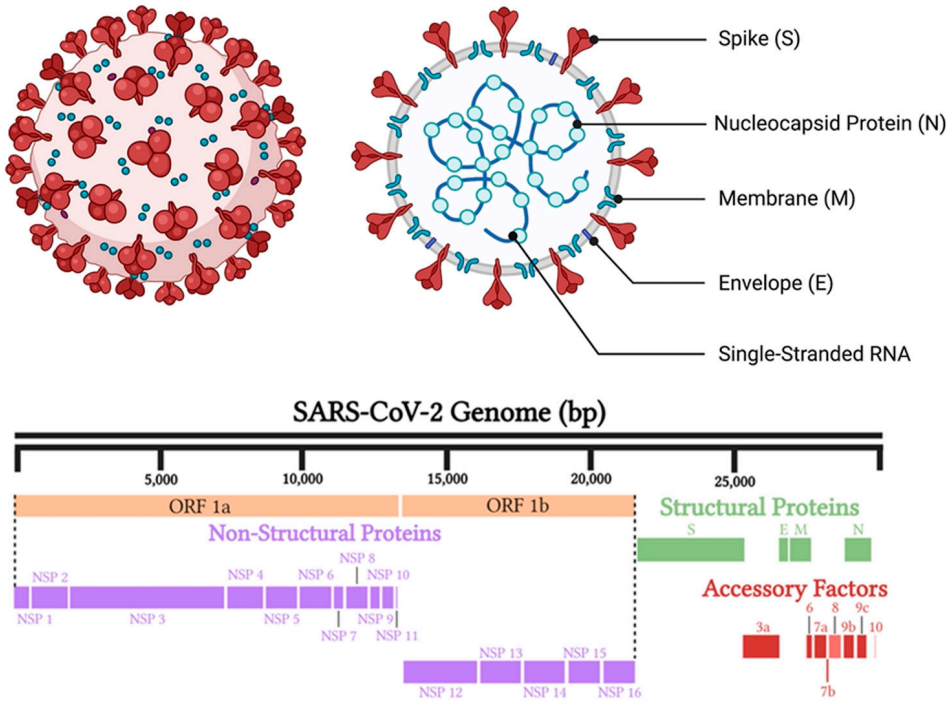

Figure 2. The structure of SARS-CoV-2. Reprinted from Jamison et al, A comprehensive SARS-CoV-2 and COVID-19 review, Part 1: Intracellular overdrive for SARS-CoV-2 infection. European Journal of Human Genetics (2022) 30:889–898 under a creative commons attribution 4.0 international license (CC BY 4.0)

The SARS-CoV-2 virus genome consists of two open reading frames, Orf1a and Orf1b, which encodes the replicas polyproteins. The last third of the genome encodes the four major structural proteins; spike, envelope, membrane and nucleocapsid (5).

The viral envelope surrounds the genome and consists of a lipid bilayer where the spike-, envelope- and membrane-proteins are attached, see figure 2. To infect a human, the corona virus attaches to the human cell-receptors via the spike-protein and then enters the cell by endocytosis. Thereafter, the RNA genome of the virus can be directly translated by the ribosomes, forming structural proteins. Via replicas proteins the viral genome can be replicated. The nucleocapsid-protein is then responsible for the genome packing and the envelope and membrane proteins form the outer layer. Following this, new viruses can assembly, be secreted through secretory vesicles and infect new human cells and start to replicate in a new lifecycle (6). For a schematic figure of the viral life cycle and immune response, see figure 3.

The most important protein for infecting human cells is the spike-protein. A trimer of the spike-protein forms the spikes on the surface of the SARS-CoV-2 virus. The spike-protein itself is divided into two subunits, S1 and S2, where S1 is the most variable and most important for host attachment. S1 forms the receptor binding domain (RBD) and S2 enables fusion with the host cell.

The viral entry into the human cell is a multistep process that starts with the binding of the RBD of the spike-protein to the ACE2-receptor (5). Cells with ACE2 are mainly found in the respiratory tract but also in other organs such as the liver and kidney.

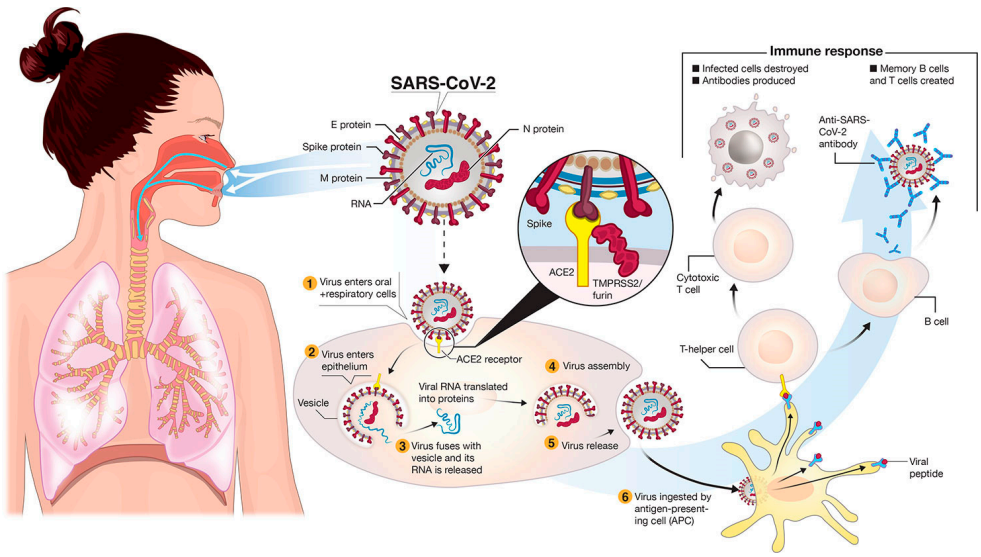

Figure 3. Transmission and life-cycle of SARS-CoV-2 causing COVID-19. SARS-CoV-2 is transmitted via respiratory droplets of infected cases to mucosal cells. The gateway to host cell entry (magnified view) is via Spike-converting enzyme 2 (ACE2) interaction. A simplified depiction of the life cycle of the virus is shown along with potential immune responses elicited. Reprinted from Funk CD, Laferrière C and Ardakani A (2020) A Snapshot of the Global Race for Vaccines Targeting SARS-CoV-2 and the COVID-19 Pandemic. *Front. Pharmacol.* 11:937. doi: 10.3389/fphar.2020.00937 under a creative common license CC-BY. Figure text shortened.

Variants of concern

The primary strain from Wuhan is called wildtype (WT) or B1, but during the pandemic new virus variants emerged. Mutations leading to changes in the spike protein can sometimes give the new variant an advantage in transmission and sometimes increased diseases severity. WHO classify new virus variants with enhanced virulence as variants of concern (VOC). In Sweden the second wave of the pandemic was dominated by the Alpha-variant (B1.1.7) and the third wave by the Delta variant (B1.617.2), see figure 1. Studies has showed that both Alpha and Delta variants had enhanced transmissibility and increased diseases severity with

higher risk of hospitalization and death (7, 8). In early 2022, the Omicron variant (B1.1.529) took over and since then this variant has dominated. The Omicron variant are more transmissible but less prone to cause severe COVID-19 and studies show a reduced risk of hospitalization when infected with Omicron compared to the former variants (9, 10).

COVID-19

Pathogenesis

Inhaled droplets that are released from an infected person by coughing, breathing or talking is the most common way of transmission of the SARS-CoV-2 virus. The virus can also be detected in blood, faeces and other body fluids but the risk of transmission via these routes is low (11). SARS-CoV-2 mainly infect and replicate in cells of the nasal cavity in the upper respiratory tract. This feature differs from SARS-CoV and MERS-CoV, which cannot replicate in the upper airways, and is probably one explanation for the wide transmissibility of SARS-CoV-2 (12).

In the upper airway, the virus causes non-severe or even asymptomatic infections. But, in the beginning of the pandemic, around 15% developed pneumonia with hypoxemia and 5% had a progression to critical diseases with ARDS. The spread of the infection to the lower airway is probably due to microaspiration of virus or direct inhalation. High viral load is associated with unfavourable outcome and progress of the infection to the lower respiratory tract seems to depend on a poor initial response from the innate immune system, especially delayed or mistimed initial interferon response (13).

Simplified, the pathogenesis can be described in three phases, viral replication, immune hyperactivity and pulmonary destruction (14). Poor viral control causes increased viral replication, which then triggers the immune system, and cause a dysregulated response with hyper inflammation and hyper coagulopathy. In the lower airways the virus binds to the ACE2-receptor of the alveolar epithelial cells. During viral replication, the viral RNA activates cytokine production and causes accumulation of various immune cells. The dysregulated immune response leads to increased permeability, resulting in alveolar and interstitial oedema (15). The cell damage is also caused by direct cytopathic effect of the virus. Altogether, it leads to increased dead space ventilation, impaired gas exchange, atelectasis and progression to ARDS, with lung failure and development of lung fibrosis. Another common feature of ARDS and severe COVID-19, is coagulopathy and endothelial damage. This vascular pathology, including thrombotic microangiopathy, do not only aggravates hypoxemia but also causes extrapulmonary manifestations such as different organ failure (12).

Later during the pandemic, when the Omicron variant took over, less severe diseases was seen and pneumonia and ARDS was, at least in Sweden, hardly seen at all. This might be due to high level of immunity from vaccination but also due to the changed characteristics of the virus. Hui *et al* have showed that Omicron has lower replication competence in human lungs than the Delta variant, compatible with lower diseases severity (16).

Symptoms

The most common symptoms of COVID-19 are fever, dry cough, fatigue and myalgia and the majority of the cases are mild to moderate in severity (17, 18). However, the range of symptoms are diverse and the clinical manifestations varied during the ongoing pandemic. Initially, severe pneumonia with ARDS was more common and severe complications like thromboembolism or haemorrhagic stroke were often seen (19). Many patients experienced gastrointestinal symptoms with diarrhoea and nausea and even skin manifestations was quite common, reported in up to 20% of patients (11). Neurological symptoms like altered mental state, dizziness and anosmia were also seen (20, 21). Patients in need of hospital care typically presented with fever, dyspnoea and respiratory failure around one week after the initial symptoms. Blood samples showed inflammatory activity with high levels of CRP, ferritin and d-dimer. Leukocyte count was often normal but sometimes lymphopenia was present (17). Common findings in radiological examinations of severe cases were widespread ground-glass opacity. Later during the pandemic, when new virus variants emerged and widespread vaccination was conducted, very few cases of severe pneumonia are diagnosed and the numbers of patients in need of hospital and intensive care have decreased dramatically. After vaccination became available, risk factors for developing severe COVID-19 are potent immune suppression, high age, obesity (BMI >40) and other severe chronic heart-, kidney-, liver- or lung disease or poorly regulated diabetes mellitus (22, 23).

Diagnosis

To diagnose COVID-19 in the acute phase, PCR-analysis on nasopharynx-specimen is recommended and displays a high sensitivity and specificity. Antigen-testing with immunochromatografic assays are widely available and easy to perform but have lower sensitivity and are not recommended for use in hospitals Serological assays are not routinely used for diagnosis but are relevant for assessment of immune status or to diagnose previous COVID-19 infection.

Treatment

In the early days of the pandemic, no antiviral treatment was available and the treatment was limited to symptomatic and supportive treatment. Clinical trials were quickly initiated to identify drugs that could prevent or improve the outcome of severe COVID-19. The REMAP-CAP trial could show that high doses of systemic

glucocorticoids reduced the mortality in patients receiving oxygen support or invasive mechanical ventilation with ≥7 days of symptoms (24). Other trials could show, in some cases, that interleukin-6 receptor antagonists or Januskinase inhibitors could further reduce the mortality in severe COVID-19 (25, 26). These drugs mainly reduce the inflammatory lung damage induced by the SARS-CoV-2 virus and should not be given in the early stages of the infection when viral replication is still high.

