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Analyse von Determinanten der Immunantwort nach SARS-CoV-2-Infektion bzw. -Impfung im Hinblick auf Risikofaktoren für Seropositivität

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List of Abbreviations

Anti-N antibody/ Anti-N	Anti-Nucleocapsid antibody
Anti-S antibody/ Anti-S	Anti-Spike antibody
BTI	Breakthrough infection
COVID-19	Coronavirus disease
CoVs	Coronaviruses
DBS	Dried blood spots
HCWs	Health care workers
KoCo-Impf	The prospective COVID-19 post-immunization serological cohort in Munich
KoCo19	The representative COVID-19 cohort Munich
MERS-CoV	Middle east respiratory syndrome coronavirus
PCR	Polymerase chain reaction test
RBD	Receptor binding domain
RKI	Robert Koch Institute
RNA	Ribonucleic acid
SARS-CoV	Severe acute respiratory syndrome coronavirus
SARS-CoV-2	Severe acute respiratory syndrome coronavirus 2
VOC	Variant of concern
VOI	Variant of interest
WHO	World Health Organization

List of Publications

List of publications not part of this doctoral project

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1. Contribution to the Publications

In the following, I present my contribution to the individual publications that form the basis of this dissertation.

1.1 Contribution to Publication I

The research presented in "*The Prospective COVID-19 Post-Immunization Serological Cohort in Munich (KoCo-Impf): Risk Factors and Determinants of Immune Response in Healthcare Workers*" aims to identify

(i) risk factors for SARS-CoV-2 infection among Health Care Workers (HCWs),
(ii) factors that influence the immune response following SARS-CoV-2 infection or vaccination, and
(iii) differences between the HCW cohort and the general population.

The analysis presented in this paper is based on the baseline visit of the KoCo-Impf study cohort: The Prospective COVID-19 Post-Immunization Serological Cohort in Munich.

The work presented in this paper resulted in a shared first authorship with Yeganeh Khazaei. This is based on a collaboration between the data analysis and study team, due to the unique recruitment process for the KoCo-Impf study. Recruitment occurred at various institutions over seven months during the COVID-19 pandemic. As a result, the progress of the pandemic, including the different infection waves in the Munich population, local outbreaks, and the duration of the development of the immune response, had to be considered in the analysis. In detail, one key element of the research is the detection of past silent and symptomatic SARS-CoV-2 infections based on the presence of anti-Nucleocapsid antibodies in blood samples. While this method identifies past infections, it cannot assess the date of infection. Given that the sample collection took place over seven months, it was necessary to correct for the different times at which the risk for infection was different. This aspect demanded specific statistical tools, that were acquired with the collaboration.

While the methodology and resulting recruitment process of the KoCo-Impf study were pre-defined, in my role as project manager of the KoCo-Impf study I took the lead in managing study procedures. My responsibility comprised the entire project administration, by leading and actively participating in coordinating the overall KoCo-Impf study procedures. This included adjusting study materials, such as questionnaires and other patient-facing material to the dynamic situation and communicating with study participants. Based on that, I led and participated in cohort recruitment procedures and data collection, including questionnaires and blood sampling during the baseline visit. Subsequently, I led the digitization of paper-based questionnaire data, ensuring its availability and quality for analysis. Last but not least I administered the KoCo-Impf study database, managing various data sources and ensuring the maintenance of the study cohort.

In my role as project manager, I coordinated and instructed the KoCo-Impf study team, which consisted of a large number of students supporting all the above-mentioned study procedures. This structured and organized administration and monitoring of collaborations within the institute, coupled with active participation and administration of study activities, facilitated robust data collection and quality control in a unique situation and time-sensitive implementation.

After project administration, I provided substantial support in data analysis, in particular contributing to data curation and variable definition for inclusion in the analysis. I also played an active

role in identifying the research questions and reviewing and discussing the conducted models. This included reviewing the R analysis code, deciding the best model to apply and identifying potential mistakes to ensure robustness in the process. Moreover, I offered critical insights for discussing and interpreting results and provided significant contributions and consultation to incorporate study-specific recruitment procedures, cohort characteristics, and variable peculiarities. This involved leading communication between study team members and co-authors. This contribution was crucial to ensure that the data could be analyzed in accordance with the data collection and that the results could be interpreted in an epidemiologically correct approach.

Upon completion of data analysis and interpretation of results, I played a major role in drafting the manuscript, including the main text, figures, references, and subsequent revisions. Being actively involved in interpreting the results based on epidemiological principles and conducting preliminary research, I ensured a comprehensive and accurate representation of our findings. After finalizing the manuscript, I submitted the manuscript and coordinated the review process and re-submission until final publication, marking the culmination of the dissemination of study findings.

1.2 Contribution to Publication II

The research presented in "*Understanding the Omicron Variant Impact in Healthcare Workers: Insights from the Prospective COVID-19 Post-Immunization Serological Cohort in Munich (KoCo-Impf) on Risk Factors for Breakthrough and Reinfections*" further explores the KoCo-Impf cohort, specifically focusing on its follow-up visit. The work aims to describe, determine, and conceptualize the SARS-CoV-2 infection risk for HCWs during the Omicron wave.

Building upon my previous contribution to the baseline analysis (i.e. Publication I), I coordinated the KoCo-Impf study procedures. It was important to monitor and reflect the dynamic nature of the pandemic and its impact on study administration. Consequently, before the start of the data collection, I led the adjustment of the study protocol and updated and adjusted the questionnaire used for data collection. My responsibilities included organizing and managing sampling rounds. I therefore conducted follow-up administration and data collection of questionnaires and blood samples during follow-up visits. This included coordinating remote sampling by organizing the shipment of self-sampling kits and questionnaires to participants and the return to the study center. Additionally, I supervised and actively participated in sample and data collection on-site. Subsequently, I oversaw and instructed the digitization of paper-based questionnaire data, ensuring its accessibility and quality for analysis. My responsibilities included the coordination and instruction of the KoCo-Impf study team that supported all the above-mentioned study procedures.

Building on this groundwork, I performed data curation and provided significant input and consultation for the subsequent data analysis. This included identifying research questions, shaping the analysis methodology, and reviewing, discussing, and adapting model choices based on interpretation. I also offered critical insights for the discussion and interpretation of results. Additionally, I facilitated communication between the data analysis and administration teams, ensuring a cohesive workflow. Upon the completion of data analysis, I contributed substantially to the thorough revision of the manuscript draft. This involved conducting literature research and providing relevant literature and interpretation. I was actively involved in conducting preliminary research to ensure a comprehensive and accurate representation of our findings. Subsequently, I played a pivotal role in the submission, revision, and resubmission of the manuscript.

Consequently, my contribution to sample and data collection, data analysis, and manuscript preparation during time-critical and accurate study-execution was crucial to the conduct of the study.

2. Introduction

2.1 Background

In the following, the background of the research questions and the resulting publications are described.

2.1.1 The COVID-19 Pandemic

The COVID-19 pandemic is one of the most recent and serious threats to global health. While disease outbreaks have occurred throughout history, COVID-19 stands out as a major global health emergency.

On 31 December 2019, Wuhan, China, reported cases of 'viral pneumonia', which was soon linked to a novel coronavirus (1). By 11 January, the virus had resulted in its first death (1). The virus quickly spread worldwide, and reached Europe with the first confirmed case in France (24 January 2020) (1). In the early phase of the pandemic, Europe was heavily affected, reporting more cases and deaths than the rest of the world combined, apart from the People's Republic of China (1, 2). In Germany, the first confirmed case of COVID-19 was identified at our Institute of Infectious Diseases and Tropical Medicine in Munich on 27 January 2020 (3).

Within a month, on 30 January 2020, the World Health Organization (WHO) declared a global Public Health Emergency of International Concern (4). This was only the sixth time an outbreak was declared as such, following H1N1 (2009), polio (2014), Ebola in West Africa (2014), Zika (2016), and Ebola in the Democratic Republic of Congo (2019) (5).
However, the outbreak quickly surpassed previous public health emergencies in spread and severity. After 118,000 cases in 114 countries, and 4,291 deaths worldwide, the global outbreak was characterized as a pandemic on 11 March 2020 (6, 7). The virus itself was identified as severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2), while the infectious disease was named coronavirus disease (COVID-19) (8, 9).

SARS-CoV-2 has likely a zoonotic origin similar to other coronaviruses and was spilled over to humans in a setting of high-density human population in close contact with living animals (10). In humans, the virus is transmitted via droplets or direct contact (5). The transmission can occur from asymptomatic individuals and before the onset of symptoms (10). COVID-19 is characterized by clinical symptoms of fever, fatigue, cough, and myalgia and can vary in severity (5, 11).

In the absence of vaccines and COVID-19 medications, non-pharmacological measures such as facemasks, physical distancing, quarantine, and lockdowns were adopted. These measures had a profound social and economic impact, leading to significant economic downturns, rising unemployment rates, school closures, remote work, and increased social isolation. Public health strategies such as widespread testing, contact tracing and surveillance were implemented for transmission mitigation and to prevent the healthcare system from being overwhelmed. Hospitals rapidly increased intensive care unit capacities, and efforts were made to stockpile essential medical supplies. Despite these efforts, resources were limited, and the healthcare systems faced overwhelming demands and high burnout rates. In addition, research was established to develop prevention and treatment strategies. (12)

As this thesis describes determinants of the immune response against SARS-CoV-2, an overview of the immunological mechanism of the SARS-CoV-2 virus is described in the following.

2.1.2 The Immunological Mechanisms of SARS-CoV-2

SARS-CoV-2 belongs to the species *Severe acute respiratory syndrome-related coronavirus* in the family *Coronaviridae* (8, 11). Coronaviruses (CoVs) have been identified since the 1960s (13). To date, seven types of coronaviruses are known to cause diseases in humans (13):

- HCoV-229E
- HCoV-NL63
- HCoV-OC43
- HCoV-HKU1
- SARS-CoV (Severe acute respiratory syndrome coronavirus)
- MERS-CoV (Middle east respiratory syndrome coronavirus)
- SARS-CoV-2 (Severe acute respiratory syndrome coronavirus type 2)

While some coronaviruses (HCoV-229E, HCoV-NL63, HCoV-OC43, and HCoV-HKU1) cause only mild symptoms, three of the seven (SARS-CoV, MERS-CoV, and SARS-CoV-2) are highly pathogenic, capable of causing severe respiratory infection and death. (11, 13)

CoVs are enveloped, single-stranded ribonucleic acid (RNA) viruses (see Figure 1). The viral genome is located within the nucleocapsid (N) protein at the core. This core is protected by a lipid bilayer, embedded with structural proteins: spike (S), membrane (M), and envelope (E). (11, 13)

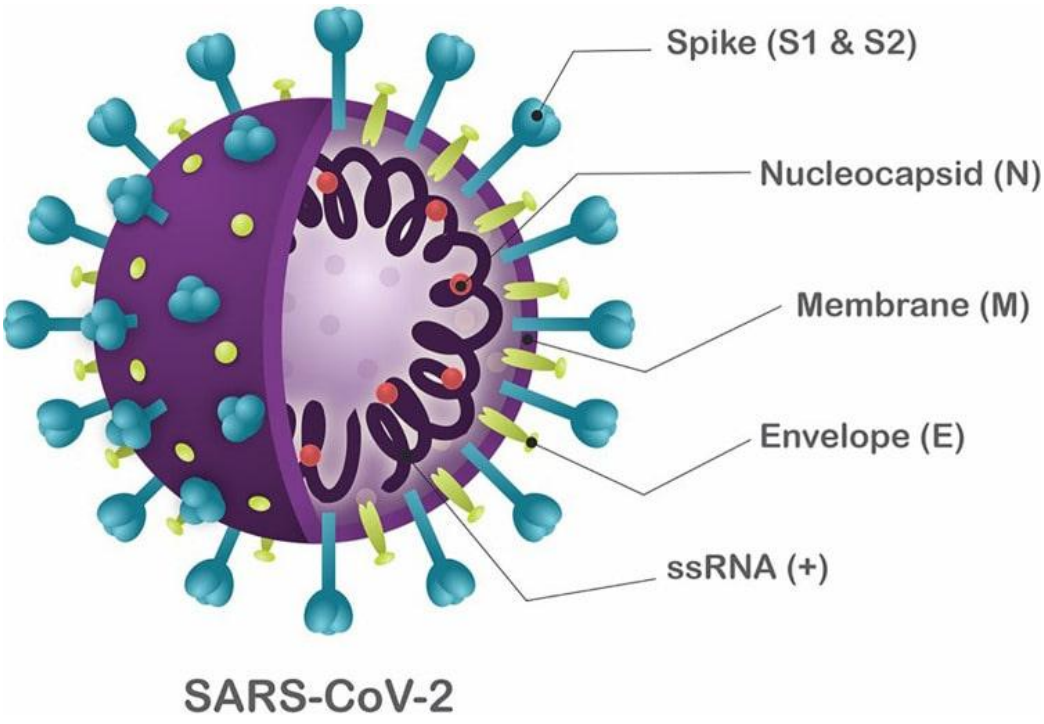

Figure 1: Schematic structure of SARS-CoV-2. The viral structure is primarily formed by the structural proteins such as spike (S), membrane (M), envelope (E), and nucleocapsid (N) proteins. Reprinted from (13)

While the membrane and envelope proteins have regulatory functions, the N and S proteins are of greater importance for SARS-CoV-2 transmission and its immune response (11, 14).

The S protein, unique to SARS-CoV-2, is essential for viral entry into the host cell and is cleaved into two functional domains, S1 and S2. S1 contains the receptor binding domain (RBD) and is critical for cell entry, while S2 provides structural support (11).
The SARS-CoV-2-infection process begins with the binding of the spike protein RBD to the human host cell. After attachment, the virus enters the cell and releases its RNA genome and N. Upon entering the host cell, SARS-CoV-2 triggers an immune response. (11, 14, 15)

The research underlying this dissertation is based on the analysis of determinants of the immune response against SARS-CoV-2 in humans. For this reason, and to understand the immunological basis of the research, the immune response induced by SARS-CoV-2 is described in the following.