The first antiviral drug that became available was remdesivir (27). In contrast to the immune modulating drugs, antivirals should be given in the early stage of the infection to stop the viral replication in order to prevent severe disease. Remdesivir has to be administrated intravenous, which limits the clinical usability of the drug. During 2022 a new antiviral was introduced, nirmatrelvir-ritonavir, which is administered as a tablet (28). Due to a change in the pandemic situation and mass vaccination, nirmatrelvir-ritonavir should only be given to selected patients with several risk factors for severe COVID-19 and not be used widely in all COVID-19 cases.

Monoclonal antibodies were used for a short period during the pandemic (29). Due to the emergence of new virus variants, monoclonal antibodies lost their effect and are no longer used. Convalescent sera has not been proved to have any effect and has no place in the treatment of COVID-19 today (30).

Treatment with anticoagulantia reduce risk for respiratory support, but the dosing has been debated and depends on whether the patient is critical ill or not. (31, 32). Other treatments, such as antibiotics for a concurrent bacterial infection, are given after individual medical assessment.

Vaccination

In a record effort, several vaccines were developed and made available in less than 1 year. The main reason behind this impressive progress was the use of a new technology for development of a vaccine using mRNA. The research by Katalin Kariko´ and Drew Weissman enabled the development of this technique and they were subsequently awarded with the Nobel Prize in Medicine in 2023. This vaccine contributed to the end of the pandemic and saved millions of lives (4).

Instead of using the classical method of vaccination using an antigen (protein), the new feature of this vaccine was to deliver an mRNA-molecule instead. The mRNA-molecules, encapsulated in lipid nanoparticles, are transported into the cells where it is used as an instruction for the cells to build and produce protein that will mimic the antigen (protein) of the virus, hence generating an immune response.

The first two vaccines that became available were mRNA-BNT162b2 (Pfizer-BionTech) and mRNA-1273 SARS-CoV-2 (Moderna). Vaccination of prioritized populations started in December 2020. Other vaccines, as ChAdOx1 nCoV-19

(Astra Zeneca), with a DNA-vector instead of the mRNA, was used early in the pandemic and later, commencing in December 2021, the protein-based vaccine NVX-CoV2373 (Novavax) became available. At the moment, a combined vaccine against COVID-19 and Influenza (Moderna) has been developed and has showed promising results in the phase 3 trial (33).

The BNT162b2 mRNA (Pfizer-BionTech) was the vaccine predominately used in Sweden. It protects against severe COVID-19 and has been proved to be safe (34, 35). As new virus variants emerged, updated versions of the vaccine were developed using the same technology and rapidly became available. The vaccine was initially given as two doses intramuscularly, with at least three weeks between doses, and with a third booster dose at a later stage. The vaccination recommendations have changed during the past years; originally all adults in Sweden were recommended to be vaccinated, but from 2023 only elderly individuals or adults with risk factors for severe COVID-19 are recommended to continue the vaccination program with repeated booster doses.

Post COVID-19 condition

In most patients with COVID-19, the symptoms are transient (23). A subset of patients, however, experience prolonged symptoms (36, 37). During the summer of 2020, long-term symptoms of COVID-19 in certain individuals became evident and increased recognition and research was initiated. To date, the exact cause of the long-term symptoms, which is called post COVID-19 condition, or the mechanism behind it, are not known and no effective treatment is available (38). The frequency of post COVID-19 condition varies in different studies, from around 10% up to almost 62% (39-41). According to WHO, post COVID-19 condition is defined as "the continuation or development of new symptoms 3 months after the initial SARS-CoV-2 infection, with these symptoms lasting for at least 2 months with no other explanation". Common remaining symptoms are headache, fatigue, sleep disturbances, anxiety, cough, and/or breathlessness (42, 43). The condition can occur even after a mild COVID-19 infection.

Patients suffering from post-COVID-19 condition are a new and large group and there is a need to increase the understanding of this condition in order to find effective treatments and minimize the consequences for these patients.

A brief overview of the immune system

The human immune system is built up by different parts, with different features, that cooperate and affect each other in a complex balance that is still not fully understood. The immune system is specific, selective, adaptive and has a memory. These characteristics mean that the immune system is able to detect substances that are foreign to the body, such as virus, bacteria or other antigens, and then adjust and improve the defence as well as creating memory cells that will give rise to an even faster reaction at following exposures.

The immune system is classically divided into two categories, based on some characteristics and the way they respond to an antigen. The innate system, or the non-specific system, reacts in the same way to all antigens. In contrast, the adaptive, or specific immune system, reacts to a specific antigen. Both systems are closely linked and cooperate. Generally, the terms immune system or immune response refers to the adaptive immune system.

The innate immune system

The components of the innate immune system attack and destroy foreign substances as soon as they enter the body. Different parts of the innate immune system can also activate and/or enhance various mechanisms of the adaptive immune system (44). The main components of the innate immune system are the epithelial barrier, phagocytes, natural killer cells (NK cells) and certain proteins, such as complement proteins and cytokines.

The skin, the respiratory and the gastrointestinal tract are lined with epithelia that serve as a first barrier against microbes. It is not only a physical barrier; the cells also produce antibiotic peptides and contains intraepithelial T-cells.

The human body has two types of phagocytes, neutrophils and the monocytes/macrophages. These cells can be activated by other infected cells via cytokines, complement proteins or other signal substances (proteins). When they recognize a microbe, the process of phagocytosis occurs. When the microbe is ingested, the formation of reactive oxygen intermediates and nitric oxide inside the phagocyte kills the microbe. Activated phagocytes also produce signal substances themselves, which mediate other host defence mechanisms (45).

The NK cell is a type of lymphocyte but it does not express immunoglobulins or any T cell receptors. It reacts against intracellular microbes, such as viruses, killing infected cells and producing IFNγ, which in turn activates macrophages.

The complement system consists of circulating proteins and can be activated by three pathways. When activated, they start a cascade reaction, which leads to coating of microbes and promotion of phagocytosis, promoting inflammation and leading to apoptosis or microbes lysis. The complement system it also a part of the adaptive

immune system, as one of the pathways for activation, the classic pathway, is mediated by antibodies. The other two pathways are the alternative pathway, where the microbe itself activates the complement cascade, and the lectin pathway, where a plasma protein binds to glycoproteins on the surface of a microbe and then activates the cascade.

Cytokines are proteins that are produced in different immune cells after microbial stimuli and they mediate many of the cellular reactions in the immune system. There are several different cytokines, with different cell sources and different cellular targets. Some functions of cytokines are to promote inflammation, activate different immune cells and stop viral infections by inhibit viral replication. Some of the cytokines can be classified into both the innate and the adaptive immune system.

The adaptive immune system

The adaptive immune system is characterized for its specificity and its memory. The capacity of a memory means, in short, that the immune system can respond faster and more effectively to repeated exposure of the same antigen. The adaptive immune system can be divided into two subgroups – the cell-mediated immunity and the humoral immunity. The two different subgroups interact and depend on each other, as well as on the innate immune system. For an overview of the primary immune response to an infection, see figure 4.

The cell-mediated immunity

Very simplified, the cell-mediated immunity consists of different T lymphocytes that can kill infected human cells, help the B-cells to produce antibodies and activate phagocytes to kill the microbe that is ingested. The lymphocytes are identified and classified according to their surface proteins, e.g. CD4+. The total population of T lymphocytes are extremely diverse and consist of clones with different receptors for different proteins (antigens). Since T cells cannot bind directly to circulating antigens, they are dependent on the antigen-presenting cells (APC), such as dendritic cells or macrophages, for recognition of the antigen and thereafter activation. Considering their function, T-cells are best at combating intracellular infections, such as viruses, but they also play an important role in the activation of B-cells and the maturation of antibodies (46, 47).

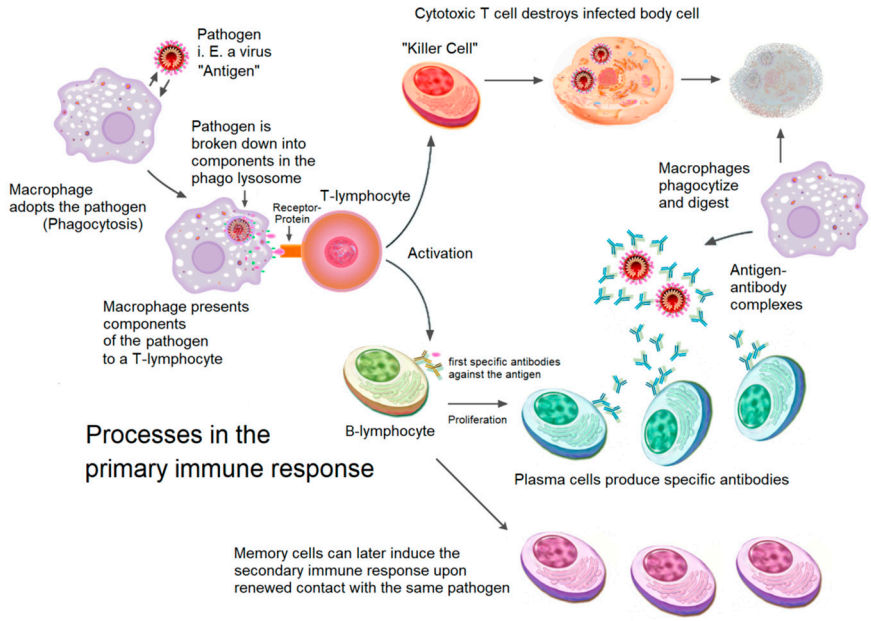

Figure 4. Overview of the primary immun response to a viral infection. By Sciencia58 an the makers of the single images Domdomegg, [1], Fæ, Petr94, Manu5 - Own Graphic using File:Macrophage.svg, File:201308 B cell.svg, File:Normal plasma blood cells.jpg, File:Aufbau einer Tierischen Zelle.jpg, File:3D medical animation coronavirus structure.jpg, source: Immune response, CC BY-SA 4.0, https://commons.wikimedia.org/w/index.php?curid=90421209

The humoral immunity

The humoral immunity is mediated by antibodies, which are produced by B-lymphocytes. Antibodies can either be circulating, as a secreted protein, or be a membrane-bound antigen receptor. In contrast to the T cell receptors, the antibodies are able to recognize many different types of molecules; proteins but also carbohydrates and lipids. The most important role of the antibodies, with respect to viral infections, is the ability to stop the virus before invasion of a human cell, e.g. to prevent the virus from establishing an infection.

For the structure of an antibody, see figure 5. An antibody, or an immunoglobulin (Ig), consists of four polypeptide chains, two heavy chains (H) and two light chains (L), which are attached to each other with disulfide bindings in a Y-shaped formation. Each chain has a variable region (V) and together with the constant region (C) they fold and form a domain. This domain, or area, is needed for antigen recognition, and is called F_{ab} (fragment antigen binding). The antigen binding site is in the V-regions and the site contains hypervariable regions (CDRs). The

fragment with only the constant regions of the heavy chains is called F_c (fragment crystalline). Different kinds of heavy chains forms different classes of Ig and the different Ig classes have different forms and different functions when secreted. IgG subclass 1-4 consists of H type γ and is secreted as a monomer (46).

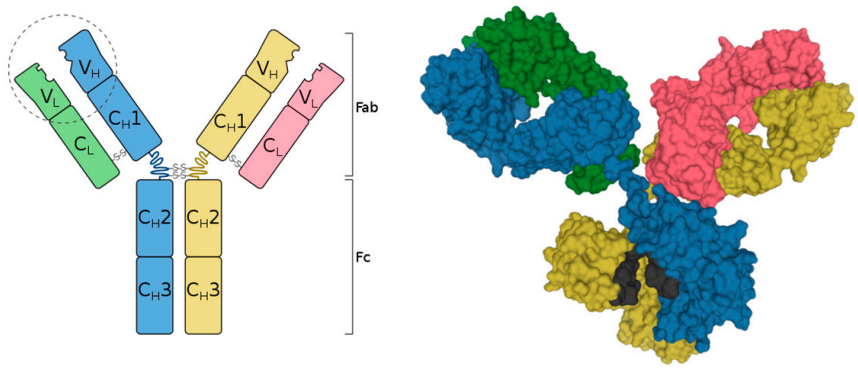

Figure 5. The structure of secreted IgG, schematic vs more accurate depiction. Pictures made by Tokozero. Reprinted from Wikipedia under creative commons attribution 4.0 international license (CC BY SA 4.0), https://commons.wikimedia.org/w/index.php?curid=93798019, https://commons.wikimedia.org/w/index.php?curid=95724513.