2.1.3 The Immune Response against SARS-CoV-2

As described above, SARS-CoV-2 activates the immune response, leading to the generation of antibodies. These antibodies typically develop about two weeks post-infection, reaching a plateau thereafter. In the event of re-exposure, these antibodies recognize the SARS-CoV-2 S protein and block the virus from entering cells. However, immunity after natural infection appears to become weak within two to three months post-infection and can be influenced by several factors (see Figure 2). (15, 16)

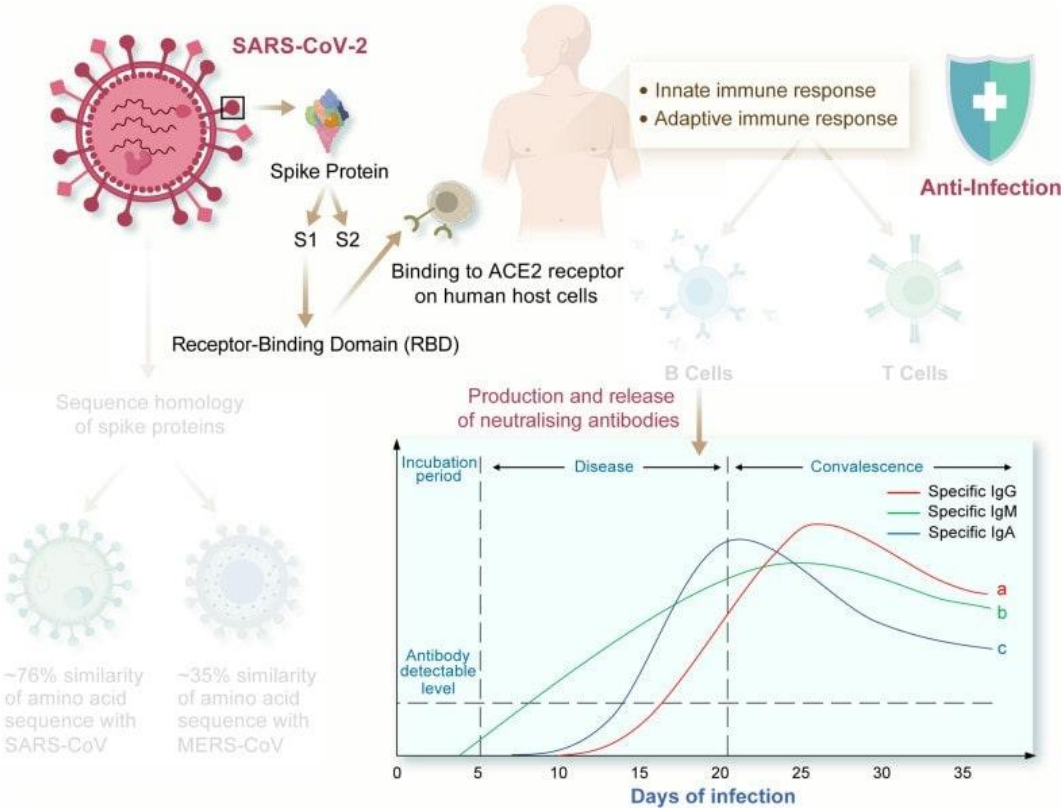

Figure 2: Illustration of SARS-CoV-2 infection process and development of humoral immune response. The immune response triggers the onset and persistence of antibodies. Reprinted and adapted from (16).

The antibody response against SARS-CoV-2 can be triggered not only by natural infection but also by vaccination (16). Vaccines against COVID-19 have been rapidly developed and deployed: On 31 December 2020, the WHO issued the first emergency use validation for a COVID-19 vaccine (1, 17). During the pandemic, more than 50 vaccines were approved worldwide, 12 of which were listed as Emergency Use Listing by WHO (18, 19). Vaccines have proven to be the central element in reducing the morbidity and mortality of the COVID-19 pandemic (12). As of 2024, more than 13.64 billion COVID-19 vaccine doses have been administered (20), the majority being mRNA vaccines (21). These vaccines focus on the stimulation of anti-S antibodies (13, 22).

Consequently, although other SARS-CoV-2 specific antibodies are prevalent, anti-S antibodies are the key element of the immune response to both naturally infected and vaccinated individuals.

Additionally, antibodies targeting the N protein are also relevant. Like the production of anti-S antibodies, anti-N antibodies develop within a few days after natural infection (23). While anti-S antibodies are developed after natural infection and/or vaccination, anti-N antibodies are only developed after natural infection and/or vaccination with nucleocapsid-containing vaccines not commonly used in the Western world (13, 22, 23). Consequently, the identification of anti-N antibodies enables a distinguishment between infection-related immune response and vaccine-induced immune response.

For these reasons, testing and monitoring antibodies, in particular anti-S and anti-N antibodies, allows for understanding and following the short-, middle- and long-term immune response to SARS-CoV-2. Antibody testing helps detect previous infections or immunizations, supports surveillance, and can inform treatment and prevention strategies (15). Thus, antibody testing is a foundational strategy for public health and pandemic response (15).

2.1.4 Monitoring in Determinants of the Immune Response against SARS-CoV-2 in Munich

Europe and Germany were heavily affected by the COVID-19 pandemic. Six infection waves have been observed in Germany until now (see Figure 3) (24).

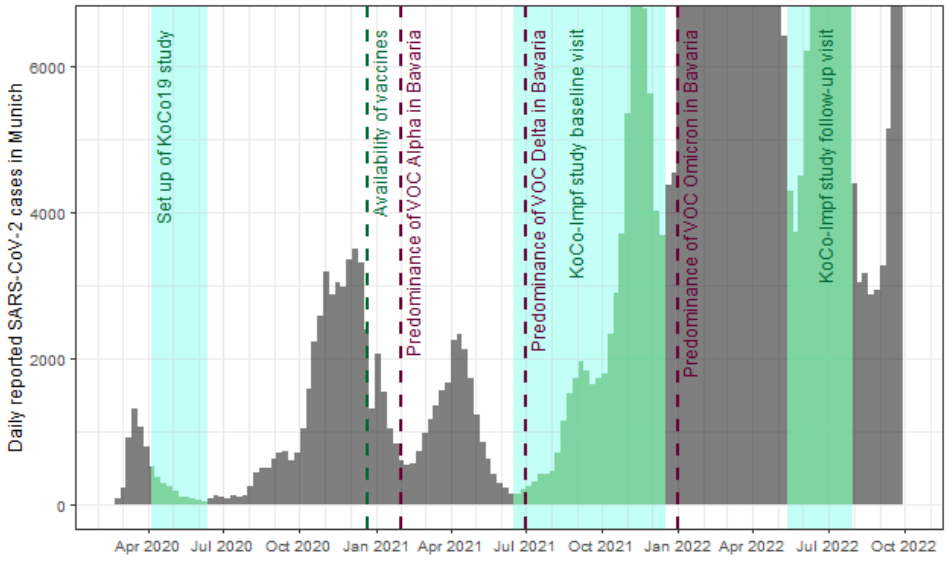

Figure 3: Development of COVID-19 infection numbers in Munich with regard to predominant Variants of Concern (VOC) and availability of vaccines. In black, daily infections as reported by the Robert Koch Institute (RKI). In blue, sampling timepoints of the KoCo19 and KoCo-Impf studies. The green line represents the introduction of COVID-19 vaccines. The red lines represent the starting point of the dominance of the respective VOCs in Munich. Adapted and reprinted from (25).

First wave: March to May 2020

After the initial outbreak, from March to May 2020 Germany implemented its first nationwide lockdown to control the rising number of infections. The measures included the closure of non-essential shops and schools, a ban on public gatherings of more than two people, and strict social distancing rules. Upon re-opening, strict hygiene protocols were implemented, and efforts were made to stockpile essential medical supplies to support the healthcare system. (26-30)

Shortly after the identification of the first COVID-19 case in Germany, a prospective, population-based cohort was established in Munich to monitor the immune response in the Munich population (31). The *Prospective Covid-19 Cohort Munich (KoCo19)*, representative of the Munich population, established a method to determine the seroprevalence of anti-N and anti-S antibodies in a large population (31-34). Seroprevalence is defined as „the frequency of specific antibodies in blood serum indicating an existing or past infectious disease“ (35, 36). Instead of blood serum, the methodology of the KoCo19 cohort is based on Dried Blood Spots (DBS), capillary blood from the fingertip collected and dried on filter paper cards (33, 34). This approach, developed as a public health tool for resource-limited settings, allows reliable, cost-effective, and accurate collection of blood samples in large-scale serosurveys and a precise analysis in a short time (33, 34).

Simultaneously, new virus variants emerged following the appearance of the SARS-CoV-2 wild type in December 2019 (37). SARS-CoV-2 Variants of Interest (VOI) are variants with genetic changes affecting transmissibility, virulence, antibody evasion, susceptibility to therapeutics, or detectability and demonstrate an emerging risk to global public health (37, 38).
SARS-CoV-2 Variants of Concern (VOC) additionally demonstrate increased detrimental change in COVID-19 epidemiology, clinical disease severity, or decreased effectiveness of public health interventions (37, 38).

While several VOIs emerged from August 2020 on, to date five VOCs have been identified by the WHO, starting with VOC Beta (B.1.351), which emerged in South Africa in May 2020, followed by VOC Alpha (B.1.1.7), which emerged in Great Britain in September 2020 (39, 40). The nomenclature was introduced retrospectively and does not reflect the chronological order.

Second wave: October 2020 to February 2021

After a „summer plateau“, infection rates increased again (24), prompting a „light lockdown“ in November 2020 (41), followed by a second „hard lockdown“ from December 2020 to spring 2021, in response to escalating cases and strain on the healthcare system (42-47). New VOCs Delta (B.1.617.2) emerged in India in October 2020 and Gamma (P.1 alias B.1.1.28.1) in Brazil in November 2020 (39, 40).

As countermeasures, the first vaccines against COVID-19 became available in Germany on 21 December 2020 (Comirnaty) and 6 January 2021 (Spikevax). Both vaccines are based on mRNA, resulting in the production of anti-S antibodies. The first vector-vaccine became available on 29 January 2021 (ChAdOx1). The immunization regime with these vaccines comprises two doses. Participants with undergone infection were recommended no vaccination (48, 49), later vaccination only 6 months after infection (50) with one dose (49, 51), or in case of vaccination and subsequent infection, a second dose only 6 months after infection (49, 50). Due to limited resources, vaccine distribution was prioritized based on age, exposure, and vulnerability. Thus, elderly persons and HCWs were vaccinated first. (50)

Due to the dynamic course of the pandemic and the introduction of vaccines, the method and experience from the KoCo19 cohort were used to establish the *Prospective COVID-19 Post-Immunization Serological Cohort in Munich (KoCo-Impf)* (52). The KoCo-Impf cohort recruited individuals from the Munich municipality and surrounding areas, mostly HCWs, aged 18 years or older, who had received at least one COVID-19 vaccination dose (52).
Subsequently, sampling rounds of the KoCo19 and KoCo-Impf cohorts were conducted in parallel. Both cohorts joined the ORCHESTRA project (Connecting European Cohorts to Increase Common and Effective Response to SARS-CoV-2 Pandemic) on 1 December 2020, enabling international collaboration involving 26 partners from 15 European countries (52, 53).

Third wave: March 2021 to May 2021

Figure 4 shows the distribution of virus variants in Germany. The third wave was characterized by the predominance of the VOC Alpha in Germany (24, 54). The vector-vaccine Jcovden became available on 11 March 2021, providing complete immunization after only one dose (55).

Subsequently, the recruitment process of the KoCo-Impf cohort began on 16 June 2021, and follow-up sampling of the KoCo19 cohort took place (25, 52).

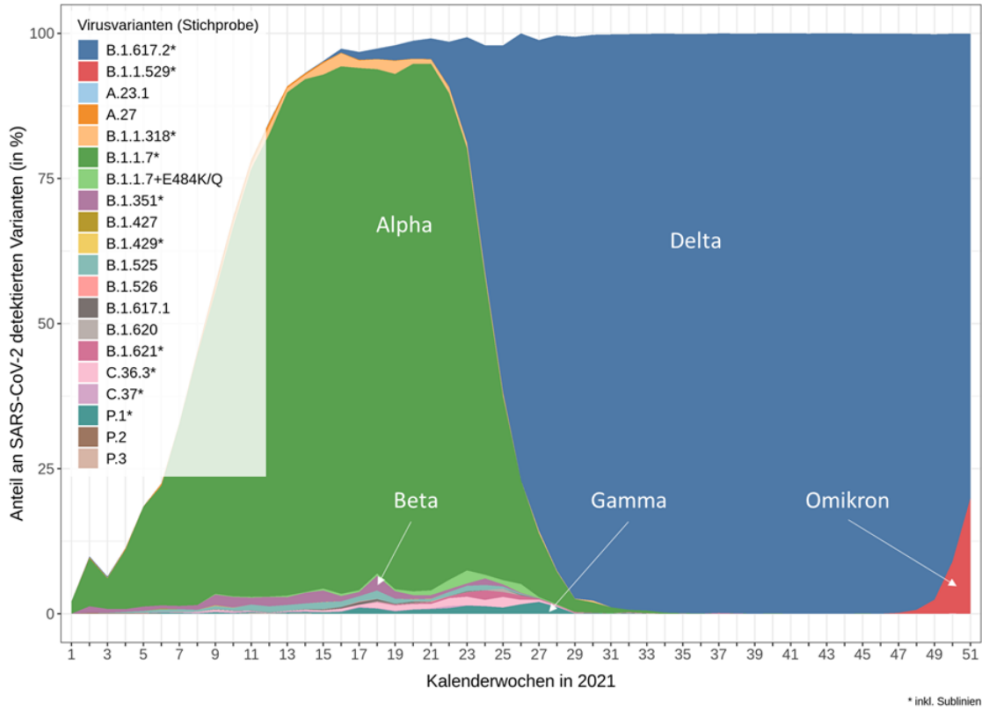

Figure 4. Percentage of VOC and VOI in relation to the genome sequences based on SARS-CoV-2-positive PCR samples. Omicron accounted only for a neglectable percentage of SARS-CoV-2 positive cases at the baseline sampling. Reprinted from (54).

Fourth wave: August 2021 to December 2021

The fourth wave was characterized by the predominance of the VOC Delta in Germany (24, 54). In response to the ongoing high number of cases, further follow-up samplings of the KoCo19 cohort took place (25). The recruitment of the KoCo-Impf cohort continued, with baseline data being collected between 16 June 2021 and 16 December 2021 (52).

The protein-based vaccine Nuvaxovid became available on 20 December 2021 (56). With increasing vaccination coverage (> 65% in November 2021 (57)), the focus of the vaccination campaign shifted from vulnerable groups to younger populations and addressing vaccine hesitancy

(58). In addition, booster vaccinations six months after completion of initial immunization were introduced first for vulnerable populations (59, 60), and later also for the general population (58), besides for individuals who had been vaccinated and infected (58-60).

Fifth wave: December 2021 to May 2022

The new VOC Omicron (B.1.1.529) emerged in Botswana and spread in South Africa in November 2021 (39, 40). The first confirmed case of the Omicron variant in Germany was registered at the end of November 2021 (61). However, the new VOC accounted for only 0.7% of cases in Germany (62) and 6.5% of cases in Bavaria (63) up to the two weeks required to develop antibodies detectable at the end of KoCo-Impf baseline sampling period in December 2021.

The Omicron variant is characterized by mutations in the S protein, including the RBD region. The variant is less pathogenic, resulting in more silent, asymptomatic infections (64). However, Omicron variants show increased transmissibility and rapid spread compared to earlier variants, resulting in a higher susceptibility to reinfections and breakthrough infections (BTI). BTI refers to infections in fully vaccinated people, in case of SARS-CoV-2 usually to infections after two doses of vaccination (65). Studies have shown that while two vaccine doses offer limited protection against infection, three doses provide better protection (64, 66, 67).

In response, in December 2021, individuals who had been infected and subsequently vaccinated were also recommended a booster dose six months after vaccination (68). Individuals who had been vaccinated at least once and subsequently infected, were also recommended a booster dose six months after infection (68). However, in January 2022, recommendations for a booster vaccination shortened the interval from six to three months (69). In addition, a second booster vaccination was recommended three months after the first booster vaccination for elderly and high-risk individuals, and six months after the first booster vaccination for healthy HCWs (70). For individuals who completed three vaccinations and one subsequent infection, no second booster dose was recommended (70).

Nevertheless, the increased transmissibility of the VOC Omicron resulted in a surge in infections and a burden on the healthcare system (71, 72).

On 13 May 2022, follow-up samplings of the KoCo19 and KoCo-Impf cohorts were initiated to monitor the dynamic changes in the immune response in the Munich general population and HCWs, demonstrating the impact of Omicron (73).

Sixth and subsequent waves: From June 2022 on

Follow-up data collection continued until 31 July 2022 (73). Diverse Omicron sublineages became dominant in Germany. By mid-2023, COVID-19 have transitioned from the pandemic to the endemic phase in Germany. Due to widespread immunity from vaccinations and previous infections, COVID-19 waves had become less distinct. (63)

2.2 Rationale and Objectives

2.2.1 Rationale

Since the emergence of the SARS-CoV-2 virus in December 2019, the resulting COVID-19 pandemic has been one of the most challenging threats to global public health, with a relevant social and economic impact (12). To date, more than 777 million cases and more than 7 million deaths have been officially reported (20), with the number of unreported cases is estimated to be much higher. Besides the death toll, many victims suffered from long-term health effects such as „long Covid“ or other permanent disabilities (74).

To assess and control such outbreaks and pandemics, it is essential to collect epidemiological data such as incidence and prevalence as well as hospitalization and mortality rates (15). Understanding the pathogen and the resulting immune response is crucial for pandemic response and public health strategies (15). More precisely, antibody studies enable the detection of undergone silent and symptomatic infections, supporting surveillance and prevention strategies (15).

To prevent COVID-19 disease and control the outbreak, vaccines have been developed and made available to the public since December 2020 (19). Antibody studies therefore also have the potential to monitor the effect of immunization and to provide guidance for vaccination strategies (15). Vaccination reduces the risk for a heavy disease course and may also reduce the risk of infection in the short term but may need to be repeated to maintain long-term protection as the immune response wanes over time (75, 76). Consequently, early vaccination could potentially increase the risk of later infection compared to early vaccinated or infected, making it crucial to monitor vaccinated individuals and their fluctuating immune response (75-77).

Vaccination strategies against COVID-19 prioritize high-risk individuals and aim to vaccinate a majority of the population of 70% to develop herd immunity, i.e. to increase the likelihood of infected people being surrounded by non-vulnerable persons and thus breaking infection chains (78-80). The global allocation of COVID-19 vaccines was planned in two phases:
The first phase aimed to cover 3% of the national population and targeted exclusively HCWs as the most exposed links in the infection chain (78, 79). Subsequent vaccination deliveries aimed to cover an additional 17% of the national population, targeting individuals at higher ages and those with underlying health conditions (78, 79). Overall, the first phase aimed to target 100% of HCWs and 100% of older populations (60+) and other priority risk groups with primary series and booster doses (80). After covering high-risk groups, the second phase aimed to provide additional doses beyond the 20% benchmark, targeting the general population (78, 79). This way, HCWs, the elderly, and individuals at significantly higher risk of severe disease or death received COVID-19 vaccines first (50, 78, 79, 81).

HCWs are defined as „all people engaged in work actions whose primary intent is to improve health. This includes health service providers, such as doctors, nurses, midwives, public health professionals, technicians (laboratory, health, medical and non-medical), personal care workers, community health workers, healers and practitioners of traditional medicine. It also includes health management and support workers, such as cleaners, drivers, hospital administrators, district health managers and social workers, and other occupational groups in health-related activities. This group includes those who work in acute care facilities and in long-term care, public health, community-based care, social care and home care and other occupations in the health and social work sectors (…)“ (78, 79).

HCWs were considered at a higher risk of getting infected by SARS-CoV-2 than the general population due to the nature of their work. In addition, infected HCWs risked transmitting the infection to their patients, including vulnerable individuals with underlying health conditions who are at high risk for severe COVID-19 disease and complications. Hence HCWs were prioritized in vaccination strategies. (78, 79)

Considering their role in the healthcare system, HCWs are of particular interest in managing the COVID-19 pandemic and require special surveillance. For these reasons, antibody studies were established to determine the immune response against SARS-CoV-2 and identify risk factors for SARS-CoV-2 infection before and after vaccination, comparing the general population (31, 82-84) with HCWs (85, 86).

As a result, the longitudinal cohort named KoCo-Impf (Prospective COVID-19 post-immunization Serological Cohort in Munich—Determination of immune response in vaccinated subjects) was established in May 2021. As described above, the cohort is mainly composed of HCWs in the municipality of Munich and aims to understand the short-, medium- and long-term immune response in vaccinated individuals. The work presented in this doctoral project is based on the research carried out as part of the KoCo-Impf study.

2.2.2 Study Aims and Objectives

The overall aim of this doctoral project is to assess determinants of the immune response against SARS-CoV-2 in vaccinated individuals. Based on the immunological mechanisms described in the above sections, the induction types of natural infection and vaccination, as well as the resulting antibody types, anti-N and anti-S were defined as determining components in the research conducted. Consequently, the specific objectives of this doctoral project were defined as follows:

Objective 1: To determine the seroprevalence in vaccinated individuals based on SARS-CoV-2 antibodies

1a: To determine the seroprevalence of anti-N-antibodies over time

1b: To determine the seroprevalence of anti-S-antibodies over time

Objective 2: To identify risk factors for SARS-CoV-2 infection

2a: … with respect to the different SARS-CoV-2 variants

2b: … with respect to the vaccination status

2c: … with respect to reinfection

Objective 3: To identify determinants of the quantitative SARS-CoV-2 anti-N serology after SARS-CoV-2 infection

Objective 4: To identify determinants of the quantitative SARS-CoV-2 anti-S serology after SARS-CoV-2 vaccination and/or infection

4a: To identify determinants of the quantitative SARS-CoV-2 anti-S serology

4b: To determine the development of the quantitative SARS-CoV-2 anti-S serology

These objectives were defined at the start of the project. In the next chapter, I will describe how the individual objectives were analyzed in the individual publications.

2.3 Analysis of Determinants of the Immune Response against SARS-CoV-2 in the KoCo-Impf-Cohort

The following describes which aspects of the doctoral project are covered by the publications.

2.3.1 Risk Factors and Determinants of Immune Response in Healthcare Workers

The KoCo-Impf study is based on the collection of Dried Blood Spots (DBS) on filter paper cards as described above. DBS samples were collected to determine the antibody status, together with questionnaire data for information on participants' characteristics including vaccination status. Overall, the KoCo-Impf cohort comprises 6,467 participants, enrolled in 16 subgroups according to occupational activities. The sampling took place between June and December 2021 as shown in Figure 3.

The results of the KoCo-Impf study "*Risk Factors and Determinants of Immune Response in Healthcare Workers"* (52) directly contribute to this doctoral project's objectives as described in the following:

Objective 1: Determination of seroprevalence in vaccinated individuals:
The research presents an estimation of undergone silent and symptomatic infections in HCWs in Munich up to December 2021 and comparison with the general population.

Objective 2: Identification of risk factors for SARS-CoV-2 infection:
As illustrated in Figure 4, the study identifies risk factors for SARS-CoV-2 infection, considering all SARS-CoV-2 variants prevalent to December 2021, which are mainly wildtype, alpha, and delta virus variants. These results improve our understanding of the susceptibility to infections among HCWs. The data on vaccination status collected at the baseline visit was used to identify risk factors for SARS-CoV-2 infections with respect to the vaccination status before infection, therefore enhancing our understanding of potential protective effects.

Objective 3: Identification of determinants of the quantitative SARS-CoV-2 anti-N serology after SARS-CoV-2 infection:
The analysis provides a better understanding of factors influencing the quantitative anti-N immune response, providing insights on susceptibility to infection.

Objective 4: Identification of determinants of the quantitative SARS-CoV-2 anti-S serology after SARS-CoV-2 vaccination and/or infection
The analysis provides a better understanding of factors influencing the quantitative anti-S immune response. The findings delved into effects of vaccination, infection, biological and behavioral factors on the immune response, enhancing our understanding of immunization.

2.3.2 Understanding the Omicron Variant Impact in Healthcare Workers: Insights on Risk Factors for Breakthrough and Reinfections

The end of the sampling period for the baseline survey also coincided with a turning point in the development of the pandemic. A novel variant emerged in Botswana and spread in South Africa, prompting the WHO to classify it as VOC on 26 November 2021, due to a dramatic increase in infections and risk of reinfection compared to other VOCs.

Given the rapid spread of the Omicron variant and the increasingly complex situation in Germany, a follow-up of the KoCo-Impf cohort was conducted between 13 May 2022 and 31 July 2022 as shown in Figure 3. The analysis presented in the KoCo-Impf study "*Understanding the Omicron Variant Impact in Healthcare Workers"* (73) described the follow-up data of the KoCo-Impf cohort. The results of this research directly contribute to this doctoral project's objectives as described in the following:

Objective 1: Determination of seroprevalence in vaccinated individuals:
The research provides an estimation of undergone silent and symptomatic infections in HCWs in Munich between the baseline and follow-up sampling. This approach illustrates the fraction of new, mostly silent SARS-CoV-2 infections, BTIs, and reinfections.

Objective 2: Identification of risk factors for SARS-CoV-2 infection:
The work identifies risk factors for SARS-CoV-2 infection across all variants, for Omicron only, and compares risk factors for Omicron to all variants. It explores reinfection and BTIs.

Objective 4: Identification of determinants of the quantitative SARS-CoV-2 anti-S serology after SARS-CoV-2 vaccination and/or infection:
The analysis determines the development of the quantitative SARS-CoV-2 anti-S serology. This approach demonstrates the vanishing effect of vaccination, as well as the fraction of reinfections and BTIs.

In conclusion, the results presented in publication II enhance our understanding of susceptibility to infections with the SARS-CoV-2 Omicron variants.

3. Summary

The COVID-19 pandemic has posed one of the most significant challenges to public health systems worldwide. To date, more than 777 million individuals have been infected with SARS-CoV-2, and more than 13.6 bn vaccine doses have been administered (20). More than 67% of the total population have been vaccinated with a series of two COVID-19 vaccine doses, as initially defined as complete primary vaccination (20). Based on this, COVID-19 vaccines have shown to be a central element in reducing the morbidity and mortality associated with the pandemic (12).

The work presented here investigates determinants of the immune response after SARS-CoV-2 infection and vaccination in a cohort comprising mostly HCWs and the general population. Capillary blood samples were collected between June 2021 and July 2022 to detect SARS-CoV-2 antibodies in two rounds, describing past asymptomatic and symptomatic infections as well as vaccination history. The approach combined a seropositivity definition based on SARS-CoV-2 antibodies with a large sample size and detailed vaccination data.

The analysis of the baseline data, collected between June 2021 and December 2021, examined factors that influence the immune response following SARS-CoV-2 infection or vaccination and explored risk factors for infection.

The results demonstrated that in this cohort, HCWs were found to have a higher risk of SARS-CoV-2 infection compared to the general population in the early phase of the pandemic. Participants reporting a past known contact with SARS-CoV-2-positives demonstrated a higher risk for infection compared to those having none or unwitting contact. The analysis also demonstrated the protective effects of vaccination, with elderly participants showing a weaker immune response compared to younger participants. Smokers compared to never smokers demonstrated a decreased risk of infection and lower immune responses after both vaccination and infection, due to potential immunosuppressive or behavioral effects. Detailed results are described in the publication (52).

In the analysis of the follow-up data, based on samples collected between May 2022 and July 2022, the research aimed to understand the impact of the Omicron variant and explored differences in risk factors for infection across virus variants as well as BTIs and determinants of reinfections.

The analysis has demonstrated the belonging institutional subgroup of HCWs as the most influential variable in all risk factor analyses. The protective effect of prior pre-Omicron infections against SARS-CoV-2 infections during the Omicron wave diminished, but the risk factor analysis for reinfection remained inconclusive besides demonstrating a negative association with age. This suggests that reinfection could potentially affect any individual or that the underlying variable indicating an increased or decreased risk for reinfection was not investigated. However, the overall analysis revealed that younger ages were associated with a higher risk for any type of infection: any infection in the study period, infections in the Omicron period, BTIs and reinfections. These results as well as the increased risk for infection in a larger household introduce rather a behavioral than biological component due to an increased exposure to the virus through more contacts at home, work or social life compared to individuals living in smaller households and older participants.

The results are described and discussed in detail in the publication (73).

In conclusion, the results described here demonstrate that in the early phase of the COVID-19-pandemic, HCWs faced an increased risk of infection compared to the general population in this cohort. However, localized outbreaks within specific institutions overshadowed the effects of broader waves seen in the general population. The results observed in this research indicate that the increased risk of infection was primarily due to occupational activities and the working environment. This risk diminished with the increasing availability of personal protective equipment and changing transmissibility of new virus variants. By the Omicron period, in our cohort, the infection risk for HCWs had become more comparable to the general population.

The effect of changing infection risk can also be seen in the role of vaccination. This research has demonstrated that before the availability of vaccines, HCWs were at significantly higher risk of infection. However, after primary vaccination as defined at the time, their risk of BTI was reduced. The analysis demonstrated that in this cohort, vaccination provided robust protection against infection during the early phase of the pandemic. Nevertheless, the effectiveness of vaccination diminished during the Omicron era, as evidenced by the high number of BTIs, and reinfections.

These insights underscore that the healthcare environment was a primary risk factor at the outset of the pandemic, but they also highlight the importance of considering the broader environmental risks HCWs face in their environments. A holistic understanding of infection transmission dynamics within individual institutions is essential, and protective measures should be tailored to the specific contexts of each institution, moving beyond generalized approaches. However, effective pandemic management must extend beyond healthcare facilities and address behavioral risk patterns. Future research should consistently integrate behavioral, institutional, and biological determinants of risk to build a comprehensive understanding of infection dynamics and improve protective strategies.

Although research results remain heterogeneous (85, 87-89), they are congruent with the results presented here in highlighting increased infection risks among HCWs, driven by transmission between colleagues and patient contact, underscoring their significant role in the pandemic's spread. Vaccination proved to be a critical preventive measure. In general, research revealed that HCWs, despite being a vulnerable group, are highly diverse, encompassing various demographics and behavioral patterns.

The findings of this thesis shed light on the long-term immune response to SARS-CoV-2 in HCWs, contributing to understanding determinants of immunization and infection determinants. On 5 May 2023, the WHO declared the end of COVID-19 as a global health emergency (90, 91). Although SARS-CoV-2 has become endemic, its continued prevalence and mutations highlight the importance of ongoing monitoring and research.