The epitope, is the part of the antigen which is recognized by the antibody. The antibody binds reversibly with non-covalent interactions to the antigen. The strength of the antigen-antibody binding is called affinity. After repeated exposure to an antigen, the strength of the binding increases and this procedure is called affinity maturation. However, since the total strength of the binding is greater than a single antigen-antibody bond the total strength, or the "functional affinity" is referred to as the avidity.

B cell selection and affinity maturation

Naïve B cells exist in the bone marrow and do not initially secrete antibodies. After stimulation from different signals, such as IL-7, they start to proliferate and mature. In the steps of maturation, B cells that are unable to express useful receptors (immunoglobulins) undergo apoptosis (negative selection) and the cells that recognize an antigen have a positive selection. This selection gives rise to immature B-cells, expressing membrane-bound IgM. When entering the peripheral lymphoid tissues the selection continues and give rise to mature B cells, which then express

membrane-bound IgM and IgD. This mature B cell can recognize an antigen but the stimuli from the antigen is not enough for activation. Stimuli from helper T cells and various other reactions are needed for activation. When activated, a clonal expansion of the B-cells occurs and the B-cells start to secrete IgM. Following this, the B-cells continue to differentiate from IgM secreting cells to IgG secreting cells. With further maturation, the B cell will start to produce high-affinity IgG and some of the B-cells will differentiate into memory B cells (46).

When B cells differentiate from secreting IgM to become an IgG secreting cell, the mechanism is called heavy chain class switching. Switching, or the ability to express different antibody isotypes, is essential for an effective defence against different microbes. The switch is initiated by signals from cytokines and helper T cells and affected by the type of microbe that started the immune response and the site of the immune response, e.g. in mucosal lymphoid tissues mainly IgA are produced.

The maturation of the antibody affinity increases with prolonged and repeated antigen-exposure and originate through mutations in the V region of the antibody. This process takes place in the germinal centres of the lymphoid follicles and helper T cells are highly involved in the process (48). In the germinal centre, there is a fast and extensive proliferation of antigen-activated B cells and numerous mutations occurs during this proliferation. This leads to different B cell clones with varying affinity to the antigen, where B cells with high affinity are selected by the ability to bind to exposed antigens and thereby avoid apoptosis.

Rationale for the studies

Since COVID-19 was a new disease caused by a novel virus, the knowledge gap in the eve of the pandemic was substantial. There was an urgent need of research for every aspect of the disease. During the first months, publications were often focused on the clinical characteristics and diagnostics, with these studies mainly reporting hospitalized and severely ill patients. Large scale randomised studies were initiated in order to test possible treatments. Due to the novelty of the virus, nothing was known about long-term complications, the development of immunity and the risk for reinfections. At that time it was not known if, or when, a vaccine would be available.

Although millions of people have suffered from severe COVID-19, the vast majority of the cases have been mild to moderate and the vast majority of cases did not need hospital care. Therefore, we wanted to explore the clinical picture and the risk of complications and long-term symptoms in the population of non-severe COVID-19. Published reports concerning HR-QoL in infectious diseases are uncommon.

Patients living with long-term symptoms/post COVID-19 condition are a new group of patients that are often misunderstood in the health-care system. The pathogenesis is not known, and there is still no treatment available. To learn more about post COVID-19 condition, we wanted to explore the patient´s experience of living with this condition in a qualitative study.

Since immunity, due to natural infection and/or vaccination, is a key factor to end a pandemic, this area is of greatest interest. Even though thousands of studies about the immune response in COVID-19 have been published, only a handful of studies focused on the avidity index. For that reason, we wanted to explore the immune response with a focus on the avidity index, after both natural infection and vaccination.

In the first year of the pandemic, the need for easy-to-perform methods or tests for predicting risk of severe diseases and to predict protection from vaccination, was obvious. In daily clinical work, the level of antibodies is used as an estimation of protective immunity and vaccine effect. Since avidity maturation is an effect of B cell selection, there is a potential for avidity to reflect protective immunity. Based on this, the role of avidity index in relation to the neutralizing capacity of the antibodies was deemed of interest to study.

Aims

The overall aim with this thesis was to explore the immune response after COVID-19 and after vaccination, focusing on avidity of anti-spike IgG, and explore the acute and long-term symptoms of COVID-19, including the impact of COVID-19 in self-reported health and quality of life.

The specific aims of each paper were:

Paper I

To explore the maturation of IgG avidity and antibody-levels over time in patients with PCR-confirmed non-severe COVID-19.

Paper II

To investigate the development of acute and long-term symptoms and evaluate the quality of life in patients with non-severe COVID-19 in a Swedish setting.

Paper III

To explore patients' health experiences of the post COVID-19 condition.

Paper IV

To explore the correlation between the avidity index and neutralization titre, examine whether the avidity index of anti-spike IgG can contribute to a better and more precise estimation of the neutralizing capacity on SARS-CoV-2 virus, and also to investigate the immune response after primary infection with SARS-CoV-2, compared to two doses of vaccine.

Paper V

To compare the immune response, focusing on anti-spike IgG levels and avidity index, after the second dose of vaccine between a cohort of COVID-19 convalescents with a COVID-19 naïve cohort. The second aim was to describe the side effects that were reported after the vaccinations.

Ethical Considerations

All studies in this thesis are approved by the Swedish Ethical Review Authority, approval number Drn: 2020–02691 and 2021–00355. Collected blood samples are stored in a biobank according to regulations. All included patients gave informed consent.

All personal data are handled according to GDPR-regulations. When presented in the studies, all data are anonymised and it is not possible to identify an individual patient.

Our intervention with repeated blood samples involves no significant medical risk. Collecting blood samples is routine work for health-care personal and all samples were collected at hospitals or at the primary healthcare centres.

Since all participants received the results of the antibody-levels in their sample, the main ethical concern in the study was how the results of the antibody analyses were going to be interpreted by the participants. We were concerned about an inappropriate assessment of the results. For example, one could theoretically use the result of detected antibodies as a guarantee of protection against infection and ignore the recommendations from the Public health authorities about physical distance, hygiene routines etc. There was also a possibility that the results could be used as a reason to avoid vaccination. To minimize this risk, we created comments on how to interpret the results and these comments were sent together with results to the patients. An email-address and telephone number for questions were also included.

Patients and Methods

The COVID-19 Symptoms and Immunity study

All papers included in this thesis were based on the study "*COVID-19 Symptoms and Immunity*", a prospective longitudinal cohort study, which started in June 2020 in the county of Halland, Sweden.

The intention of the study was to follow patients with non-severe COVID-19 during at least two years and focus on clinical aspects, such as symptoms, sequelae and impact on health-related quality of life, and immunological aspects, such as immune response after infection, vaccination and risk of reinfection. Approximately 10 000 positive PCR-tests for COVID-19 were noticed in the county of Halland during March 2020 to January 2021. About 7000 of the tests were taken as a part of the large-scale testing, where the test were taken upon the patient´s initiative and no medical appointment was needed. Based on surveillance data (unpublished), B1 and B1.1 were the dominating virus variants in the county of Halland during June 2020 until January 2021. Those variants were spread throughout Europe from the large outbreak in Italy

We used a digital system for sending out the invitations to the study as well as for all further communications with the patients. The system (Entermedic®, Entergate AB), approved for health-care and research data, sent mobile phone text messages that contained a link to the study information, the questionnaire or other instructions such as call for blood samples.

Part I

Patients with a positive PCR-test for SARS-CoV-2 during four different weeks in June-August 2020 and during four different weeks in October 2020-January 2021 were noticed by the local laboratory system or the regional surveillance system. Within 2 days after the positive PCR-test, invitations to the study were sent to patients who were resident in the county of Halland, aged >15 years, had an available mobile phone number and who was not hospitalized by the time of the diagnosis. When an informed consent was given, the patient was included in the prospective cohort of the study. In total 318 invitations was sent and 154 patients were included.

Immediately after inclusion, a questionnaire about medical history and present symptoms was sent. During the first week after inclusion a questionnaire about present symptoms was sent on daily basis and after the first week the same questionnaire was sent once a week until the patient reported to be healthy again. After that, during the following two years, weekly questionnaires about symptoms were sent in order to notice reinfections.

Blood samples were collected at 1, 3 and 6 months after the diagnosis of COVID-19. At the same time points, questionnaires about self-reported health and health-related quality of life were sent.

In June 2020, a retrospective cohort was added to the study. Health-care workers at Hallands hospital in Halmstad or Varberg, who had a PCR-confirmed COVID-19 during March-May 2020, who had not been hospitalized and had an available mobile phone number, was invited to the study. If giving an informed consent the person was included in the retrospective cohort of the study. In total 190 invitations were sent and 139 was included in this cohort. Immediately after inclusion a questionnaire about medical history, symptoms of the previous COVID-19 and duration of the symptoms, was sent. Thereafter, the same weekly questionnaires for symptoms as for the prospective cohort were sent. Blood samples were collected at 3 and 6 months after the infection and at the same time points, questionnaires about self-reported health and health-related quality of life was sent.

Part II

When vaccine became available in beginning of 2021, the part II of the study, the vaccine substudy, could start. From the included patients in part I of the study, health-care personal were sought and invited to the vaccine substudy/part II. If giving an informed consent, they were included in this part of the study as well and received questionnaires about the vaccination (date, type of vaccine, side-effects) and blood samples were collected 1, 3 and 6 months after the second and after the third dose of vaccine. In total 169 invitations were sent and 145 was included in this cohort.

In March 2021, one more cohort was added to study – a COVID-19 naïve cohort. We searched for participants among health-care workers who had not had COVID-19, by advertising at Hallands hospital Halmstad. Inclusion criteria were informed consent, no sign of a present or former COVID-19 and a negative test for nucleocapsid-antibodies. The same vaccine-questionnaires were sent and blood samples were collected at 1, 3 and 6 months after the second and after the third dose of vaccine. Also, weekly questionnaires about symptoms of infections was sent. In total, 46 patients contacted us and 43 of them were included in this COVID-19 naïve cohort.

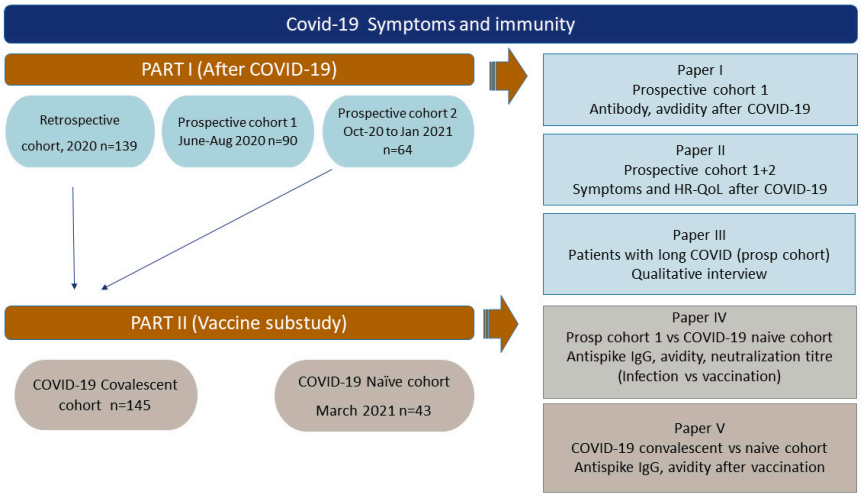

Figure 6. Description of the study "COVID-19 Symptoms and Immunity" and the relation to the papers.

Paper I

Study population

Patients with at least two blood samples from different time points were sought from the prospective cohort that included during June-August 2020 in the study *COVID-19 Symptoms and Immunity*. In total, 75 out of 90 patients could be included.

SARS-CoV-2 antibody detection

Two different kind of antibodies were measured, nucleocapsid-antibodies (n-antibodies) and anti-spike IgG.