4. Zusammenfassung

Die COVID-19-Pandemie war eine der größten Herausforderungen für die Gesundheitssysteme weltweit. Bis heute haben sich mehr als 777 Millionen Menschen mit SARS-CoV-2 infiziert und über 13,6 Mrd. Impfstoffdosen wurden verabreicht (20). Über 67% der Gesamtbevölkerung hat mindestens zwei Impfdosen, und damit eine zu dem Zeitpunkt so definierte Grundimmunisierung, erhalten (20). COVID-19-Impfstoffe waren damit ein zentrales Element bei der Reduktion der Morbidität und Mortalität (12).

Die hier vorgestellte Arbeit untersucht Determinanten der Immunantwort nach SARS-CoV-2-Infektion bzw. -Impfung in einer Studienpopulation, die hauptsächlich aus medizinischem Personal (Health care workers, HCWs) und der Allgemeinbevölkerung besteht. Zwischen Juni 2021 und Juli 2022 wurden in zwei Runden Kapillarblutproben gesammelt, um SARS-CoV-2-Antikörper nachzuweisen, die Rückschlüsse auf asymptomatische und symptomatische Infektionen sowie auf Impfverhalten geben. Mit diesem Ansatz wurde die Definition von Seropositivität auf der Grundlage von SARS-CoV-2-Antikörpern mit einer großen Studienpopulation und detaillierten Angaben zu Impfungen kombiniert.

In der Analyse der ersten Erhebungsrunde, die zwischen Juni 2021 und Dezember 2021 durchgeführt wurde, wurden Faktoren, die die Immunantwort nach einer durchgemachten SARS-CoV-2-Infektion oder -Impfung beeinflussen, sowie Risikofaktoren für eine Infektion untersucht.

Die Ergebnisse zeigten, dass in dieser Studienpopulation das Risiko einer SARS-CoV-2-Infektion bei HCWs in der frühen Phase der Pandemie höher war als in der Allgemeinbevölkerung. Teilnehmende, die Kontakt mit SARS-CoV-2-positiven Personen angaben, zeigten ein höheres Infektionsrisiko im Vergleich zu denen, die keinen oder unwissentlichen Kontakt angaben. Die Analyse zeigte auch die protektiven Auswirkungen einer Impfung, wobei ältere Teilnehmende im Vergleich zu jüngeren Teilnehmenden eine schwächere Immunreaktion zeigten. Raucher wiesen verglichen zu Nichtrauchern ein geringeres Infektionsrisiko und sowohl nach Impfung als auch nach Infektion eine schwächere Immunreaktion auf, was auf mögliche immunsuppressive oder verhaltensbedingte Effekte zurückzuführen sein kann. Die detaillierten Ergebnisse sind in der Publikation (52) beschrieben.

Die Analyse der zweiten Datenerhebung zwischen Mai 2022 und Juli 2022 zielte auf ein besseres Verständnis der Auswirkungen der Omikron-Variante ab und untersuchte Unterschiede in den Risikofaktoren für Infektionen mit den verschiedenen Virusvarianten sowie Durchbruchsinfektionen und Determinanten von Reinfektionen.

Die Analyse hat gezeigt, dass die institutionelle Subgruppe, also die Eingruppierung der HCWs nach Gesundheitseinrichtungen, die einflussreichste Variable in allen Risikofaktoranalysen war. Die protektive Wirkung von früheren SARS-CoV-2-Infektionen gegen Infektionen während der Omikron-Periode nahm ab, die Risikofaktoranalyse für Reinfektionen lieferte abgesehen von einer negativen Assoziation mit dem Alter jedoch keine eindeutigen Ergebnisse. Das lässt darauf schließen, dass Reinfektionen möglicherweise jeden betreffen könnten, oder dass die zugrundeliegende Variable, die Hinweise auf ein erhöhtes oder reduziertes Risiko geben könnte, in dieser Analyse nicht erfasst wurde. Die Analyse ergab jedoch grundsätzlich, dass ein jüngeres Alter mit einem höheren Risiko für jede Art von Infektion assoziiert war: Infektionen im gesamten Untersuchungszeitraum, Infektionen während der Omikron-Periode, Durchbruchsinfektionen und Reinfektionen. Diese Ergebnisse sowie das erhöhte Infektionsrisiko in einem größeren Haushalt lassen eher auf eine zugrundeliegende Verhaltenskomponente als auf eine biologische Komponente schließen, z.B. durch eine erhöhte Exposition durch mehr Kontakte zu Hause, in der Arbeit oder

auch durch ein ausgeprägteres Sozialleben im privaten Umfeld im Vergleich zu Personen in kleineren Haushalten und älteren Personen. Die Ergebnisse werden ausführlich in der Publikation (73) beschrieben und diskutiert.

Zusammenfassend lässt sich aus den Ergebnissen der Analyse schließen, dass in dieser Kohorte HCWs in der Anfangsphase der Pandemie einem höheren Infektionsrisiko ausgesetzt waren als die Allgemeinbevölkerung. Lokale Ausbrüche in den individuellen Gesundheitseinrichtungen überschatteten jedoch die Auswirkungen der breiteren Infektionswellen in der Allgemeinbevölkerung. Die Ergebnisse dieser Studie deuten darauf hin, dass das erhöhte Infektionsrisiko in erster Linie auf berufliche Tätigkeiten und das Arbeitsumfeld zurückzuführen ist. Dieses Risiko nahm mit der zunehmenden Verfügbarkeit von Schutzausrüstung und der veränderten Übertragbarkeit neuer Virusvarianten ab. Bis zur Omikron-Periode hat sich das Infektionsrisiko für HCWs an das der Allgemeinbevölkerung angeglichen.

Die Auswirkung eines veränderten Infektionsrisikos zeigt sich auch in der Rolle der Impfung. Die Ergebnisse dieser Untersuchung lassen darauf schließen, dass vor der Verfügbarkeit von Impfstoffen das Infektionsrisiko für HCWs deutlich höher war. Nach einer zu dem Zeitpunkt definierten Grundimmunisierung verringerte sich jedoch ihr Risiko für eine Durchbruchsinfektion. Diese Ergebnisse demonstrieren, dass in der hier vorgestellten Kohorte eine Impfung in der Anfangsphase der Pandemie einen Schutz vor SARS-CoV-2 Infektionen bot. Diese Wirksamkeit der Impfung nahm jedoch während der Omikron-Welle ab, was sich in einer hohen Zahl an Durchbruchsinfektionen und Reinfektionen zeigt.

Diese Erkenntnisse demonstrieren, dass eine Tätigkeit im Gesundheitswesen zu Beginn der Pandemie ein wesentlicher Risikofaktor war. Sie betonen aber auch die Bedeutung weiterer Risikofaktoren, denen die HCWs in ihrem persönlichen Umfeld ausgesetzt sind. Aus diesem Grund ist ein ganzheitliches Verständnis der Infektionsübertragungsdynamik in den einzelnen Gesundheitseinrichtungen essenziell. Schutzmaßnahmen sollten daher über allgemeine Ansätze hinausgehen und auf die spezifischen Gegebenheiten der einzelnen Einrichtungen eingehen. Darüber hinaus muss ein wirksames Pandemiemanagement jedoch über die einzelnen Gesundheitseinrichtungen hinausgehen und auch verhaltensbedingte Risikomuster adressieren. Künftige Forschung sollten daher verhaltensbedingte, institutionelle und biologische Determinanten integrieren, um ein umfassendes Verständnis der Infektionsdynamik zu entwickeln und Schutzstrategien zu verbessern.

Obwohl die Forschungsergebnisse nach wie vor uneinheitlich sind (84-87), stimmen sie mit den hier vorgestellten Ergebnissen insoweit überein, dass sie auf ein erhöhtes Infektionsrisiko bei HCWs aufgrund von Übertragungen zwischen Personal und Patientenkontakten hinweisen und damit deren signifikante Rolle bei der Ausbreitung der Pandemie betonen. Impfungen haben sich jedoch als entscheidende Schutzmaßnahme erwiesen. Grundsätzlich lässt aus den bisherigen Forschungsergebnissen darauf schließen, dass HCWs, obwohl sie grundsätzlich eine besonders vulnerable Gruppe darstellen, sehr heterogen sind und verschiedene demografische Merkmale und Verhaltensmuster aufweisen.

Die Ergebnisse dieser Doktorarbeit geben Aufschluss über die langfristige Immunantwort gegen SARS-CoV-2 in HCWs und tragen somit zum Verständnis von Determinanten der Immunantwort und des Infektionsgeschehens bei. Am 5. Mai 2023 erklärte die WHO das Ende von COVID-19 als globalen Gesundheitsnotstand (90, 91). Obwohl SARS-CoV-2 inzwischen endemisch geworden ist, unterstreichen die anhaltende Prävalenz und Mutationen die Notwendigkeit eines kontinuierlichen Monitorings sowie weiterer Forschung.

5. Publication I

viruses

MDPI

Article

The Prospective COVID-19 Post-Immunization Serological Cohort in Munich (KoCo-Impf): Risk Factors and Determinants of Immune Response in Healthcare Workers

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Abstract: Antibody studies analyze immune responses to SARS-CoV-2 vaccination and infection, which is crucial for selecting vaccination strategies. In the KoCo-Impf study, conducted between 16 June and 16 December 2021, 6088 participants aged 18 and above from Munich were recruited to monitor antibodies, particularly in healthcare workers (HCWs) at higher risk of infection. Roche Elecsys® Anti-SARS-CoV-2 assays on dried blood spots were used to detect prior infections (anti-Nucleocapsid antibodies) and to indicate combinations of vaccinations/infections (anti-Spike antibodies). The anti-Spike seroprevalence was 94.7%, whereas, for anti-Nucleocapsid, it was only 6.9%. HCW status and contact with SARS-CoV-2-positive individuals were identified as infection risk factors, while vaccination and current smoking were associated with reduced risk. Older age

correlated with higher anti-Nucleocapsid antibody levels, while vaccination and current smoking decreased the response. Vaccination alone or combined with infection led to higher anti-Spike antibody levels. Increasing time since the second vaccination, advancing age, and current smoking reduced the anti-Spike response. The cumulative number of cases in Munich affected the anti-Spike response over time but had no impact on anti-Nucleocapsid antibody development/seropositivity. Due to the significantly higher infection risk faced by HCWs and the limited number of significant risk factors, it is suggested that all HCWs require protection regardless of individual traits.

Keywords: COVID-19; SARS-CoV-2; health care workers; vaccination; immunologic response; antibodies; seroprevalence; breakthrough infections; ORCHESTRA

1. Introduction

The first report of the severe acute respiratory syndrome Coronavirus 2 (SARS-CoV-2) causing COVID-19 was on 31 December 2019 in the city of Wuhan (Hubei province, China) [1]. The World Health Organization (WHO) declared COVID-19 as a pandemic on 11 March 2020, after more than 118,000 cases in 114 countries and 4291 deaths occurred [2]. Since then, there have been outbreaks worldwide, with approximately 767 million confirmed cases and more than 6.9 million deaths as of June 2023 [3]. The first COVID-19 cases in Germany were observed in the municipality of Munich in late January 2020 [4]. Several vaccines were promptly developed and have been available in Germany since 27 December 2020 [5]. The first individuals to receive vaccinations were healthcare workers (HCW: people engaged in work actions whose primary intent is to improve health [6]) (HCWs), the elderly, and those who were at a high risk of severe illness to prevent the healthcare system from collapsing from overwhelming case numbers or lack of personnel [6–9]. HCWs are of particular interest and require careful investigation regarding SARS-CoV-2 infections. As vaccine protection diminishes over time, receiving an early vaccination reduces the risk of early infection but may increase the risk of later infection. This has been noted in several studies [9–11].

Many cohort studies have been set up since the beginning of the pandemic to analyze risk factors for infection before and after vaccination in both the general population [12–16] and HCWs [17,18].

Considering the role of antibody levels in protection against infection, most studies also analyze antibody titers over time. Anti-nucleocapsid (anti-N) antibodies develop only after natural infection (or vaccination with nucleocapsid-containing vaccines not commonly used in the Western world), while anti-spike (anti-S) antibodies develop after natural infection or/and vaccination [19].

Collatuzzo et al. [17] analyzed the predictors for a longer duration of the anti-S immune response at 9 months after the first COVID-19 vaccination in a multicentric European cohort of HCWs. A part of these data was fed into their analysis following the European-wide Consortium ORCHESTRA (Connecting European Cohorts to Increase Common and Effective Response to SARS-CoV-2 Pandemic). Female gender, young age, a previous infection, two vaccine doses, and mRNA and heterologous vaccination were found to determine higher anti-S antibody levels.

Moncunill et al. [20] analyzed determinants of antibody responses to COVID-19 mRNA vaccines in a cohort of exposed and naïve HCWs. Comparing previously SARS-CoV-2 infected versus uninfected individuals, the first ones were found to have higher anti-S IgA, IgG, and IgM levels, independently of the brand of the vaccine. At the same time, non-infected individuals developed significantly higher antibodies, depending on the brand of the vaccine. Interestingly, despite the clear impact of SARS-CoV-2 exposure on vaccine response, time since infection did not have a major effect on antibody response. Moreover, age and sex were not significantly associated with anti-S IgG levels in multivariable models.

Notarte et al. [21,22] analyzed determinants of antibody responses after COVID-19 mRNA vaccines in different populations. Regardless of the vaccine brand used, older age, male sex, seronegative status prior to vaccination, and presence of major comorbidities were associated with lower antibody titers (total antibodies, IgG, and/or IgA), supporting the findings of Yang [23].

Other factors leading to lower anti-S antibody titers were smoking [20,24] and homologous vaccination schemes [25–27].

In April 2020, the prospective Munich COVID-19 cohort (KoCo19) began to better evaluate the true case numbers [12,28,29]. Latest results show that vaccination prevents infection: anti-N seroprevalence was greater in the non-vaccinated population compared to the vaccinated one. At the same time, anti-N seroconversion rates (incidence) among vaccinated subjects did not show any statistical difference compared to the non-vaccinated group. Breakthrough infections (BTIs) may thus contribute relevantly to community spread, also considering the fact that the vaccinated population is much larger than the non-vaccinated population. The sub-cohort with jobs having a high contact risk with COVID-19 cases (e.g., HCWs) was found to have an increased risk for infection [30].

In May 2021, a new longitudinal cohort named KoCo-Impf (Prospective COVID-19 post-immunization Serological Cohort in Munich—Determination of immune response in vaccinated subjects) was established at the Division of Infectious Diseases and Tropical Medicine, comprising mostly HCWs with high contact risk with the SARS-CoV-2 virus. The analysis presented here aimed to identify risk factors for infection among HCWs, factors that influence the immune response following infection or vaccination, and differences between HCWs and the general population. The analysis utilized multivariable logistic regression analysis to identify risk factors for infection based on qualitative anti-N antibody results. Additionally, multivariable generalized linear models (GLM) were employed to determine the factors that raise antibody titers following infection and/or vaccination, using quantitative anti-N and anti-S antibody values.

The KoCo-Impf study was recruited concurrently with the third and fourth follow-ups of KoCo19 in Munich. This allowed for a comparison of the general population of Munich (KoCo19) with their HCWs. Although the crude rates for anti-N seroprevalence were similar, a direct comparison was challenging. However, it was confirmed in both the KoCo19 and the KoCo-Impf that HCWs had a higher risk of infection. Sex, age, household size, and intake of immune-suppressing drugs were not found to be significant risk factors for infection in either cohort, but being a current smoker was.