For detection of total n-antibodies (IgM plus IgG), we used Elecsys Anti-SARS-CoV-2 on Roche Cobas e801 (Roche Diagnostics). Results were reported as cut-off index (signal sample/cut-off, s/co) with values >1 considered as positive. Samples generating values above the linear range were diluted and reanalysed. Serum samples collected in 2016 from unidentified blood donors (n = 100) were used as negative controls.

For detection of anti-spike IgG, we used Anti-SARS-CoV-2 QuantiVac ELISA (Euroimmun) manually. Results were reported in RU/ml according to manufacturer. Samples ≥11 RU/ml were considered as positive, <11 to ≥8 RU/ml as borderline and <8 RU/ml as negative, according to manufacturer. Samples generating values above the linear range were diluted and reanalysed.

Avidity index

The avidity index was analysed for anti-spike IgG. Basically, the same ELISA as previously described for detection of anti-spike IgG was used. Samples were added in duplicates to the antigen coated wells but, with the addition of a chaotropic agent (4M urea) to one well and pure buffert to the other well (reference well). The samples were then incubated for 10 minutes and then analysed according to the manufacturer´s instructions with the exception of adding one extra washing cycle. The avidity index was described as $OD_{urea}/OD_{reference}$ and multiplied with 100 to be expressed as percentages. If necessary, samples were diluted and reanalysed to fit in the linear range of the assay.

The use of 4M urea was based on initial experiments on 4 pairs of samples collected 2 and 4 weeks after PCR-confirmed COVID-19. In the optimization experiments, the 8 samples were analysed for avidity index with 1, 2, 3, 4 and 5M urea. Since the clearest differentiation in avidity index between the samples taken at 2 weeks after infection compared to samples taken 4 weeks after infection was seen with 4M urea, this concentration was then chosen and used throughout the study.

Statistics

For descriptive analysis, median values were used. Wilcoxon Signed Rank test was used to analyse differences over time and for differences between groups, Mann-Whitney was used. $p<0.05$ was considered significant. All statistical analysis were performed with IBM SPSS statistics 27.

Paper II

Study population

All patients (n=154) from the prospective cohort of *COVID-19 Symptoms and Immunity* was included.

Questionnaires for symptoms

During the first week after inclusion, daily questionnaires about symptoms or recovery were sent, thereafter the questionnaires was sent on weekly basis. The questionnaire contained a list of 25 specified symptoms but also a possibility to write in free text. When recovery from the symptoms was reported, another weekly questionnaire was sent with questions about the health status. The questionnaires during the first year after the PCR-confirmed COVID-19 were analysed.

EQ5D-VAS

EQ5D-VAS is validated and commonly used to evaluate health-related quality of life (HR-QoL) and self-reported health (49). It has five questions covering five

dimensions of health (mobility, self-care, usual activities, pain/discomfort and anxiety/depression). Each question has three levels of response (no problem, moderate problem or severe problem) and in the last question, the patient rates the overall health on a 0-100 visual analogue scale (VAS). An index score from EQ5D-VAS is calculated by using an algorithm, and for this study the European weights was used (50, 51).

The patients received the EQ5D-VAS in a digital questionnaire at 1, 3 and 6 months after COVID-19. In the initial questionnaire, which the patients received immediately after inclusion, the EQ5D-VAS was retrospectively rated for the month before COVID-19 (time point 0).

The BIPQ

The brief illness perception questionnaire is a nine-item scale used to evaluate the patient´s perception of the diseases (52). Five of the items capture the cognitive dimensions (consequences, timeline, personal control, treatment control and identity), two items assess the emotional perception (concerns and emotions) and one items assess illness comprehensibility. Since the last item is an open question it was not included in this study. For each item, the patients score their perception from 0-10. A higher score represents a more negative perception except for the items of personal and treatment control and comprehensibility where the scores are reversed. By adding up the scores from all items, the BIPQ index is calculated. The BIPQ was carried out in the initial questionnaire, thus during the first days of the COVID-19 infection, and a BIPQ index was calculated (53).

Statistics

Bivariate Pearson correlation were computed for BIPQ and EQ5D-VAS scores. For investigating the differences in EQ5D-VAS scores between men and women and the difference in BIPQ index between patients with short or long-term symptoms independent samples t-test was used. Levene´s test was used to evaluate the equality of variance between groups. For pairwise mean differences in EQ5D-VAS scores between the different time points, both on the full sample as well as the sub-group with long-term symptoms, paired samples t-test was used. Results were considered statistically significant at p-value <0.05. All statistical analysis was performed with IBM SPSS Statistics 27.

Paper III

Study population

From the weekly questionnaires in the prospective cohort of the study *COVID-19 Symptoms and Immunity*, 18 patients with symptoms lasting more than 8 weeks after COVID-19 could be identified. All 18 patients were asked to participate in the interview study by written letter; the digital system was not used for this study. Totally 14 patients gave an informed consent and were included.

Data collection

A researcher with experience from qualitative methodology (Ingrid Larsson) performed individual semi-structured interviews by telephone or digitally (Microsoft Teams®). Three main questions were asked, "How do you describe your experiences of being affected by COVID-19?", "What does health mean to you?" and "How do you experience your health after the disease"? Two pilot interviews were conducted and since no changes in the method were done those interviews were included in the study. The interviews lasted for 45-120 minutes. All interviews were audio-recorded and transcribed verbatim.

Data analysis

Data were analysed with qualitative content analysis (54, 55). The interviews were read and listened to several times. Thereafter, the content that was related to the aim of the study was extracted to form units of analysis. These units were then condensed into smaller meaning units (n=221), which were labelled with a code. The codes were assessed and interpreted, giving rise to six sub-categories. These sub-categories were grouped into three categories, which reflects the manifest content. The underlying meaning, the latent content was formulated as an overall theme.

Paper IV

Study population

Patients were sought from the prospective cohort of *COVID-19 Symptoms and Immunity*, included during June – August 2020, and from the COVID-19 naïve cohort from part II of the same study, included during March 2021. Serum samples were taken at 1, 3 and 6 months after COVID-19 or after the second dose of vaccine. Due to logistic reasons, there was a limitation of 80 samples for the neutralisation assay and the 6 months samples from the COVID-19 naïve cohort were not available at the time for inclusion. Patients with blood samples from all time points were prioritized and the ambition was to have equal numbers of samples from the prospective COVID-19 cohort and from the COVID-19 naïve cohort to the neutralisation assay. In total 34 patients (92 blood samples) were included, 21 from the COVID-19 naïve cohort and 13 from the COVID-19 cohort.

SARS-CoV-2 antibody detection and avidity index

For detection of anti-spike IgG the ELISA assay Anti-SARS-CoV-2 QuantiVac ELISA (Euroimmun), previously described in paper I, was used manually.

The method used for avidity index is described more in detail in paper I. Briefly, the same ELISA assay as for anti-spike IgG is used but with the addition of an extra step with a chaotropic agent (4M urea) in one of the duplicates. The avidity is then defined as $OD_{urea}/OD_{reference}$ and multiplied by 100 to be expressed as percentage.

Neutralization assay

A cytopathic effect (CPE)-based microneutralisation assay was used according to Varnaite *et al* (56). A mix of the serum samples and living virus is incubated for 1 h and then added in duplicates and different dilutions to Vero E6 cells. After 5 days of incubation, each cell are examined with optical microscopy and categorised as either neutralizing (<50% CPE) or non-neutralizing. The reciprocals of the highest neutralizing dilution for the two duplicates is assessed as the result.

Statistics

For correlation analysis, Spearman correlation was used. For analysing differences over time within the cohort, Wilcoxon signed rank test was used and for differences between the cohorts, Mann-Whitney was used. $p<0.05$ was considered statistically significant. All statistics was computed in IBM SPSS statistics 29.

Paper V

Study population

Patients from the part II of the *COVID-19 Symptoms and Immunity*, the vaccine substudy, were included. The vaccine sub-study consists of a COVID-19 convalescent cohort (health-care workers with PCR-confirmed non-severe COVID-19 during March 2020 to January 2021 (n=139)), and a COVID-19 naïve cohort (health-care workers without a former COVID-19 (n=43)). Serum samples were taken at 1, 3 and 6 months after the second dose of vaccine. Inclusion criteria to paper V was at least two blood samples from two different time points after vaccination and vaccination with mRNA vaccine BNT162b2 (Pfizer/BioNTech). In total 61 patients were included, 31 from the COVID-19 convalescent cohort and 30 from the COVID-19 naïve cohort.

SARS-CoV-2 antibody detection and avidity index

For the detection of anti-spike IgG, Anti-SARS-CoV-2 QuantiVac ELISA (Euroimmun), previously described in paper I and paper III, was used manually.

For the avidity index the same method as in paper I and paper III was used. Briefly, the same antibody assay as for anti-spike IgG is used but with the addition of a chaotropic agent (4M urea) in one of the duplicates. The avidity is defined as $OD_{urea}/OD_{reference}$ and multiplied by 100 to be expressed as percentage.

Vaccine Questionnaire

Seven days after the first and after the second dose, a vaccine questionnaire was sent using the digital platform. The questionnaires included questions about the occurrence of side effects. The patients could tick from a list of nine common side effects and/or write in free text. If side effects occurred, there was questions about the duration and the need of sick leave.

Statistics

For analysing differences in antibody levels and avidity index between the cohorts, Mann-Whitney was used, and for analysing differences over time within the cohorts, Wilcoxon signed ranks test was used. For analysing differences in proportions of reported side effects between the cohorts and between the doses, chi squared test was used. $p<0.05$ was considered statistically significant. All statistics were computed in IBM SPSS statistics 29.

Results

Paper I

In total, 75 patients could be included. The median age was 50 years (range 19-76) and 65% (n=49) were women. Comorbidity was reported by 28% (n=21) and obesities (BMI >30), hypertension and chronic headache were most common and reported by 16%, 11% respectively 7%. Only 1 patient reported immunosuppressive treatment. 23% were health-care workers and 16% reported long-term symptoms (>2 months).

After COVID-19 the levels of nucleocapsid-antibodies increased significantly from 1 to 3 months and then the levels significantly decreased between 3 to 6 months (median value at 1, 3, 6 months: 28,3 s/co, 39,3 s/co, 17,1 s/co, $p<0.001$). 89% of the patients developed detectable n-antibodies three months after the infection.

The anti-spike IgG level after COVID-19 was at is highest at 1 month and then significantly decreased (median value at 1, 3, 6 months: 37,6 RU/ml, 24,1 RU/ml, 18,2 RU/ml, $p<0.001$). 88% of the patients had detectable spike-antibodies after the infection.

No significant differences in n-antibodies nor anti-spike IgG were seen between health-care workers and non-health-care workers or between the patients with symptoms longer than 2 months compared to those with symptoms less than 2 months. For n-antibodies there was a small but significant difference between men and women, with higher levels in men at 3 and 6 months (median value men vs women at 3 and 6 months:99,0 s/co vs 23,1 s/co, $p=0.025$; 55,3 s/co vs 11,4 s/co, $p=0.018$). For anti-spike IgG no difference due to sex was seen.

The avidity index of anti-spike IgG increased significantly from 1 to 6 months after COVID-19 (median value at 1, 3, 6 months: 52%, 66%, 71%, $p<0.001$) and the avidity index did not correlate with the levels of anti-spike IgG ($p>0.05$). The avidity index did not differentiate for health-care workers or the patients with symptoms longer than 2 months. At 3 months there was a small, but statistically significant, difference between men and women (median value men vs women at 3 months: 61% vs 67%, $p=0.015$).