2. Materials and Methods

2.1. The KoCo-Impf Cohort: Cohort Design, Inclusion Criteria, and Setting

The objective of KoCo-Impf is to investigate the short-, medium- and long-term immune response to SARS-CoV-2 vaccination. This study is funded by the European Union's Horizon 2020 research and innovation program, as part of ORCHESTRA (Connecting European Cohorts to increase common and Effective SARS-CoV-2 Response), and also by the Division of Infectious Diseases and Tropical Medicine's own resources [31].

Between 16 June and 16 December 2021, a total of 6467 participants aged 18 years or older, who had received at least one COVID-19 vaccination, were recruited for this study from the Munich municipality and surrounding areas. The recruitment campaign was carried out through three different paths (Figure 1, top):

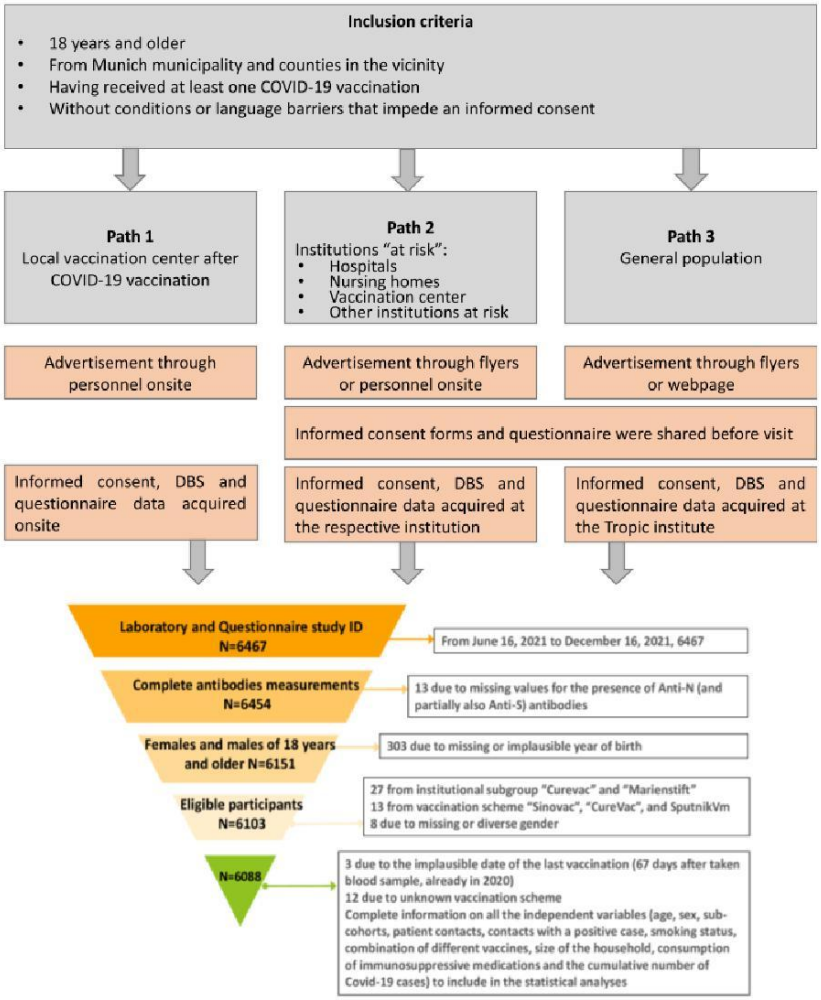

Figure 1. Recruitment paths and criteria for inclusion into the analysis. Gray boxes: inclusion criteria and places of recruitment. Orange boxes: information on advertisement modalities for recruiting participants; modalities of the acquisition of informed consent, questionnaire data, and capillary blood samples (acquired in person by study personnel). A triangle diagram describing the exclusion criteria and the final information of the analyzed participants.

Path 1: At the local vaccination center Riem, where individuals were approached with this study's information after their vaccination,

Path 2: At hospitals and nursing homes in the Munich area, targeting particularly exposed or vulnerable individuals (HCWs), and

Path 3: Via brochures and on the website of the Division of Infectious Diseases and Tropical Medicine for the general population.

Participants with language barriers (insufficient knowledge of the German language) or inability to provide informed consent were excluded.

Recruitment strategy, acquisition of informed consent, capillary blood samples, and questionnaire data occurred in different ways depending on the recruitment pathway:

Path 1: Directly after vaccination,

Path 2: By study teams making appointments on specific days to visit the sites, catching participants in the building during their working time, and

Path 3: Posting advertisements on the webpage of the Division of Infectious Diseases and Tropical Medicine, Klinikum der Universität München; participants could make an appointment for a personal visit via a hotline.

After data cleaning, 6088 participants were included in the analysis (Figure 1, bottom). Capillary blood samples were taken from participants to determine their antibody status, and questionnaire data were collected to obtain information on participants' characteristics. The recruitment of employees from the University Hospital of Munich (LMU) was conducted simultaneously with the RisCoin HCWs cohort study, which studies risk factors for COVID-19 vaccine failure among HCWs [32].

2.2. Specimen Collection and Laboratory Analyses

Teams of trained field workers collected capillary blood samples (also known as a dry blood spot or DBS) following proper infectious disease control and blood sampling procedures to conduct laboratory analysis. The process for analyzing a DBS is explained in detail [33]. Two types of assays were used: the Roche Elecsys® Anti-SARS-CoV-2 assay anti-Spike (anti-S) test, referred to as Ro-RBD-Ig, and the Roche Elecsys® Anti-SARS-CoV-2 anti-Nucleocapsid (anti-N) test, referred to as Ro-N-Ig. The Ro-RBD-Ig detects antibodies after infection and vaccination, while the Ro-N-Ig test is used to differentiate between antibodies resulting from infection (both anti-S and anti-N present) and those due to vaccination (only anti-S present). The Ro-N-Ig test determines if an individual has previously had an infection but cannot provide information on the infection date. The Ro-RBD-Ig test has a cut-off value of 0.115 for DBS-seropositivity, while the Ro-N-Ig test has a cut-off value of 0.105. For both assays, a cross-reaction with viral infections predating the COVID-19 era could be excluded. This was achieved by analyzing samples obtained from blood donors prior to the emergence of COVID-19 [34,35].

2.3. Questionnaire Data

This study used questionnaires to gather information from participants about

- recruitment (institutional subgroup; recruitment date);
- demographic (date/year of birth; sex; level of education; household size);
- health-related behavior (smoking status; pre-existing medical conditions; medication scheme (intake of immunosuppressive drugs; others));
- employment-related behavior (occupational status; working conditions);
- COVID-19-related health status (vaccination status such as the date and type of first, second, and third vaccination if applicable; infection status, only Polymerase chain reaction (PCR)-confirmed COVID-19-diagnosis; diagnosis period; diagnosis date, month, and year; diagnosis in relation to vaccination and immunization status; diagnosis date after first vaccination; diagnosis date after full immunization (Two doses of Comirnaty, Spikevax or Vaxzevria or one dose of Jcovden at the time of data collection); severity of SARS-CoV-2-infection; previous contact with SARS-CoV-2 infected person; testing frequency; symptoms suggestive for COVID-19).

In the course of this study, three different versions of the questionnaire were used: Questionnaire 1 was provided on paper and used at the beginning of this study. Questionnaire 2 (used after 15 October 2021) was also provided on paper and included questions about the possibility of a third COVID-19 vaccination, as well as additional information that had emerged as potentially relevant during the course of this study (e.g., educational attainment, occupation, the presence of pre-existing conditions, and the course of COVID-19

disease). Questionnaire 3 was completed online by LMU employee hospital participants and requested the same information as Questionnaires 1 and 2.

Participants in Path 1 received Questionnaire 1 on the day of recruitment and filled it out during the recruitment procedures. Participants in Paths 2 and 3 were given the option to fill out Questionnaires 1 and 2 beforehand and bring them to the recruitment session or to fill them out on the day of recruitment. Participants in Path 2 also received Questionnaire 3 on the day of recruitment and were asked to fill it out during the recruitment procedures or as soon as possible thereafter.

Paper-based questionnaires were digitized using the software FormPro (version 3.1, OCR System GmbH, Leipzig, Germany, 2021).

2.4. Variables Definition

The variables that were used for the analysis are described in Table 1 and were selected following medical relevance. While most of the variables were obtained directly from the questionnaire, some of them were derived from other variables. The latter includes the vaccination scheme, time since the second vaccination, the occurrence of BTIs, time since infection, and the combination of the vaccination scheme and former infection, which is referred to as "immunity" hereafter. The recruitment process for KoCo-Impf was unique as it took place at various institutions over a period of seven months during the pandemic. Since a positive anti-N antibody level indicates a past infection, which could have occurred a long time ago, it is essential to take the different waves of the pandemic into account and correct for the different times at risk. Therefore, the cumulative number of new COVID-19 cases from the beginning of the pandemic to each date of recruitment was added as a covariate based on a weekly rolling window. A time lag of two weeks was applied, as anti-N and anti-S antibodies often need two weeks to develop after infection. [36,37].

Unlike most studies, we defined a SARS-CoV-2 infection by looking at anti-N antibody positivity instead of just considering PCR-positive tests. This approach ensures that asymptomatic and previously undiagnosed infections are more likely to be detected. Infection and vaccination by those vaccines used in our cohorts can be differentiated by serology, detecting both anti-S and anti-N antibodies. This analysis neglects information on symptoms. This choice was made due to the fact that many infections resulted in being asymptomatic, and the severity of symptoms does not necessarily indicate a different change in the antibody response.

2.5. Statistical Analyses

Before conducting statistical analysis, data were cleaned and secured. Categorical variables are presented as frequencies and percentages, while continuous variables are presented as mean values and standard deviations (SD). Mean values, SDs, and crude associations were calculated for all quantities and are presented in Table 2.

Table 1. Variables description with color-coded allocation to the three statistical models used in the analysis. Covariables of: all three models, green; only anti-N qualitative model, pink; only anti-S quantitative model, gray; anti-N quantitative model and anti-S quantitative model, blue; anti-N qualitative model; anti-N quantitative model, gold.

Variable Name	Definition (Type of Variable)
Quantitative anti-N/S	The detected amount of Ro-N-Ig/Ro-RBD-Ig from DBS (continuous)
Qualitative anti-N/S	A positive anti-N/S result is defined when the amount of Ro-N-Ig/Ro-RBD-Ig is ≥0.105/0.115 (positive/negative)
Age ****	Age of participants in years (continuous)
Cumulative cases	Cumulative number of COVID-19 cases from the beginning of the pandemic till the recruitment date (continuous)
Intake of immunosuppressive drugs ****	Current intake of medications that may suppress the immune system (yes, no)
Sex ****	Sex of the participant (male, female)
Smoking status ****	Current smoking status (never smoker, current smoker, past smoker)
Contact with patients ****	Direct contact with patients (yes, no)
Contact with positives ****	Previous contact with COVID-19 affected/SARS-CoV-2 infected person (yes, no, or unwittingly)
Household size ****	Number of household members including participant (1, 2, 3, 4, 5, >5)
Institutional subgroup	Categorization according to the institution of recruitment (Hospitals *: Medical center of LMU, Tropical Institute **, MK Bogenhausen, MK Harlaching, MK Neuperlach, MK Schwabing, MK Thalkirchner Straße, Barmherzige Brüder, Seefeld, Institutions of long-term care: Eichenau, MS Heilig Geist, MS Rümannstraße, Obersendling Others: Vaccination center Riem, Friedenheimer Brücke, General population ***)
Breakthrough Infection (BTI) ****	An infection happened at least 2 weeks after the second dose (yes, no, not applicable)
Time since infection ****	Time between the sampling date and the positive PCR (infected in less than 3 months, infected between 3 and 6 months, infected between 6 and 12 months, infected after 12 months, no infection)
Combination of vaccination scheme and former infection (immunity)	A composite variable containing information on the previous infection (based on anti-N result) and the undergone vaccination scheme (infection yes, not vaccinated, infection yes + one vaccination, infection yes + two vaccinations, infection yes + three vaccinations, infection no + one vaccination, infection no + two vaccinations, infection no + three vaccinations)
Time since second vaccination ****	Time between the second vaccination and the sampling date (continuous)
Vaccination scheme ****	A combination of types of vaccination and number of vaccinations, including BioNTech/Pfizer, Moderna, AstraZeneca, Johnson & Johnson/Janssen (no vaccination, one vaccination, two vaccinations, three vaccinations)

* Includes study participants from Path 2. ** Division of Infectious Diseases and Tropical Medicine of LMU. *** Includes study participants from Path 1 and Path 3. **** Based on self-reported questionnaire data.

Table 2. Cohort description with data before imputation.