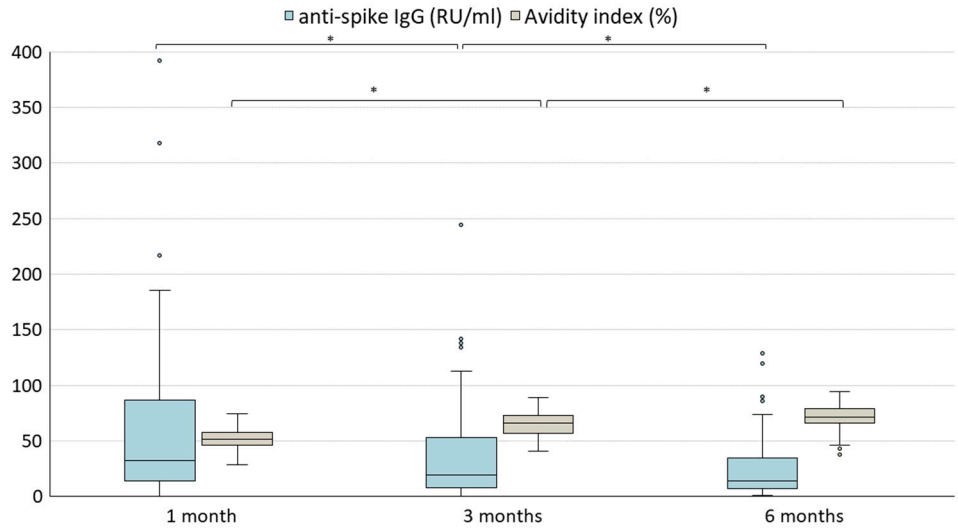

Figure 7. Anti-spike IgG (RU/ml) and avidity index (%) after PCR-confirmed COVID-19. *p<0.05.

Paper II

All 154 patients from the prospective cohort could be included. Mean age was 46 years (range 18-79 years). 69% were females and 30% reported any comorbidity and most common was obesities (13%), hypertension (11%) and chronic headache/migraine (11%).

The most common symptoms during the first week of COVID-19, reported by more than 50% of the patients, were malaise, fatigue, headache, fever and cough. The same initial symptoms, but also soared throat and arthralgia, were reported by more than 50% of the patients that later experienced prolonged symptoms. Skin tenderness and paraesthesia, unusual symptoms of a viral infection, was reported by 19% respectively 11% of all patients. The was no significant difference between the numbers of reported symptoms between those who later experienced long-term symptoms and those who did not (median numbers of reported symptoms: 8 vs 9, p=0.65).

The duration of symptoms were 1-4 weeks for the majority of the patients (65%) and 19% had symptoms for less than a week. 12% reported long-term symptoms (>8 weeks) and 7% still reported symptoms six months after the infection.

Among those who reported long-term symptoms, 56% had not recovered after 12 months. The most reported symptoms during 6-12 months after the infection were impaired physical condition, fatigue, anosmia and headache.

The BIPQ was included in the start questionnaire and all but one of the patients answered. The mean value of BIPQ index was 35.1 and the mean values of the different dimensions is presented in figure 8. In patients with long-term symptoms the mean value of BIPQ was higher, but not significantly higher (41 vs 34, p=0.09). There was a significant negative correlation of BIPQ index and EQ5D-VAS at 1, 3 and 6 months but no correlation was seen prior to the infection (correlation at 1 month: r=-0.35, p<0.001).

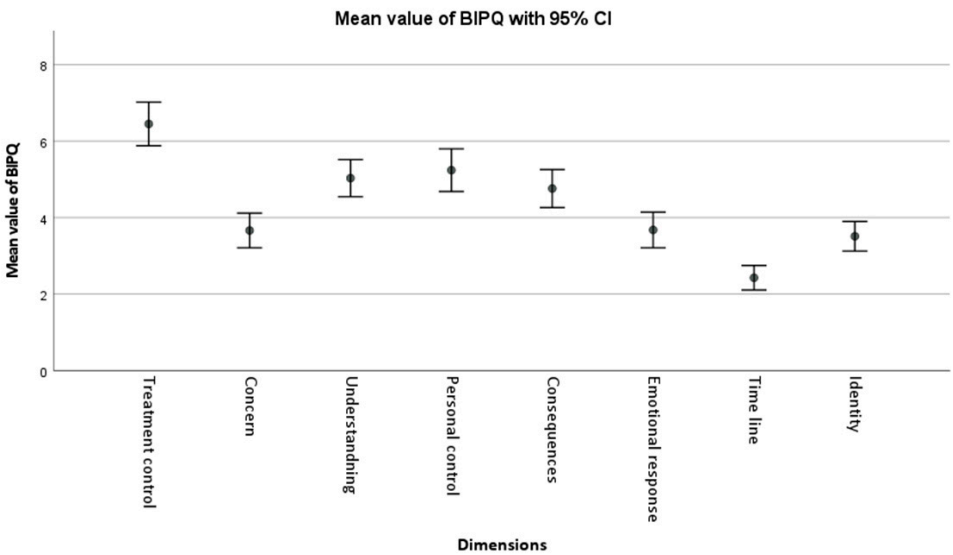

Figure 8. Mean value of BIPQ with 95% CI.

The response rate for the EQ5D-VAS questionnaires at 1, 3 and 6 months was high. Five patients did not answer any of the questionnaires and 8-14 patients did not answer at the different time points.

The EQ5D-VAS score was significantly lower at all the time points after the infection compared to the score before the infection. The same pattern was seen in the sub-group of patient with long-term symptoms, but the mean difference of EQ5D-VAS score prior infection compare to 1 month after, was higher than for the whole population (0.14 vs 0.03), see figure 9. The score did not correlate with age and there was a small, but significant, difference between men and women at all the time points except at 3 months.

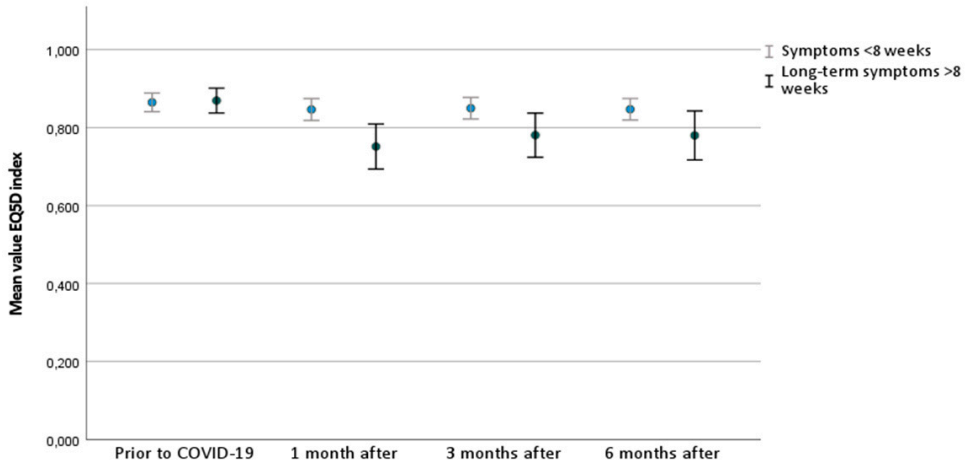

Figure 9. Mean value of EQ5D-VAS index, with 95% CI, at different time points after COVID-19, in the cohorts with symptoms less or longer than 8 weeks.

Paper III

Of the 14 included patients, 71% were females, median age was 58 years (range 26-76 years). The majority had a civil status of co-living and all patients were working or were retired. The most frequent symptoms were fatigue, anosmia/taste disorder and dyspnoea.

The results from the qualitative content analysis with theme, categories and sub-categories are presented in table 1. For extended description of the results with quotes, see the original paper in the appendix.

Table 1. Result from the qualitative content analysis

Theme	**Moving between uncertainty and new insights**		
Categories	Loss of abilities	Loss of control	Revaluation of life
Sub-categories	Losing smell and taste	Being foreign to oneself	Accepting the transformed body
	Lacking energy	Seeking answers	Prioritinzing health

Paper IV

In total 34 patients were included, 38% (n=13) in the infection cohort and 62% (n=21) in the vaccine cohort. Mean was age 46 years (range 21-69 years). The mean age between the cohorts did not differ but the proportion of women was higher in the vaccine cohort (76% vs 54%).

After the second dose of vaccine BNT162b2 (Pfizer/BioNTech) high levels of anti-spike IgG developed with maximum levels after 1 month and then a significant decline over time. The median levels of anti-spike IgG at 1, 3 and 6 months after vaccination were 628 RU/ml, 229 RU/ml and 60 RU/ml in the vaccine cohort. After natural infection (infection cohort) the pattern was the same but antibody-levels were significantly lower (median level at 1, 3 and 6 months 51 RU/ml, 22 RU/ml and 15 RU/ml), see figure 10.

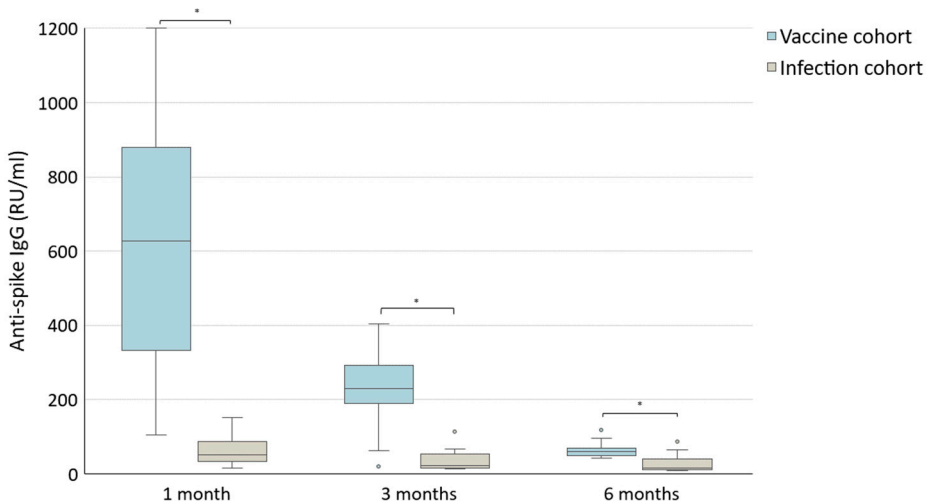

Figure 10. Anti-spike IgG (RU/ml) levels after vaccination compared to after PCR-confirmed COVID-19. *p <0.05

In the vaccine cohort, avidity index of anti-spike IgG was at a high level already after 1 month and there was no significant change during six months of follow-up (median value at 1, 3 and 6 months: 86%, 87%, 87%, p >0.05). In the infection cohort, the avidity index was significantly lower (median value: 50%, 57% and 61%) but increased over time (p<0.05), see figure 11.

The neutralization titre after vaccination declined significantly from 1 to 3 months (median titre at 1 and 3 months: 540, 180, p<0.05). Neutralization titres in the infection cohort decreased significantly (p<0.05) from 1 to 3 months (median titre at 1, 3 and 6 months: 75, 60, 60), see figure 12.

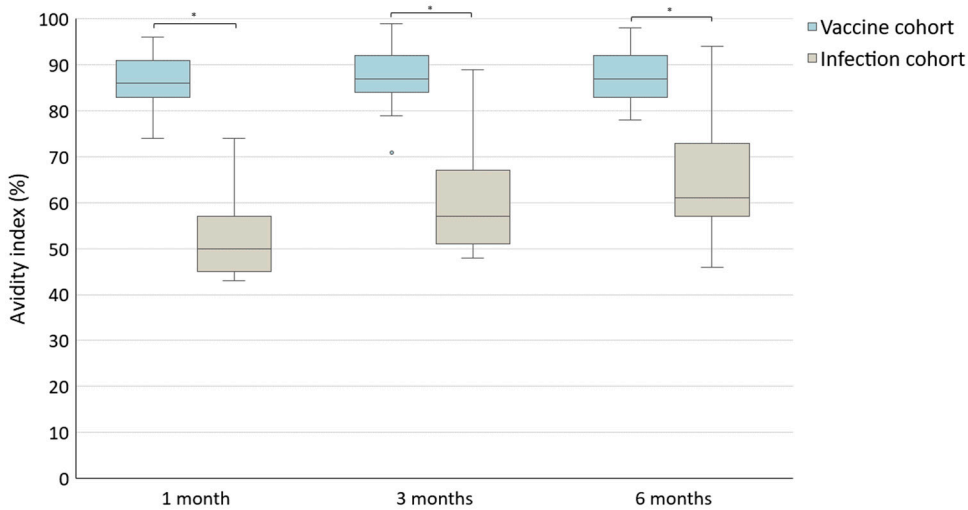

Figure 11. Avidity index after vaccination compared to after PCR-confirmed COVID-19. *p <0.05.

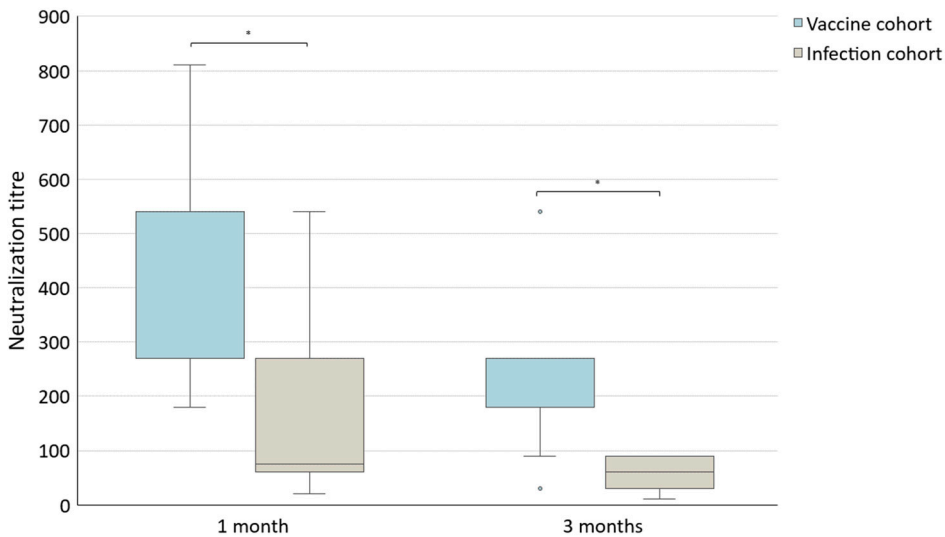

Figure 12. Neutralization titre after vaccination compared to after PCR-confirmed COVID-19. *p <0.05.

There was a strong correlation between neutralization titre and anti-spike IgG (spearman r=0.88, p<0.05), a significant but weaker correlation between avidity index and neutralization titre (spearman r=0.62, p<0.05). In the subgroup with anti-spike IgG <40 RU/ml the correlation with neutralization titre was weaker than the correlation including anti-spike IgG >80 RU/ml (spearman r: 0.49 vs 0.66).

Paper V

In the COVID-19 convalescent cohort, 31 patients were included and 30 patients were included in the COVID-19 naïve cohort. Mean time between infection and first dose of vaccine was 8,5 months. The mean age was 51 years in both cohorts and the total range of age was 24-65 years. The vast majority in both cohorts were women (90% vs 73%) and 23% respectively 37% reported comorbidity. Hypertension was most common, 10% in both cohorts. Only one patient in the COVID-19 naïve cohort reported immunosuppressive treatment.

In the COVID-19 naïve cohort, the levels of anti-spike IgG at 1, 3 and 6 months after vaccination were 558 RU/ml, 214 RU/ml and 59 RU/ml. The decline was significant ($p<0.05$). For patients in the COVID-19 convalescent cohort, the levels of anti-spike IgG were significantly higher ($p<0.05$). The levels of anti-spike IgG at 1, 3 and 6 months after vaccination in the COVID-19 convalescent cohort were 700 RU/ml, 352 RU/ml and 193 RU/ml, see figure 13.

The avidity index in the COVID-19 convalescent cohort was high at all the time points but with a small, but statistically significant, increase from 3 to 6 months (median value at 1, 3 and 6 months: 91%, 91%, 94%). When compared to the COVID-naïve cohort there was a significant difference in avidity index at 1and 6 months after vaccination ($p<0.05$) even though the naïve cohort also had high avidity index with a median value of 87% at all the time points, see figure 14.

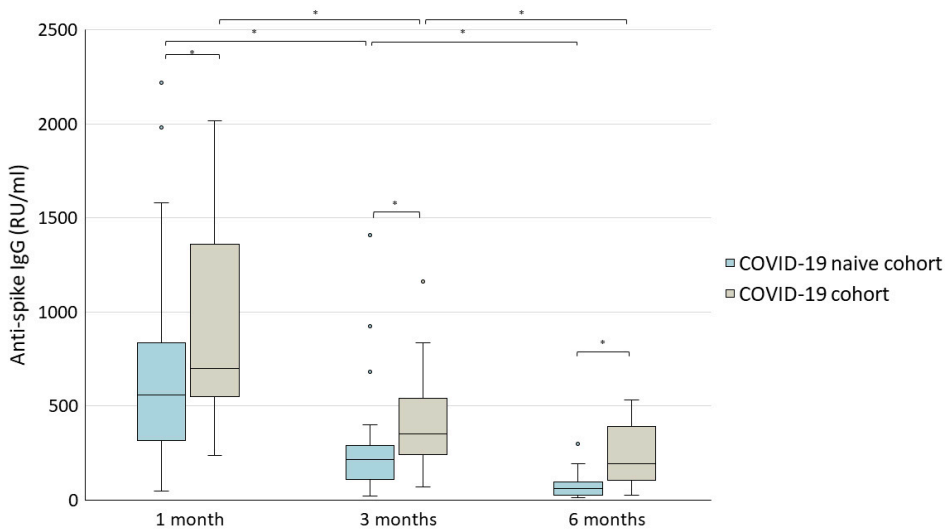

Figure 13. Anti-spike IgG (RU/ml) after vaccination, compared between a COVID-19 naïve cohort and a COVID-19 convalescent cohort. *p <0.05.

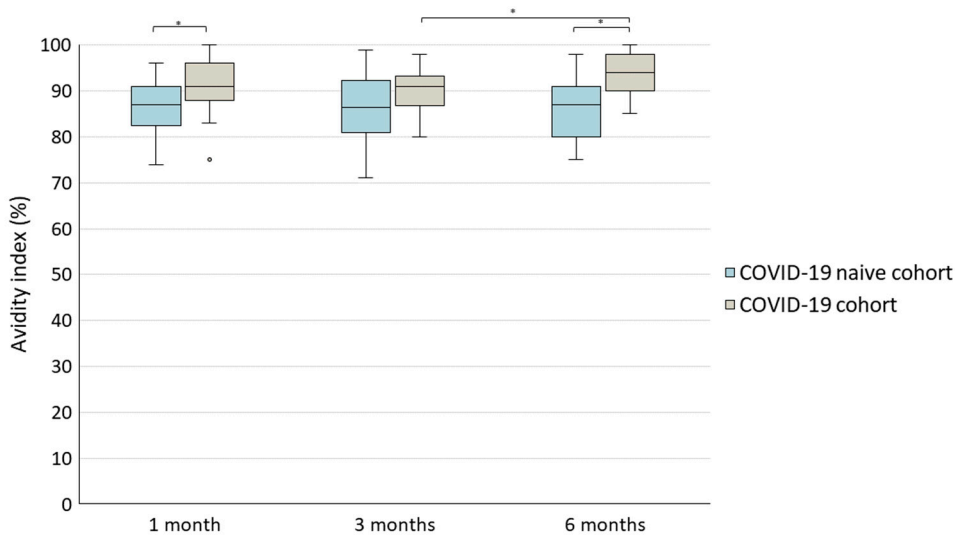

Figure 14- Avidity index (%) after vaccination, compared between a COVID-19 naïve cohort and a COVID-19 convalescent cohort. * p<0.05.

Side effects of the vaccine were reported in 52% and in 60% after the first respectively the second dose of vaccine. The most common side effects was pain at the injection site, fatigue and headache. All symptoms were of short duration, the majority only 1-2 days. After the second dose, 10% reported a need of sick-leave.

Discussion

Symptoms in non-severe COVID-19 and HR-QoL

In paper II, we could show that the most commonly reported symptoms during the first week of non-severe COVID-19 were malaise, fatigue, headache, fever and cough. This is in line with other studies (5, 17, 18). These are the similar initial symptoms reported in patients that later deteriorate to severe disease. The initial type of symptoms do not seem to differ, but the frequency of the symptoms are higher in the population with severe COVID-19 (9, 12). Thus, the initial symptoms cannot be used to predict progress to severe disease; instead, other risk factors, such as comorbidity, are more useful.

The fact that some patients experienced very few and mild symptoms or even asymptomatic infections complicated the national testing strategies and the workload concerning infection control, which likely contributed to the transmission (20, 57). In our cohort, 6% reported absence of symptoms when tested and during follow-up (unpublished data).

Nearly 40% of our cohort experienced anosmia and 19% and 11% respectively, experienced skin tenderness and paraesthesia during the first week. These symptoms can indicate an involvement of the nervous system in the pathophysiology of COVID-19, thus even in mild COVID-19 (58).

A limitation in our study is that there was no grading of the reported symptoms, only the presence of symptoms was documented in the questionnaire. A strength of the study is the rigorous follow up during a long observation period (>12 months).

HR-QoL and perception of the disease

Compared to the numerous studies regarding symptoms and immune response in COVID-19, the number of reports focusing on health-related quality of life are far less common. Generally, studies of different infectious diseases seldom have a focus in HR-QoL, reasonably because most infectious diseases have a short course and seldom exhibit a chronic stage. Since the pandemic was a unique period and COVID-19 a completely new disease, we were interested in what way the infection would affect the quality of life.

In paper II we analysed the EQ5D-VAS score at the different time points, showing that the infection had a negative effect on the self-rated HR-QoL. After the infection, the EQ5D-VAS score was established at a lower level that was retained during the follow-up. Other studies have also showed a negative impact on HR-QoL after COVID-19, but most of these studies have focused on hospitalized patients or severe COVID-19 (59-63). Our results indicate that even a mild non-severe infection can affect people's daily life.

Unfortunately, we did not have the possibility to examine the consequences of the decline in self-reported HR-QoL. Did this decline cause increased periods of sick-leave or increased numbers of health-care contacts? That would have been interesting to find out and is an area for future investigation, especially as a preparation for next pandemic. Another limitation was the lack of a control group, which means we cannot rule out that the pandemic situation itself affected the HR-QoL. Studies have shown that the pandemic situation did affect the HR-QoL in the general population but Larsson *et al* could still show a larger impact on self-rated health after COVID-19, when compared to a PCR-negative control group (64-66). An additional limitation was that the EQ5D-VAS score prior to the infection was reported retrospectively in the initial questionnaire; hence, this assessment was done in a different way than the rest of the EQ5D-VAS questionnaires.

The BIPQ is a scoring system of the perception of the disease. It is not validated for infectious diseases in general, nor COVID-19 and no other studies can be found were the BIPQ has been examined in COVID-19. Still, it reveals some interesting aspects. Overall, the results of BIPQ in paper II showed that most of the patients were confident of a fast recovery and had no major concerns about the infection. This aligns well with a mild, non-severe infection. However, we believe that the uncertainty of being infected with a novel virus, together with the pandemic situation itself, were reflected in the scores for the dimension of understanding, personal control and consequences. There was also substantial individual variations and we could see a negative correlation of BIPQ index and EQ5D-VAS score. This implies that patients that experienced their symptoms to a greater extent and were more concerned also showed a greater negative impact in the HR-QoL. This can be a valuable aspect for clinicians to consider, when identifying patients in need of extra support.

The post-COVID condition

In paper II we found that around a fifth of the patients had symptoms less than a week and the majority had symptoms for 1-4 weeks. However, we also found that 7% of the patients continued to report symptoms more than six months after the infection. We could conclude that even a mild illness could have great impact on

the health and the daily life for a long time. Around this time, other studies and reports about long-term symptoms started to emerge. In the beginning, there was no clear definition of this condition but later WHO stated that the post COVID-19 condition, or long COVID, is "the continuation or development of new symptoms 3 months after the initial SARS-CoV-2 infection, with these symptoms lasting for at least 2 months with no other explanation" (67).

Sleeping disorders, depression and problems managing work and daily life are described already after the Spanish flue (68). In other infections prolonged symptoms, especially fatigue after EBV-infection, are reported but not to the same extent as in long COVID (69, 70). However, long-term symptoms as a condition or phenomena after an infection has not gained so much attention and the pathophysiology is not known.