Covariate	Category	Number of Participants *N* (%)	Qualitative Anti-N *N* (%)		Qualitative Anti-S *N* (%)		Quantitative Anti-N Mean Value (SD)		Quantitative Anti-S Mean Value (SD)	
			Positive	Negative	Positive	Negative	Positive	Negative	Positive	Negative
Overall cohort		6088 (100.0)	424 (6.9)	5664 (93.1)	5767 (94.8)	321 (5.2)	0.94 (1.52)	0.06 (0.01)	83.54 (200.35)	0.03 (0.02)
Sex	Female	4379 (72.0)	296 (6.7)	4083 (93.3)	4199 (95.9)	180 (4.1)	0.88 (1.33)	0.06 (0.01)	82.39 (199.08)	0.03 (0.02)
	Male	1709 (28.0)	128 (7.4)	1581 (92.6)	1568 (91.8)	141 (8.2)	1.10 (1.86)	0.06 (0.01)	86.68 (204.17)	0.03 (0.02)
Institutional subgroup	Barmherzige Brüder	188 (3.0)	40 (21.2)	148 (78.8)	187 (99.5)	1 (0.5)	0.98 (1.04)	0.07 (0.008)	55.02 (106.23)	0.06 (NA)
	Eichenau	34 (0.5)	5 (14.7)	29 (85.3)	34 (100.0)	0 (0.0)	1.59 (2.00)	0.07 (0.004)	447.20 (427.47)	- *
	Friedenheimer Brücke	34 (0.5)	1 (2.9)	33 (97.1)	34 (100.0)	0 (0.0)	0.88 (NA)	0.08 (0.006)	82.45 (122.71)	-
	General population	671 (11.0)	50 (7.5)	621 (92.5)	366 (54.6)	306 (45.4)	1.33 (2.25)	0.07 (0.02)	43.84 (121.03)	0.03 (0.02)
	Medical Center of LMU	3689 (60.6)	213 (5.7)	3476 (94.3)	3680 (99.8)	9 (0.2)	0.86 (1.53)	0.06 (0.01)	85.62 (205.49)	0.04 (0.04)
	MK, Bogenhausen	238 (3.9)	23 (9.6)	215 (90.4)	238 (100.0)	0 (0.0)	1.42 (1.78)	0.07 (0.01)	62.67 (172.21)	-
	MK, Harlaching	154 (2.5)	14 (9.1)	140 (90.9)	154 (100.0)	0 (0.0)	0.87 (1.19)	0.07 (0.006)	43.20 (60.97)	-
	MK, Neuperlach	112 (1.8)	5 (4.4)	107 (95.6)	112 (100.0)	0 (0.0)	0.45 (0.38)	0.07 (0.005)	33.44 (32.95)	-
	MK, Schwabing	281 (4.6)	13 (4.7)	268 (95.3)	281 (100.0)	0 (0.0)	0.36 (0.35)	0.07 (0.009)	48.08 (128.11)	-
	MK, Thalkirchner Straße	67 (1.1)	4 (5.9)	63 (94.1)	67 (100.0)	0 (0.0)	2.15 (2.27)	0.07 (0.006)	40.60 (46.19)	-
	MS, Heilig Geist	60 (0.9)	14 (23.3)	46 (76.7)	60 (100.0)	0 (0.0)	0.61 (0.69)	0.06 (0.02)	140.81 (380.16)	-

Table 2. *Cont.*

Covariate	Category	Number of Participants *N* (%)	Qualitative Anti-N *N* (%)		Qualitative Anti-S *N* (%)		Quantitative Anti-N Mean Value (SD)		Quantitative Anti-S Mean Value (SD)	
			Positive	Negative	Positive	Negative	Positive	Negative	Positive	Negative
	MS, Rümannstraße	36 (0.5)	2 (5.5)	34 (94.5)	36 (100.0)	0 (0.0)	0.58 (0.67)	0.06 (0.005)	531.93 (574.09)	-
	Obersendling	27 (0.4)	4 (14.8)	23 (85.2)	27 (100.0)	0 (0.0)	0.88 (0.66)	0.08 (0.004)	54.03 (113.73)	-
	Seefeld	83 (1.3)	5 (6.1)	78 (93.9)	83 (100.0)	0 (0.0)	1.26 (0.52)	0.06 (0.01)	138.71 (285.03)	-
	Tropical Institute	48 (0.8)	2 (4.1)	46 (95.9)	46 (95.9)	2 (4.1)	0.16 (0.05)	0.07 (0.01)	78.37 (115.27)	0.05 (0.02)
	Vaccination center Riem	366 (6.0)	29 (7.9)	337 (92.1)	363 (99.2)	3 (0.8)	0.76 (0.85)	0.07 (0.007)	101.04 (148.18)	0.06 (0.04)
Contact with patients	Yes	3505 (57.5)	261 (7.4)	3244 (92.6)	3493 (99.7)	12 (0.3)	0.90 (1.42)	0.06 (0.01)	94.39 (227.33)	0.03 (0.03)
	No	1833 (30.2)	111 (6.1)	1722 (93.9)	1647 (89.9)	186 (10.1)	0.89 (1.39)	0.06 (0.02)	65.44 (140.44)	0.03 (0.02)
	Unknown **	750 (12.3)	52 (6.8)	698 (93.2)	627 (83.8)	123 (16.2)	1.26 (2.09)	0.07 (0.02)	70.64 (167.82)	0.03 (0.02)
Contact with positives	Yes	2804 (45.9)	278 (9.9)	2526 (90.1)	2747 (97.9)	57 (2.1)	1.00 (1.62)	0.06 (0.01)	89.99 (215.54)	0.03 (0.02)
	No or unwittingly	3284 (54.1)	146 (4.4)	3138 (95.6)	3020 (91.9)	264 (8.1)	0.84 (1.28)	0.06 (0.01)	77.70 (185.37)	0.03 (0.02)
Smoking status	Never smoker	4177 (68.5)	315 (7.5)	3862 (92.5)	3967 (94.9)	210 (5.1)	0.96 (1.57)	0.06 (0.02)	86.29 (205.12)	0.03 (0.02)
	Current smoker	1062 (17.5)	49 (4.6)	1013 (95.4)	1009 (95.1)	53 (4.9)	0.52 (0.61)	0.06 (0.01)	73.95 (188.65)	0.03 (0.02)
	Past smoker	798 (13.1)	56 (7.1)	742 (92.9)	740 (92.8)	58 (7.2)	1.20 (1.71)	0.07 (0.01)	82.21 (190.29)	0.03 (0.02)
	Unknown	51 (0.9)	4 (7.8)	47 (92.2)	51 (100.0)	0 (0.0)	0.91 (0.65)	0.06 (0.007)	80.02 (201.80)	-

Table 2. *Cont.*

Covariate	Category	Number of Participants *N* (%)	Qualitative Anti-N *N* (%)		Qualitative Anti-S *N* (%)		Quantitative Anti-N Mean Value (SD)		Quantitative Anti-S Mean Value (SD)	
			Positive	Negative	Positive	Negative	Positive	Negative	Positive	Negative
Vaccination scheme	No vacc. ***	353 (5.7)	40 (11.3)	313 (88.7)	53 (15.0)	300 (85.0)	1.65 (2.64)	0.07 (0.02)	13.25 (50.72)	0.03 (0.02)
	One vaccination	380 (6.1)	123 (32.5)	257 (67.5)	367 (96.6)	13 (3.4)	1.15 (1.53)	0.07 (0.01)	98.05 (226.56)	0.04 (0.04)
	Two vaccinations	5001 (82.2)	245 (4.9)	4756 (95.1)	4997 (99.9)	4 (0.1)	0.75 (1.23)	0.06 (0.01)	55.40 (136.23)	0.06 (0.03)
	Three vaccinations	354 (5.8)	16 (4.4)	338 (95.6)	350 (98.9)	4 (1.1)	0.79 (1.07)	0.06 (0.01)	480.65 (416.65)	0.04 (0.04)
Household size	One person	1586 (25.9)	117 (7.3)	1469 (92.7)	1477 (93.2)	109 (6.8)	1.01 (1.57)	0.06 (0.01)	80.86 (197.26)	0.03 (0.02)
	2 people	2219 (36.5)	140 (6.3)	2079 (93.7)	2107 (94.9)	112 (5.1)	1.08 (1.65)	0.06 (0.01)	84.91 (209.09)	0.03 (0.02)
	3 people	969 (15.8)	68 (7.1)	901 (92.9)	924 (95.4)	45 (4.6)	0.89 (1.53)	0.06 (0.01)	82.79 (172.72)	0.04 (0.03)
	4 people	890 (14.8)	67 (7.6)	823 (92.4)	859 (96.6)	31 (3.4)	0.70 (1.13)	0.06 (0.01)	83.94 (213.37)	0.02 (0.02)
	5 people or more	331 (5.4)	23 (6.9)	308 (93.1)	314 (94.9)	17 (5.1)	0.50 (0.67)	0.06 (0.01)	92.08 (205.55)	0.04 (0.03)
	Unknown	93 (1.5)	9 (8.8)	84 (91.2)	86 (93.2)	7 (6.8)	1.15 (2.29)	0.07 (0.01)	68.55 (163.15)	0.04 (0.03)
Intake of immunosup-pressive drugs	Yes	178 (2.9)	11 (6.1)	167 (93.9)	166 (93.3)	12 (6.7)	1.09 (1.21)	0.06 (0.02)	103.35 (234.73)	0.03 (0.02)
	No	5855 (96.0)	406 (6.9)	5449 (93.1)	5550 (94.8)	305 (5.2)	0.94 (1.53)	0.06 (0.01)	82.39 (199.94)	0.03 (0.02)
	Unknown	55 (1.1)	7 (10.9)	48 (89.1)	51 (93.8)	4 (6.2)	0.81 (0.64)	0.06 (0.008)	144.25 (233.67)	0.01 (0.01)

Table 2. *Cont.*

Covariate	Category	Number of Participants *N* (%)	Qualitative Anti-N *N* (%)		Qualitative Anti-S *N* (%)		Quantitative Anti-N Mean Value (SD)		Quantitative Anti-S Mean Value (SD)	
			Positive	Negative	Positive	Negative	Positive	Negative	Positive	Negative
Time since infection	Less than three months ago	11 (0.1)	7 (63.6)	4 (36.4)	10 (90.9)	1 (9.1)	0.74 (1.53)	0.03 (0.03)	835.43 (653.70)	0.04 (NA)
	Three to less than six months ago	10 (0.1)	3 (30.0)	7 (70.0)	10 (100.0)	0 (0.0)	0.74 (1.00)	0.05 (0.03)	184.22 (387.26)	-
	Six to twelve months ago	81 (1.3)	57 (70.3)	24 (29.7)	81 (100.0)	0 (0.0)	1.04 (1.75)	0.06 (0.03)	357.00 (500.08)	-
	More than twelve months ago	118 (1.9)	71 (59.6)	47 (40.4)	116 (98.4)	2 (1.6)	0.76 (1.10)	0.06 (0.02)	221.56 (301.96)	0.05 (0.05)
	No infection	5582 (91.8)	0 (0.0)	5582 (100.0)	5268 (94.4)	314 (5.6)	-	0.06 (0.01)	67.39 (166.41)	0.03 (0.02)
	Unknown	286 (4.8)	286 (100.0)	0 (0.0)	282 (98.7)	4 (1.3)	0.98 (1.56)	-	220.78 (323.30)	0.05 (0.03)
Breakthrough Infection (BTI)	Yes	63 (1.1)	28 (46.4)	35 (53.6)	62 (98.6)	1 (1.4)	0.58 (0.85)	0.05 (0.03)	546.24 (532.41)	0.09 (NA)
	No	6018 (98.8)	396 (6.5)	5622 (93.5)	5698 (94.8)	320 (5.2)	0.97 (1.55)	0.06 (0.01)	78.58 (187.13)	0.03 (0.02)
	Not applicable	7 (0.1)	0 (0.0)	7 (100.0)	7 (100.0)	0 (0.0)	-	0.07 (0.02)	21.87 (19.57)	-
Vaccination scheme and infection (immunity)	Infection yes, not vaccinated	40 (0.7)	40 (100.0)	0 (0.0)	36 (90.0)	4 (10.0)	1.65 (2.64)	-	18.30 (60.95)	0.05 (0.03)
	Infection yes + one vaccination	123 (2.0)	123 (100.0)	0 (0.0)	123 (100.0)	0 (0.0)	1.15 (1.53)	-	238.20 (341.43)	-
	Infection yes + two vaccinations	245 (4.0)	245 (100.0)	0 (0.0)	245 (100.0)	0 (0.0)	0.75 (1.23)	-	294.99 (398.29)	-
	Infection yes + three vaccinations	16 (0.3)	16 (100.0)	0 (0.0)	16 (100.0)	0 (0.0)	0.79 (1.07)	-	437.20 (462.30)	-

Table 2. *Cont.*

Covariate	Category	Number of Participants *N* (%)	Qualitative Anti-N *N* (%)		Qualitative Anti-S *N* (%)		Quantitative Anti-N Mean Value (SD)		Quantitative Anti-S Mean Value (SD)	
			Positive	Negative	Positive	Negative	Positive	Negative	Positive	Negative
	Infection no, not vaccinated	313 (5.1)	0 (0.0)	313 (100.0)	17 (5.5)	296 (94.5)	-	0.06 (0.02)	2.56 (7.37)	0.03 (0.02)
	Infection no + one vaccination	257 (4.1)	0 (0.0)	257 (100.0)	244 (94.9)	13 (5.1)	-	0.07 (0.01)	27.40 (62.10)	0.04 (0.03)
	Infection no + two vaccinations	4756 (78.3)	0 (0.0)	4756 (100.0)	4752 (99.9)	4 (0.1)	-	0.06 (0.01)	43.06 (90.88)	0.06 (0.02)
	Infection no + three vaccinations	338 (5.5)	0 (0.0)	338 (100.0)	334 (98.9)	4 (1.1)	-	0.06 (0.01)	482.71 (414.94)	0.03 (0.04)

* (-) indicates NA(NA); ** The values for the "unknown" category of the corresponding variables have been imputed for the modeling process; *** These participants were vaccinated on the day of blood sampling and thus considered as "not vaccinated".

To evaluate the risk of infection (anti-N seropositivity) based on qualitative binary anti-N results, a multivariable logistic regression model was used. Odds ratios (OR), 95% confidence intervals (CI), and *p*-values were computed. For the quantitative analyses, only participants with positive anti-N/S antibody values were included since the negative region is just affected by noise measurement and has no biological meaning. Two multivariable generalized linear models (GLM) with gamma distribution were fitted, with exponentiated coefficients representing the expected multiplicative changes in anti-N/S antibodies, 95% CIs, and *p*-values as output. To stabilize the anti-N model, fitting values greater than 10 were set to 10 (5 participants).

The covariate representing the cumulative number of COVID-19 cases detected in Munich (log-transformed to address the skewed distribution) was incorporated into all three models. This adjustment considered the different durations of potential exposure during the recruitment period. The covariables used in the three models are listed in Table 1, color-coded by model affiliation, and selected based on medical relevance. The missingness in the covariables was corrected by multiple imputations with m = 5 iterations. The response variables were also used in the multiple imputation procedure to obtain unbiased regression coefficients [38]. The total variance of the coefficient estimates over the repeated analyses was computed using Rubin's rules [39]. The model evaluation was performed using (i) the area under the receiver operating characteristic curve (AUC) value obtained from a ten-fold cross-validation for the qualitative analysis of binary anti-N and (ii) diagnostics plots for the quantitative analyses (Supplemental Figure S1).

All statistical analyses and visualization were performed using the R software (version 4.1.1, R Development Core Team, 2021). The models were estimated using the R package mgcv [40], and the visualization was conducted using the package APCtools [41].

3. Results

3.1. Cohort Description

Of a total of 6467 participants who were recruited for this study, 379 had to be excluded because of

- missing or incomplete antibody measurements (n = 13);
- missing or implausible self-reported year of birth (n = 303);
- participation in clinical vaccination trials or recruitment after 16 December 2021 n = 27);
- vaccination with brands not authorized in Germany (n = 13);
- missing or diverse information on sex (n = 8);
- implausible vaccination dates (n = 3)
- unknown vaccination scheme (n = 12).

The final dataset that was analyzed included 6088 participants who were enrolled in 16 different institutional subgroups. All of these participants had complete measurements of anti-S/anti-N antibodies and self-reported questionnaire data (as shown in Figure 1). In total, 6088 participants were included in the qualitative binary anti-N model, 424 participants in the quantitative anti-N model, and 5750 participants in the quantitative anti-S model.

A description of the final cohort can be found in Table 2. Participants were aged from 18 to 96 years, with a mean/median age of 41.8/41.0. Thereof, 72.0% (4379/6088) were female, and 28.0% (1709/6088) male. The majority of study participants were HCWs in hospitals (79.8%, 4860/6088) or of other HC institutions (9.1%, 557/6088), while 11.0% (671/6088) were non-HCWs but from the general population. A total of 94.8% (5676/6088) of the participants were anti-S positive, while only 6.9% (424/6088) were anti-N positive. When the analysis was limited to HCWs, 6.9% (374/5417) were found to be anti-N positive.