In our study, the prevalence of long COVID was 12% (defined as symptoms lasting more than 8 weeks) and is lower compared to other studies, which report long-term symptoms in up to 62% of patients (39, 41, 71). The divergence in the results probably reflects variations of study design and selection bias, with most studies including patients with more severe disease. Persistent symptoms are reported at the same level in other studies with a prospective design and WHO states that around 10-20% of patients typically develop long COVID (40). The most reported persistent symptoms in our cohort were impaired physical condition, fatigue, anosmia and headache; in line with other studies (72-74). The course of long COVID resembles the ME/CSF, which also is a complex condition with similar symptoms that is also poorly understood (75).

In paper II we could not find that the patients who developed long COVID had different types or numbers of initial symptoms, nor different Ct values than the other patients. According to this, the initial symptoms cannot be used to assess the risk of long COVID. The number of patients in our cohort were unfortunately too small to properly analyse other risk factors for long COVID. Other studies shows that female gender, obesity and even some specific symptoms are associated with long COVID (42, 43, 76). These risk factors may be useful in future research in order to identify the potential mechanism of this condition. Research about possible biomarkers as well as effective treatment is also needed.

In our results, we could not see any difference in the BIPQ index in the patients with long COVID and there was no correlation to the EQ5D-VAS score prior to the infection. This strengthens our conclusion that it is difficult to foresee who will suffer from long COVID.

The group of patients with long-term symptoms had the same overall pattern of EQ5D-VAS score as the general population. However, the reduction was even more pronounced in the patients with long-term symptoms and this shows that long COVID has an impact on HR-QoL. Other studies also show reduction in HR-QoL, even in non-hospitalized patients, consistent with our results (77-79).

Since patients could only indicate the type of symptoms in our questionnaires, but not grade the severity or describe the consequences of the prolonged symptoms, we invited the patients with long COVID to the interview study. Through this we had the possibility to gain important insights of the patients' experiences of living with long COVID. We could include both men and women in different ages and the majority had symptoms for at least 16 months. The most common prolonged symptoms in this cohort were anosmia/taste disorder, fatigue, dyspnoea and impaired physical condition, which are in line with the clinical characteristics of long COVID (80-82).

The qualitative content analysis is a method suitable to obtain knowledge about how individuals understand and experience their health. The trustworthiness is defined from four criteria – credibility, dependability, confirmability and transferability. For the credibility of the study, the data was analysed until consensus was reached and all results was based on the patients' quotations. For dependability, an interview guide was used and an experienced qualitative researcher performed all the interviews. The authors were both men and women, from various professions and with different academic grades, hence the analysis included diverse perspectives. Confirmability refers to the neutrality on the data and this was strengthened by the analysis process described in the method section. There was a saturation of the results with no new subcategories after the seventh interview. Each subcategory was represented by content from five to ten of the patients, which demonstrates richness of the data. Regarding transferability the limitations are that all patients were born in Sweden and living in the same region. To increase the transferability both men and women participated and the age span was wide and they lived under different conditions.

As reported, the overall theme of the patients' experiences of long COVID was "moving from uncertainty and new insights" with sub-categories characterized of losses and sufferings, but also acceptance and revaluation. The patients clearly described the loss of abilities and the lack of energy due to losing smell and/or taste and due to fatigue. This loss of abilities had changed their lives and many former activities could no longer be done. Since many of the patients had been very active before the COVID-19, this had an impact on the social, existential, physical and mental health. The symptoms influenced everyday life, affecting work performance and the social life.

The patients did also describe the loss of control when they could no longer recognize themselves and when there was no answer to why or how long the symptoms would persist. Many patients shared the experience of the ignorance from the health-care systems and expressed a hesitation to contact health-care services. This is an important feedback for both clinicians and other health-care workers.

Even though they described being out of control in this new state of health most patients adapted to the new situation. They tried to seek answers, they increased

their ability to listen to the body´s need and they tried to fight their symptoms. Engaging in alternative activities was one strategy to create health. Many patients expressed an appreciation that it did not get worse.

The symptoms and the new situation forced the patients to prioritize their health. As some of them described, the condition forced them to think about what health means since they had previously taken health for granted. Despite having a difficult time struggling with long COVID, the patients gained new, important insights and it became important for them to live in the present. They described insights about what creates health, insights about their new priorities and an acceptance of the situation and the fact that illness can affect anyone. The adaptations, the new insights, the strive for finding a new context and thoughts about the meaning of life when suffering from a diseases has been showed in other research (83).

Still, no evidence-based management of long COVID exists. Patients with long COVID, which are a new and heterogeneous group, need to be treated seriously, based on each patient's individual condition, with a multidisciplinary rehabilitation effort (84).

Immune response

SARS-CoV-2 antibody detection

In paper I, we wanted to investigate the antibody kinetics after natural infection of COVID-19. At that time, we were in the eve of the pandemic of a completely new disease. Although other studies regarding antibody kinetics were published around the same time, we believe our results still contribute to the knowledge of the immune response after non-severe COVID-19.

We could show that the majority of patients seroconverted and had detectable antibodies against both the nucelocapsid- and the spike-protein. Approximately 10% did not develop any antibodies and remained seronegative. One possible explanation for this may be that the innate immune system has the capability to clear all virus particles, without activating the humoral immune system (85).

Six months after the infection, antibodies declined significantly but were still detectable, in line with other studies (86-89). The kinetics of the various antibody differed. For nucleocapsid-antibodies, the maximum level was reached after 3 months but for spike-antibodies the maximum level in our study was reached at the first measurement, one month after the infection. The difference in the kinetics probably depends on the different classes of antibodies since we used different assays. For n-antibodies the total IgM and IgG was measured but for anti-spike only IgG was measured. Overall, the dynamics of the antibodies was as expected for a viral infection (90).

Others have showed higher levels of antibodies in severe ill and hospitalized patients (91, 92). This might be due to larger viral load and/or a prolonged time of viremia compared to non-severe cases. Since no standardization for quantification of the antibodies was available at the time for analysis, a comparison of the absolute levels between studies or assays was not possible. Therefore, in our cohort, with only non-severe cases, we could not compare results with severe cases. Still, a valid conclusion is that even with very mild symptoms, the antigen exposure is enough to induce a detectable humoral immune response in the majority of the patients.

Some studies demonstrates a difference between men and women in humoral immune response, but with inconsistent results, whether men or women have higher levels of antibodies (93-95). We saw a significant difference with higher levels of n-antibodies for men at 3 and 6 months after the infection, but we did not see any difference in spike-antibodies. It is difficult to conclude how much impact this difference has, especially in cases of non-severe COVID-19, and since there was a limited number of men included in the vaccine substudy, we could not analyse sex-differences in the immune response after vaccination. It is proved that men have a higher risk of severe COVID-19 and sex-related differences in autoimmune diseases as well as in malignancies have been showed (5, 22, 96, 97). Taken together this demonstrated that there are differences in the immune response between men and women. However, if this difference has an impact on the risk of reinfection and/or on the duration of the immunity for COVID-19 is not yet clear.

A study of health-care workers in Region Stockholm, Sweden reveals that the seroprevalence of IgG antibodies was higher in health-care workers and there was a correlation with patient contact implying an occupational risk for COVID-19 (98). When our study started we had the hypothesis that health-care personal might be more exposed to the virus and therefore the immune system might be boosted and this might lead to higher levels of antibodies. However, when it comes to the level of antibodies, the results in our study could not show any differences between health-care personal and the rest of the cohort.

Since the mechanism behind prolonged symptoms of COVID-19 is unknown, it was interesting to see if there was any difference in the immune response after the infection. Peghin *et al* demonstrated a significant association for both the prevalence and the level of IgG antibodies with post COVID-syndrome (71). With the limitation of few patients in our cohort of long-term symptoms (n=12), we could not confirm this result since the kinetics of the antibodies was the same and there was no significant difference in the level of any antibody, when comparing to the patients with symptoms of shorter duration.

In paper IV we could show that after the second dose of vaccine, the levels of anti-spike IgG were significantly higher than after natural infection. This is in line with other studies (99-102). Most likely, the repeated exposure to the antigen causes the stronger immune response after vaccination. This is strengthen by the studies which

shows that only one dose of vaccine does not yield as high antibody levels as two doses (103-106). Additionally, in paper V we could show that having a COVID-19 infection before vaccination resulted in even higher antibody-levels. Others have also showed that a previous infection enhances the immune response after vaccination (107-110).

Avidity index

Since the avidity index reflects a selection and maturation of B-cells it is likely that formation of high-avidity antibodies are essential to establish long-lasting and protective immunity (111-113). For several other viral infections, it has been shown that high-avidity is of importance for immunity and that low avidity of IgG antibodies is associated with risk of repeated infection. Freitas *et al* showed that high-avidity antibodies in RSV led to less risk of severe infection and Mercader *et al* showed that low-avidity antibodies against mumps led to higher risk of reinfection (114, 115). Incomplete avidity maturation is seen in infections with seasonal corona virus (116). It might be a feature of corona virus in general and the failure of avidity maturation might be one of the factors that enables non-sustained immunity and the possibility of repeated infections in seasonal corona virus.

In paper I, we showed an increase in maturation of the avidity during at least six months after natural infection, as others also have found (117-119). This indicates an ongoing process in the immune response well after the virus is cleared and no antigen is circulating. Although the avidity index increased over time, natural infection failed to induce high avidity antibodies, as seen in other studies (99). In paper IV, we could clearly demonstrate the difference in avidity between infection and vaccination, with higher avidity index after vaccination. The higher avidity index after two doses of vaccine compared to infection is also showed in other studies (100, 103, 120). One explanation for the difference might be that a non-severe infection just have virus in the respiratory tract and no or very low levels of antigen in the blood, in contrast to severe infection or vaccination (116). Most likely, not only the level of antigen matters, but also repeated exposure to virus antigens are of importance. In concordance with this, studies show that both antibody levels and avidity index after only one dose of vaccine is considerably lower than after two doses and a COVID-19 infection before vaccination causes higher avidity index (113, 120-123).

We could not see any differences in avidity index after COVID-19 between health-care workers and non-health care workers or between those who developed long-term symptoms or not. Between men and women, we could see a significant, but minor difference at 3 months after the infection. Since the difference was within the level of the measurement uncertainty for the assay, we assessed the difference as not clinically relevant. In other studies, no difference in avidity index between men and women is reported (101, 124). Worth noticing, is the wide range of the avidity index after COVID-19, also seen by other groups (125). As previously mentioned,

the level of virus antigen, time of exposure and degree of severe infection affect the development of avidity, but reasonably also individual factors of the immune system contribute to the immune system development and thereby the observed variation.

After two doses of vaccine, we found that the avidity index was high already after one month. This is in contrast to other studies where an increase is reported first six months after the second dose (101, 126). This discrepancy might be due to methodological issues, e g different concentrations of urea. In our avidity assay, the concentration of urea was chosen from optimization experiments where serum after infection was used. Since we have showed that vaccination results in clearly higher avidity index, with median values around 90%, compared to infection, it is possible that a use of higher urea concentration had demonstrated an increase in the avidity index after vaccination instead of a plateau close to 100% already after 1 month. Overall, studies show concordant findings of high avidity antibodies after vaccination (100-102, 104, 126, 127).

Another limitation when comparing and discussing avidity index is not only different concentrations of urea, or different chaotropic agents, it is also the lack of definition, or cut-off, for high-avidity and low-avidity. This means that comparing the absolute number of the avidity index between studies is difficult and that the avidity index has to be put in a context and that the dynamics of the avidity index might be more assessable. In our study *COVID-19 Symptoms and Immunity*, we have followed the numbers of reinfections in the patients and had the ambition of relating the avidity index to risk of reinfections. However, until November 2021 only 4 reinfections were noticed. With so few reinfections during the first year, it was not possible to define a reliable cut-off for protecting immunity in our avidity assay. Then, in late November 2021 until February 2022, Omicron emerged and more than 80 reinfections were noticed in our study (unpublished data). However, since the mutations of the spike regions in the Omicron variant were so numerous (128), it can be questioned to define those infections as reinfections and a cut-off based on our avidity assay, which is targeting the ancestral virus variant, is not useable.

Since the avidity maturation is time dependent, the avidity index might be used as a diagnostic tool for estimating the time since the infection and based on that, discriminate an acute, current infection from a past infection (129). Even though the role of such assays has subsided when NAATs are widely used, it can sometimes be useful in selected patients. Such assays are available and used in clinical practice for cytomegalovirus-infections in immunocompetent patients and was suggested as a complement of the diagnostic tools in COVID-19 (130, 131). Since there was a considerable individual variation of the avidity index after COVID-19 infection (paper I and IV) there was no clear cut-off to define a past infection (> 3 or > 6 months ago). According to this, the avidity index cannot be used to discriminate a current from a past infection.

The role of avidity index for protecting immunity

To attain protective and long-lasting immunity a variety of mechanisms in the immune system are involved. The innate immune system plays in important role as a first line defence against infections and to activate the specific immune system for further protection. Several other mechanisms for selection and maturation of immune cells are important and a sufficient amount of neutralizing antibodies are needed. The immune system can be assessed and examined in various ways. In order to assess protective immunity from the humoral immune response, both after natural infection and after vaccination, the gold standard is to measure the neutralizing capacity of the appropriate virus (48). Such cell-based assays measure the actual capacity of the antibodies to neutralize living viruses. However, since living viruses are used, these assays require biosafety level 3 laboratories. In addition, they are time consuming and expensive, and this makes them unusable as a routine diagnostic test. Instead, the level of an antibody, targeting a surface antigen, is often used as an estimation of the protective immunity and for vaccine efficacy in the daily clinical practice.

As mentioned, the formation of high-avidity antibodies is a necessity for protective immunity. Therefore, we wanted to examine the correlation between the avidity index and the neutralizing capacity. If we could find a strong correlation between the avidity index and the neutralizing capacity, we could be able to get a surrogate test for neutralizing capacity. A surrogate test, which is easy to perform and which could be used in every clinical laboratory.

In paper IV, serum samples taken after COVID-19 as well as samples taken after vaccination in COVID-19 naïve patients were analysed in a cytopathic cell-based neutralization assay. When looking at the correlation we saw a strong correlation between the levels of anti-spike IgG and the neutralizing titre, which is seen in other studies (117, 127, 132). We also found a correlation between the avidity index and the neutralizing capacity but it was considerably weaker. When combining the avidity index and the anti-spike IgG level we could again conclude that the avidity index did not contribute to a better estimation of the neutralizing capacity than the anti-spike IgG level alone. Even in the subgroup of patients with high-avidity antibodies, the correlation was lower compared to anti-spike IgG correlation. According to this result, the avidity index is not useful as an everyday diagnostic tool for estimating the protective immunity or evaluate vaccine response, at least not for COVID-19. Nevertheless, this does not mean that the avidity is inessential for the development of immunity, it only implies that it cannot be used alone as a measure of the neutralizing capacity. There are also other aspects to consider for long-lasting immunity. As for COVID-19, several new virus variants have emerged and it is therefore desirable with a broad immunity. In our work we have not investigate this aspect of immunity, but Wratil *et al* have showed that high avidity antibodies have a broader SARS-CoV-2 epitope recognition and a better capacity to neutralize SARS-CoV-2 variants of concern (113, 133).

Worth to notice, is that the strong correlation between the levels of anti-spike IgG and the neutralizing capacity justifies the clinical use of the antibody levels as a rough estimate of immunity and vaccine response. The challenge for the clinician is still to assess when the level of antibodies is sufficiently high to assure protection. It is showed that seronegativity is a major risk factor for severe COVID-19, but no cut-off for protective immunity or sufficient vaccine response for COVID-19 exists (134, 135). We could also see a clearly weaker correlation in the subgroup with low level of anti-spike IgG. This implies that it is more difficult and uncertain to estimate the neutralizing capacity when the antibody levels are low. In addition, for viral infection there are many other mechanisms, especially function of the innate immune system and T-cell response that is important for clearing the virus. Finding a cut-off for protective immunity based solely on antibody levels might therefore be very difficult and maybe not even suitable for a viral infection.

In line with the results of antibody levels and avidity index, we found a very low neutralizing capacity after natural infection but significantly higher neutralizing titres after vaccination. Already at 3 months after the vaccination, the titres had substantially decreased. This fast decline of the neutralizing titres have been shown by others and indicates a fast decline in immunity, emphasizing the importance of vaccine booster (93, 94, 100, 106, 136).

In conclusion, a natural infection with SARS-CoV-2 does not yield a strong humoral immune response and this might enable repeated infections. Even though vaccination gives rise to a strong immune response with antibodies with high neutralizing capacity, the levels decline already after a few months. Although high avidity antibodies develop after vaccination, and although the avidity seems to be preserved, the substantially decline of the anti-spike IgG levels indicates a difficulty of gaining a long-lasting immunity against SARS-CoV-2. With this characteristics of the immune response together with the fact that the existing virus variants seems to be more transmissible, it is reasonably to believe that we cannot eradicate COVID-19, nor gain protective long-lasting immunity. Instead, COVID-19 might become a seasonal infection where focus must be on vaccination of the patients with high risk of severe COVID-19 and early antiviral treatment in selected cases.

Vaccination and side effects

Vaccinating against COVID-19 was one of the most important tool to limit the impact of the disease and end the pandemic. Still, it is important to investigate and gain knowledge about possible side effects in order to get a correct benefit-risk analysis, especially when new vaccine technology was used and large scale vaccination was initiated (137).

In paper V we analysed what kind of side effects the patients reported, both after the first and second dose of BNT162b2 (Pfizer/BioNTech). The most reported side effects was, as expected, pain at the injection site, fatigue and headache, and no

severe side effects. This is in agreement with other studies (35, 138). It is established that in rare cases, the vaccine can cases severe side effects, like myocarditis (139). The limitation in our study, with a small cohort and limited time for follow-up might be a reason why we did not find any severe side effects. An interesting result, which needs to be considered when planning for large scale vaccination, is the quite big need of sick leave related to the vaccination. In our cohort, 10% reported need of sick leave after the second dose, and this is relatively low numbers compared to other studies, which reports sick leave in up to 35% (140-142).

Conclusion

- For the majority of patients, COVID-19 caused symptoms for 1-4 weeks and the most commonly reported symptoms were malaise, fatigue, headache, fever and cough.
- COVID-19 had a negative impact on HR-QoL, especially in patients with a greater disease burden (high BIPQ score) and in patients with prolonged symptoms.
- Long-term symptoms (>8 weeks) were reported by 12% of patients with non-severe COVID-19, with 7% reporting persistent symptoms after 6 months. The most commonly reported long-term symptoms were impaired physical condition, fatigue, anosmia and headache.
- Patient experiences of long term COVID-19 can be described as "moving between uncertainty and new insights" with loss of abilities, loss of control but also a revaluation of life. The symptoms influenced daily life by affecting social life, work performance and physical health.
- The immune response after non-severe COVID-19 is characterised by declining levels of anti-spike IgG and neutralization titres after 1 month. Even though the avidity index increased up to six months after infection, a natural infection failed to form high avidity antibodies.
- Two doses of vaccine gave a strong immune response; significantly stronger than after natural infection, with high levels of anti-spike IgG, high neutralization titres and high avidity index. The immune response was even stronger in patients with COVID-19 infection previous to the vaccination.
- Six months after vaccination, levels of anti-spike IgG and neutralization titre showed a substantial decline, emphasising the need of vaccine boosters.
- Reported side effects of the vaccine were mild and of short duration.
- There was a strong and significant correlation between the anti-spike IgG levels and the neutralizing titre. Although a correlation between avidity index and neutralizing titre was seen, this correlation was weaker than with anti-spike IgG and hence did not add any predictive information.

Future perspectives

COVID-19 is today a different clinical disease compared to the first years of the pandemic. It is now a disease that primarily affects elderly, fragile patients with comorbidities, but very few studies have included this population. How can prevention of the disease, e.g. vaccination, be optimized in this group? Are the available treatments effective and safe in this population? Studies of this population are important in order to further reduce the burden of the disease. Globally, aspects related to social science, economics and global justice of access to testing and vaccination are of importance, especially in order to be prepared for the next pandemic.

Aspects more related to this thesis, the immune response after both infection and vaccination, have been sparsely studied in the elderly population. In addition, the duration of immunity and the protection against emerging variants or reinfections, will continue to be an important field of research, as well as monitoring new virus variants. Overall, many processes in the immune system are not fully understood. Efforts to find clinically useful biomarkers to predict risk of severe infection or determine levels for protection are warranted, not only in COVID-19 but even in other types of infections.

Since the available vaccines seem to protect against severe COVID-19, but not against transmission, further studies on how to minimize transmission and make vaccines that are even more effective are of interest.

The large group of patients suffering from long COVID needs to be further explored. The areas concerning pathophysiology, diagnosis and treatment of long COVID needs to be studied, since the mechanism is not known and there are no defined biomarkers nor any established treatments.

Acknowledgements

I wish to thank all the patients that participated in the study!

There are many persons who have supported my work, and I would like to thank all of them and in particular:

Johan Undén, my supervisor, for getting me into this, for the help of managing all the impossible obstacles on the way and for your support and encouragement! Thanks for all the interesting discussions about research and for guiding my in the life as a doctoral student. Your way of combining research and clinical work is a great inspiration!

Jens Nygren, my co-supervisor, for all support and for so willingly share your knowledge about qualitative research and health in a wider perspective. **Johan Tham** and **Markus Lingman**, my co-supervisors, for enthusiasm and valuable feedback on study design and on the papers.

Arne Kötz, my former boss, now my colleague. Without you, this thesis would not exist! Thanks for you massive support, for encouraging me and for always having time to discuss anything or just listen to my complaints about whatever. Your genuine interest and enthusiasm in microbiology is truly an inspiration.

Anna Eringfält, for excellent laboratory work, nice talks and collaboration. Thanks for the beautiful painting to the book cover! **Fredrik Wickbom** for helping me out with the digital system. **Stefan Kunkel**, for statistic help and thanks for the patience with my questions, it was invaluable. **Ingrid Larsson**, experienced qualitative researcher, and **co-authors**, thanks for great cooperation and interesting discussions about health. **Jonas Klingström**, for making the neutralization analysis possible.

Thanks to all my colleagues at the Clinic of microbiology, Halmstad, especially **Anna**, **Ingela**, **Ali**, **Reman**, **Irene**, **Annelie**, **Eva** and **Christina** for your work with the serum samples and administration, **Ingegerd**, **Karin** and **Ellinor** for covering for me and **Terese** for giving me time to finish this thesis. Thanks to my former **colleagues at the Clinic of Infectious diseases** in Halmstad for support at work and all the entertaining discussions during coffee breaks.

Hanna Svensson, FoU, for the administrative support, always answering my questions and helping.

I am grateful for the financial support from Region Halland vetenskapliga rådet, Sparbanksstiftelsen Varberg and Södra sjukvårdsregionens doktorandmedel.

To my friends, **Åsa, Mia, Malin, Greta, Sophia, Maria, Christine, Maja, Hanna, Stina, Maria**, **Stina, Elle**, you are all such strong and incredible women! I am so happy for the time we have spent together during all the years - talking, training, travelling, studying, drinking wine, discussing life in general and making nice dinners in particular.

To **Mom** and **Dad**, thanks for your love and support. My dear siblings, **Sofia, Simon** and **Julia**. **Farmor Gun** for showing me the love of books and reading, I am so sad you are no longer with us.

Ulf, the love of my life, thanks for putting up with my monologues on covid and for believing in me. **Frans** and **Mårten**, ni betyder allt för mig och äntligen är mammas bok färdig!

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Appendices, Paper I-V

Printed by Media-Tryck, Lund 2024 NORDIC SWAN ECOLABEL 3041 0903

LUND UNIVERSITY

FACULTY OF MEDICINE

Anesthesiology and Intensive Care
Department of Clinical Sciences, Lund

Lund University, Faculty of Medicine
Doctoral Dissertation Series 2024:112
ISBN 978-91-8021-608-1
ISSN 1652-8220