3.2. Risk Factor Analysis for Anti-N Seropositivity

To determine the risk factors for contracting SARS-CoV-2, the qualitative anti-N serology test was used in conjunction with different covariables in a multivariable logistic regression model. The variables were selected following medical relevance and are described in Table 1. The results are presented in both Figure 2, where they are displayed as ORs, and in Supplemental Table S1, where they are displayed as logarithms of the ORs.

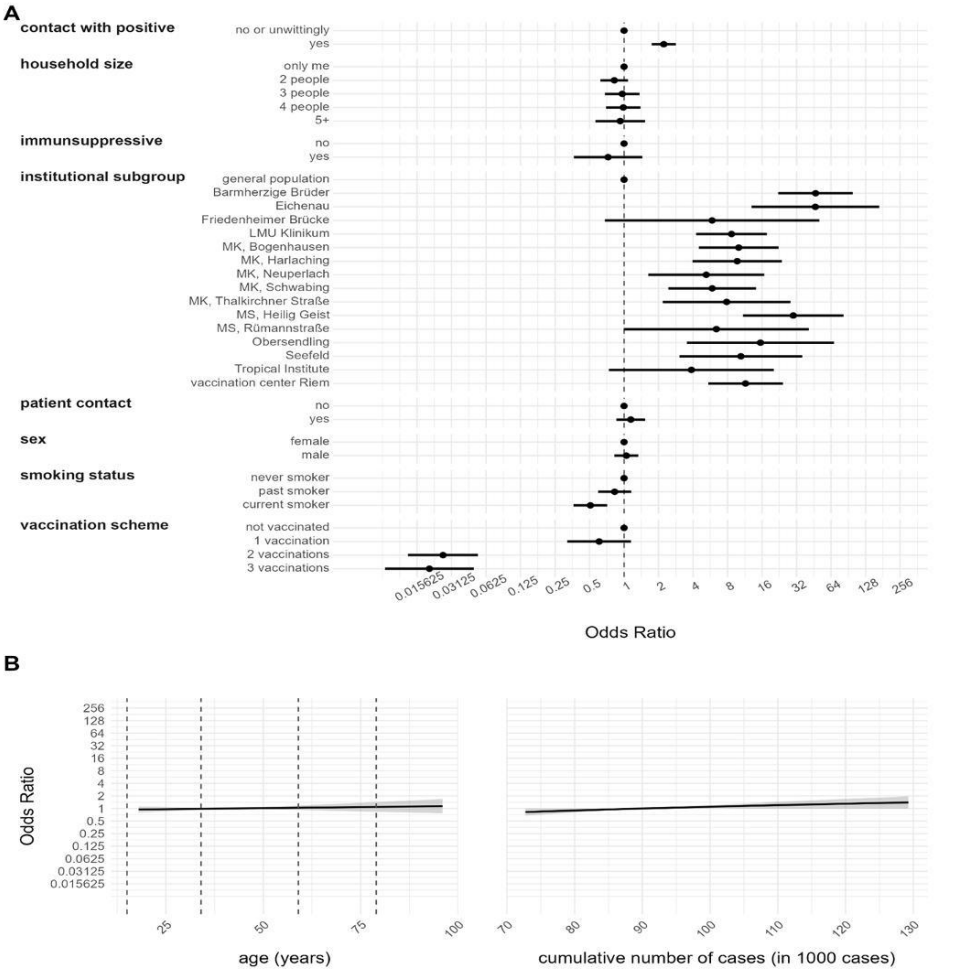

Figure 2. Risk factor analysis for SARS-CoV-2 infection, based on positive anti-N serology. Results are based on a logistic regression model and are given as ORs with a 95% CI. The obtained value of model evaluation using pooled AUC was 0.7398. (**A**) Estimates for categorical variables. (**B**) Estimates for continuous variables with 95% CI represented by the grey shadowed region.

The results indicate that compared to the general population, there is a statistically significant positive association between being an HCW employed in a hospital and an increased risk of contracting the virus (Barmherzige Brüder 46.8 [22.1, 99.1], LMU Klinikum 8.6 [4.2, 17.6], MK Bogenhausen 10.0 [4.4, 22.2], MK Harlaching 9.7 [3.9, 23.8], MK Neuperlach 5.2 [1.6, 16.6], MK Schwabing 5.8 [2.4, 14.1], MK Thalkirchner Straße 7.8 [2.1, 28.3], MS Rümannstraße (6.3 [1.0, 40.9] and Seefeld 10.4 [3.0, 35.8]). This was also the case for HCWs employed in institutions of long-term care (Eichenau 46.6 [12.9, 168.3], MS Heilig Geist 29.9 [10.9, 82.1] and Obersendling 15.4 [3.5, 67.9]) and for HCWs employed in the vaccination center Riem (11.4 [5.4, 24.2]). Interestingly, two centers did not show a statistically significant association between being an HCW and an increased risk of infection (Tropical Institute (3.8 [0.7, 20.2]) and Friedenheimer Brücke (5.8 [0.6, 50.5]). The vaccination scheme analysis revealed a strong negative association for individuals vaccinated with two (0.03 [0.01, 0.05]) or three (0.02 [0.008, 0.04]) doses compared to unvaccinated individuals. Compared to non-vaccinated participants (353 individuals), no significant effect for a vaccination with one dose (380 individuals) could be found (0.6 [0.3, 1.1]). Participants reporting a past known contact with SARS-CoV-2-positives demonstrated a strong positive association with anti-N antibody seropositivity (2.2 [1.7, 2.8]) compared to those having none or unwitting contact. Interestingly, compared to non-smokers, a strong negative association could be detected only for current smokers (0.5 [0.3, 0.7]) (former smokers not significant 0.8 [0.5, 1.1]). Age (1.0 [0.9, 1.0]), sex (male 1.0 [0.8, 1.3]), household size (2 people 0.8 [0.6, 1.0], 3 people 0.9 [0.6, 1.3], 4 people 0.9 [0.6, 1.3], 5 people or more 0.9 [0.5, 1.5], intake of immunosuppressive drugs (yes 0.7 [0.3, 1.4]) and having had contact with patients (yes 1.1 [0.8, 1.5]) were not statistically significant associated with anti-N seropositivity. The cumulative cases in the Munich municipality, indicating the development of the pandemic, were also shown to be non-significant (2.5 [0.8, 7.5]).

3.3. Determinants of Antibody Response after SARS-CoV-2 Infection

To identify the factors that influence antibody responses following infection with SARS-CoV-2, the quantitative anti-N serology was associated with different covariables in a multivariable GLM with gamma distribution. The variables were selected following medical relevance and are described in Table 1. The findings of this analysis are presented in Figure 3 as the expected multiplicative changes in anti-N/S antibodies (exponentiated coefficients) and in Supplemental Table S2 as coefficients of the model. The vaccination scheme analysis revealed that individuals with two (0.4 [0.2, 0.9]) and three vaccination doses (0.3 [0.1, 0.9]) had lower anti-N antibody levels compared to unvaccinated ones. No significant effect was found for participants with one vaccination dose (0.6 [0.3, 1.2]). A negative association could be detected for current smokers (0.6 [0.4, 1.0]), compared to non-smokers (former smokers not significant 1.1 [0.7, 1.7]). Age as a continuous variable was found to be a significant determinant, with older participants demonstrating higher anti-N antibody levels compared to younger ones (1.0 [1.003, 1.02]). Sex (male 1.2 [0.9, 1.6]), intake of immunosuppressive drugs (yes 1.1 [0.4, 2.8]), time since infection (three to less than six months ago 1.9 [0.1, 36.2], six to twelve months ago 1.3 [0.5, 3.2], more than twelve months ago 0.9 [0.3, 2.5]), BTI (yes 0.9 [0.4, 1.9]) and cumulative cases (1.9 [0.5, 6.9]) were not significant.

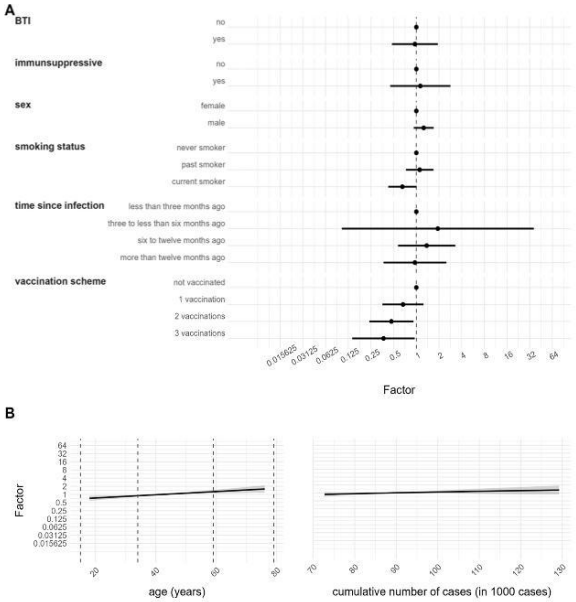

Figure 3. Anti-N antibody level after infection. Association between quantitative anti-N serology and determinants of antibody response. Results are based on a GLM with gamma distribution and are given as the expected multiplicative changes in anti-N/S antibodies (exponentiated coefficients) with a 95% CI. (**A**) Estimates for categorical variables. (**B**) Estimates for continuous variables with 95% CI represented by the grey shadowed region.

3.4. Determinants of Antibody Response after SARS-CoV-2 Vaccination and/or Infection

To ascertain the determinants that impact the antibody response after SARS-CoV-2 vaccination and infection, the quantitative anti-S serology was associated with different covariables in a multivariable GLM with gamma distribution. The results are presented in Figure 4 as the expected multiplicative changes in anti-N/S antibodies (exponentiated coefficients) and Supplemental Table S3 as coefficients of the model. Compared to unvaccinated but infected individuals, a strong positive association could be found for participants who were vaccinated one (4.4 [1.6, 12.2]), two (23.4 [8.4, 64.8]), or three (469.5 [162.9, 1352.8]) times but did not undergo an infection. An even stronger positive association was found for participants who were vaccinated one (15.9 [6.3, 40.0]) or two (51.0 [20.9, 124.8]) times and underwent an infection. The group that received three vaccinations in addition to a past infection had a lower estimate (81.9 [20.6, 325.0]) compared to the group with three vaccinations but no previous infection. However, the estimate was still higher than the group that had received two vaccinations and had a history of infection. Moreover, days since the second vaccination and thus completion of the primary vaccination schedule revealed a high negative association (0.994 [0.993, 0.995]). Participants with BTI (infection occurring two weeks after the second vaccination) demonstrated a positive association compared to non-BTI infections (infection prior to or within two weeks after the second vaccination) (4.0 [2.2, 7.4]). Interestingly, the cumulative cases in the Munich municipality, indicating the development of the pandemic, were also shown to be significant (2.5 [1.6, 3.8]). Age was found to be a significant determinant, with older participants demonstrating a negative association with anti-S antibody quantity compared to younger participants (0.987 [0.983, 0.992]). Compared to non-smokers, a negative association could be detected for

current smokers (0.8 [0.6, 0.9]) (former smokers not significant 1.0 [0.8, 1.1]). Time since infection (three to less than six months ago 0.7 [0.2, 2.6], six to twelve months ago 1.5 [0.6, 3.8], more than twelve months ago 1.3 [0.5, 3.4], no infection 0.4 [0.1, 1.2]), as well as sex (male 0.9 [0.8, 1.0]) and intake of immunosuppressive drugs (yes 1.1 [0.8, 1.5]) were not statistically significantly associated with quantitative anti-S serology.

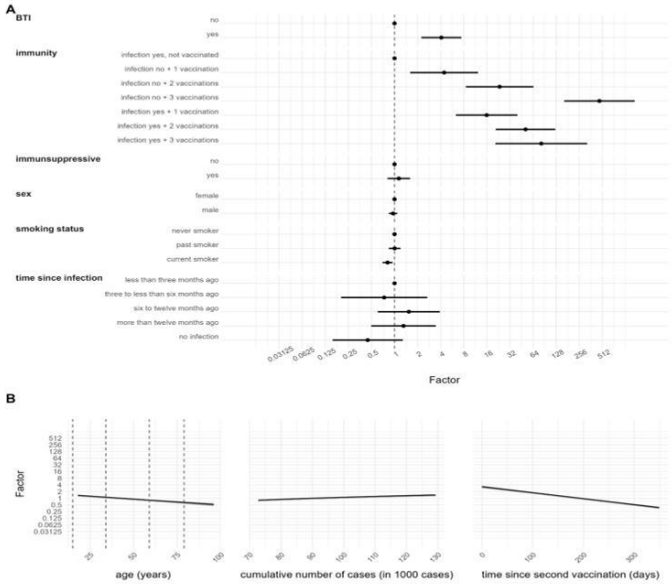

Figure 4. Anti-S antibody level after infection and vaccination. Association between quantitative anti-S serology and determinants of antibody response. Results are based on a GLM with gamma distribution and are given as the expected multiplicative changes in anti-N/S antibodies (exponentiated coefficients) with a 95% CI. (**A**) Estimates for categorical variables. (**B**) Estimates for continuous variables with 95% CI represented by the grey shadowed region.

4. Discussion

In this study, we explore the factors contributing to COVID-19 infections in a cohort comprising both the general population and HCWs, who face an increased risk of exposure to the SARS-CoV-2 virus. We utilized capillary blood samples to detect the presence of SARS-CoV-2 antibodies, which are indicative of previous infections, including both symptomatic and asymptomatic cases, as well as vaccination history. Moreover, our analysis aimed to identify factors that influence the immune response following infection or vaccination.

The recruitment process for KoCo-Impf took place over a period of seven months during the waves of the pandemic. To consider the changing time under risk, we included the overall cumulative number of cases in Munich at the respective recruitment time as a continuous covariate in our analysis. Our analysis showed that this variable has a positive though not significant, effect on anti-N seropositivity, indicating that HCWs were only weakly affected by the infection waves of the general population. One possible explanation is that since most of the reported infections occurred between six and twelve months prior to blood sampling, they mostly occurred in the first half of 2021. As a result, any association between the cumulative number of cases and anti-N seropositivity in the second half of 2021 may not be evident. Another reason could be that localized outbreaks within

specific institutions strongly influence the observed differences. This could potentially overshadow the effects of broader waves occurring within the general population. Other reasons could be that there was increasing availability of personal protective equipment (PPE) [42] and changes in risk behavior in 2021 [43]. In Bavaria, wearing protective FFP2 masks became mandatory in January 2021. Additionally, restrictions on access to public life were introduced in August 2021, based on vaccination, infection, and testing status, to reduce transmission rates [43]. As PPE has been shown to reduce the risk of infection [44], the increasing use of PPE may have compensated for any emerging outbreaks in 2021. In contrast, we found that the cumulative cases had an impact on anti-S antibody response, which could be explained by the different immune solicitations during the different waves. The dominant virus variant in Germany changed from alpha to delta in June 2021 [45], and a heterologous vaccination scheme was recommended from July 2021 onward [25–27,46,47]. Vaccination with Comirnaty rather than Spikevax was recommended for individuals younger than 30 years in November 2021 [48].

Age was found to be a statistically significant factor in anti-N immune response, with older participants showing higher levels after infection compared to younger ones. This is consistent with previous research that found a correlation between higher levels of the anti-N antibody and older age, male gender, ethnicity, and prior symptom history [49–51]. This suggests that infections in elderly individuals could lead to a more severe course of the disease and higher production of antibodies. In contrast to the anti-N immune response, our study showed that older age results in a decreased anti-S immune response, which is consistent with previous studies [21,22,52,53]. This suggests that the stimulation caused by vaccinations is more effective in younger individuals when compared to older ones.

Another aspect to consider when examining the pattern of higher anti-N levels after infection but generally lower anti-S levels in non-infected individuals of higher age is the longitudinal development of the immune response in relation to the time since vaccination. Since older individuals are considered a "high-risk" group, they were vaccinated earlier than younger individuals [6–8]. Considering that anti-S antibodies follow a pattern of rising, peaking, falling, and eventually reaching a plateau [53], the earlier timing of vaccination could have led to a decrease in the anti-S antibody titer at the time of blood collection, resulting in a lower overall level. Consequently, the protection against a second infection is considered to be lower in this group, posing an increased risk of SARS-CoV-2 infection and a stronger immune response against the N protein compared to younger individuals who were recently vaccinated and had a higher anti-S antibody titer shortly after vaccination.

However, it is worth noting that a systematic review and meta-analysis conducted by Cheng et al. (2022) focused on prime-boost immunization with the COVID-19 vaccine but only analyzed studies with non-infected participants [27]. Subgroup analyses by age did not find a significant difference in antibody concentrations between young and old populations. Nevertheless, this finding may be attributed to the selection bias of only analyzing non-infected individuals. Young and elderly people who were most affected by the pandemic were excluded, and the definition of non-infected might vary between studies (RT-PCR and serology).

Our analysis has shown that individuals who currently smoke have a lower prevalence of anti-N SARS-CoV-2 antibodies compared to those who never smoked. It is important to note that the current smoker group in our cohort had significantly fewer participants compared to the non-smoker group (1 to 4 ratio). This discrepancy in sample size raises concerns about the comparability of the two groups, as the underrepresentation of current smokers may introduce bias to the results. However, the lower risk of infection among current smokers aligns with similar findings from the analysis of the KoCo19 cohort [30]. Additionally, a recent study by Günther et al. (2022) supports these findings, as it demonstrated that current smokers were nearly half as likely to test positive for SARS-CoV-2 antibodies compared to non-smokers [54]. That study did not observe any differences in antibody levels between smokers and non-smokers who had been infected with or vaccinated against SARS-CoV-2, suggesting that the lower prevalence of antibodies in

smokers may be attributed to lower infection rates rather than reduced antibody response. In contrast, our results show a significantly reduced response to both the anti-S and anti-N antibodies in current smokers compared to non-smokers, consistent with previous studies by Reusch (2023), Ferrara (2022), and Moncunill (2022) [20,24,52]. Smoking may induce an immunosuppressive effect, as reported by Haddad (2021) and Sopori (2002) [55,56]. The lower anti-N antibody levels in current smokers compared to never-smokers may indicate not only a reduced development of antibodies but also a faster seroconversion to negative levels. Therefore, the anti-N seropositivity in current smokers may not be directly comparable to the never-smoker group, assuming a similar decrease and subsequent non-detection of past cases. It is also worth considering that smoking has been identified and communicated through the media as a risk factor for severe COVID-19 infections, leading to increased morbidity and mortality. Hence, it cannot be excluded that current smokers may have taken more precautions to avoid contact compared to non-smokers. The effect of current smoking on the risk of infection remains controversial and should be interpreted with caution [57].

The risk factor analysis showed that HCWs had an increased risk of infection compared to the general population, which is interestingly consistent with previous research on the KoCo19 cohort and other studies that have identified HCWs as a vulnerable group for infection [30,44,54]. However, the use of PPE has been shown to reduce the risk of infection [44], possibly leading to a change in the risk of infection in HCWs over time. Since our definition of infection is based only on positive anti-N, which remains positive for a long period of time [58], this baseline analysis of our study is not designed to detect this aspect. Recent research by Vivaldi et al. (2022) identified a change in the risk of infection due to time and vaccination status, with HCWs being at a higher risk of infection before vaccination but a reduced risk of breakthrough infection after primary vaccination [14]. Since the inclusion criteria for the KoCo-Impf study required at least one vaccination, it is impossible to correct this effect here. However, a follow-up analysis with the KoCo19 and the KoCo-Impf cohort may provide more insight into this aspect.

Another approach to determining whether HCWs have an increased risk of SARS-CoV-2 infection than the general population is by comparing anti-N seropositivities. In November 2021, the KoCo19 cohort, which represents the general Munich population, conducted its fourth follow-up in parallel with the KoCo-Impf recruitment. To compare the anti-N seroprevalence of both cohorts, we focused on the estimates for vaccinated persons in the KoCo19 cohort. The seropositivity was estimated to be 11.8% (9.8–13.8%) [30]. When we restricted the KoCo-Impf analysis to only HCWs, we observed a seroprevalence of 6.9% (6.2–7.6%), which is considerably lower than the seroprevalence of the vaccinated KoCo19 participants at the same time point. However, it is important to note that while the KoCo19 cohort is population-based and representative of the Munich population after statistical weighting, the KoCo-Impf cohort can be considered a convenience sample since it was not randomly selected. Therefore, it might be very complicated to compare both seroprevalences. This further emphasizes the importance of representative study designs. As the risk factor analysis for both KoCo19 and KoCo-Impf indicated a statistically significant higher risk of infections among HCWs, the lower seroprevalence in KoCo-Impf could be attributed to variations in infection and vaccination timing compared to the general population. Due to their higher risk, it is possible that HCWs were infected more frequently during the period when the general population was receiving their first two vaccinations. As HCWs, they had better access to testing facilities, which allowed them to become aware of their infection and receive vaccinations later in accordance with vaccination policies. On the other hand, in the general population, it is likely that more individuals were unknowingly infected and still received vaccinations despite their recent infection. The relatively lower underreporting probability among HCWs likely resulted in fewer cases where individuals were vaccinated despite having been recently infected, leading to lower seroprevalence among HCWs.

The risk of SARS-CoV-2 anti-N seropositivity was found to be higher among all HCWs except for those working in two specific institutions: Friedenheimer Brücke and Tropical Institute. While HCWs at Friedenheimer Brücke and the Tropical Institute have regular patient interactions, their work environment differs from that of HCWs in hospitals and long-term care facilities. Friedenheimer Brücke specializes in prenatal diagnostics, while the Tropical Institute primarily focuses on travel counseling and vaccinations. As a result, both facilities have a smaller patient population, and if symptomatic, these patients can choose to stay at home, thereby reducing the risk of infection for the personnel. The analysis did not find a significant effect of patient contact on SARS-CoV-2 anti-N seropositivity, suggesting that the increased risk of infection may be due to occupational activities and the working environment. This is consistent with recent research identifying occupational activities (tracheal intubation) as a risk factor for HCWs [44]. In addition, differences in infection frequency and spread between institutions can lead to variations in seropositivity rates.

As the institutional subgroup was found to have the strongest effect as a covariate, a sensitivity analysis was performed to evaluate how it impacted the overall risk factor analysis (Supplemental Table S4). However, no remarkable difference was observed.

Upon studying the kinetics of the anti-S antibody response, we found that the level increases with the number of COVID-19 vaccinations but decreases after days since the second vaccination. These results are consistent with previously published studies [52]. When individuals with one or two doses of vaccination were additionally infected, our analysis showed that they presented significantly higher anti-S values compared to only vaccinated individuals. Interestingly, with three vaccinations, the effect was reversed. While other studies with one or two vaccinations have shown similar behavior, we could not find comparable studies in the literature on the analysis of three vaccinations [21,22,52,53]. The combination of three vaccinations and one infection suggests that either the infection occurred in the early phases of the pandemic or recently (an infection between the vaccination scheme can be excluded in the time before Omicron), but the effect might be smaller due to the passage of time or ongoing immune response. This can be confirmed by the similarities with the estimate of two vaccinations with or without infection.

Our findings also indicate that the sequence of the triggers is important, with BTIs showing higher anti-S antibody titers but a non-significant tendency towards lower anti-N. This is in line with the other literature where the interpretation is that the immune system is solicited with vaccination (higher anti-S) so that a severe disease can be prevented (lower anti-N, since less reaction is needed) [59–61].

It is interesting to note that even though SARS-CoV-2 infection clearly affects the anti-S immune response, the duration since infection did not have a significant effect in any of our models. This finding is consistent with results that have already been published [20]. Numerous studies have demonstrated that the longitudinal development of both anti-N and anti-S antibodies follows a pattern of increasing, peaking, decreasing, and ultimately plateauing [53,62,63]. In our data, most of the reported infections occurred between six and twelve months prior to blood sampling, during which time most participants had already reached the plateau phase. Therefore, the lack of statistical significance is likely due to the fact that the only trajectory that can be fitted to these data is the plateau phase.

The analysis presented in this study encompasses the time period starting from the onset of the pandemic until December 2021. Therefore, the conclusions derived from this analysis specifically pertain to SARS-CoV-2 infections caused by the wild-type to delta variants of concern. A follow-up was carried out in May 2022 to include the circulation of the omicron variant of concern. A follow-up manuscript will present the findings of this follow-up study and compare them with the results obtained from the initial analysis.

The number of individuals who tested positive for anti-N antibodies but were unvaccinated (40) is lower than the number of individuals who tested positive for anti-S antibodies (53), even though their antibody response can only be attributed to a natural infection. This difference of 13 samples is likely due to the recruitment process rather than the assays them-

selves. Our cohort recruitment includes individuals at various stages of infection (recently infected, infected long ago, etc.) and at different time points of vaccination. Consequently, it is possible that a recently infected individual may only exhibit one type of antibody since their development requires time. On the other hand, someone who was infected a long time ago may have already seroconverted back, although not completely for both antibodies. Furthermore, information regarding vaccination status relies on self-reported questionnaire data, which may be influenced by bias or incomplete responses. Therefore, the discrepancy in this small number of samples is likely a combination of these factors.

After more than a year since the onset of the pandemic, we established the KoCo-Impf cohort to examine antibody development following vaccination and infection. Considering the significant findings already observed with KoCo19, we primarily focused on recruiting HCWs who face a specific risk of infection due to their frequent contact with multiple individuals, some of whom may be infected. It is important to note that our study population represents a convenience sample consisting solely of non-randomly selected vaccinated individuals. This aspect makes it more challenging to compare our results directly with those of the general population. However, the unique combination of our definition of seropositivity (based on anti-N and anti-S values) and the large sample size with detailed vaccination information makes our cohort unique in the world. We also found that vaccination protects against infection, but elderly people tend to have weaker immune responses and present higher anti-N but lower anti-S values compared to younger participants. Interestingly, smokers had a decreased risk of infection and lower immune responses after both vaccination and infection. HCWs were found to have a higher risk of SARS-CoV-2 infection in both the KoCo19 and the KoCo-Impf studies. However, only a few risk factors, such as age, vaccination status, contact with SARS-CoV-2 positive cases, and smoking status, were found to be statistically significant. As a result, no specific subgroups of HCWs requiring greater protection were identified. Instead, it is crucial to ensure the protection of all HCWs regardless of individual characteristics.

5. Conclusions

HCWs had a higher risk of SARS-CoV-2 infection in both the KoCo19 and KoCo-Impf studies. Multiple vaccinations and diverse vaccination schedules reduced infection risk while influencing the anti-N and anti-S immune response. Age impacted immune response, with older individuals exhibiting differences compared to younger ones. Interestingly, smokers had a lower infection risk, but their immune response weakened after vaccination and infection. The limited number of significant risk factors indicates that no specific HCW subgroups require heightened protection but that the protection of all HCWs remains crucial, regardless of individual characteristics.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/v15071574/s1, Figure S1: Model check for Anti-N quantity, based on positive anti-N serology. Results are based on a generalized linear model (GLM) with gamma distribution. Figure S2: Model check for Anti-S quantity, based on positive anti-S serology. Results are based on a generalized linear model (GLM) with gamma distribution. Table S1: Values to the risk factor analysis for SARS-CoV-2 infection, based on positive anti-N serology. Results are based on a logistic regression model and are given as the logarithms of the ORs with a 95% CI. Table S2: Values of the association between quantitative anti-N serology and determinants of antibody response. Results are based on a GLM with gamma distribution and are given as coefficients of the model with a 95% CI. Table S3: Values of the association between quantitative anti-S serology and determinants of antibody response. Results are based on a GLM with gamma distribution and are given as coefficients of the model with a 95% CI. Table S4: Sensitivity analysis of the risk factor analysis for SARS-CoV-2 infection, based on positive anti-N serology, excluding the variable "institutional subgroup". Results are based on a logistic regression model and are given as the logarithms of the ORs with a 95% CI.

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6. Publication II

viruses

MDPI

Article

Understanding the Omicron Variant Impact in Healthcare Workers: Insights from the Prospective COVID-19 Post-Immunization Serological Cohort in Munich (KoCo-Impf) on Risk Factors for Breakthrough and Reinfections

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Abstract: This study analyzes immune responses to SARS-CoV-2 vaccination and infection, including asymptomatic cases, focusing on infection risks during the Omicron wave, particularly among high-risk healthcare workers. In the KoCo-Impf study, we monitored 6088 vaccinated participants in Munich aged 18 and above. From 13 May to 31 July 2022, 2351 participants were follow-uped. Logistic regression models evaluated primary, secondary, and breakthrough infections (BTIs). Roche Elecsys® Anti-SARS-CoV-2 assays detected prior infections (via anti-Nucleocapsid antibodies) and assessed vaccination/infection impact (via anti-Spike antibodies) using dried blood spots. Our findings revealed an anti-Nucleocapsid seroprevalence of 44.1%. BTIs occurred in 38.8% of participants, with reinfections in 48.0%. Follow-up participation was inversely associated with current smoking and non-vaccination, while significantly increasing with age and receipt of three vaccine doses. Larger household sizes and younger age increased infection risks, whereas multiple vaccinations and older age reduced them. Household size and specific institutional subgroups were risk factors for BTIs. The anti-Nucleocapsid value prior to the second infection was significantly associated with reinfection risk. Institutional subgroups influenced all models, underscoring the

importance of tailored outbreak responses. The KoCo-Impf study underscores the importance of vaccination, demographic factors, and institutional settings in understanding SARS-CoV-2 infection risks during the Omicron wave.

Keywords: COVID-19; SARS-CoV-2; health care workers; vaccination; immunologic response; antibodies; seroprevalence; breakthrough infections; reinfections; ORCHESTRA

1. Introduction

The initial documentation of the emergence of COVID-19, attributed to the severe acute respiratory syndrome Coronavirus 2 (SARS-CoV-2), dates back to 31 December 2019, in Wuhan, located in the Hubei province of China [1]. Recognizing the widespread impact, the World Health Organization (WHO) officially declared COVID-19 a pandemic on 11 March 2020, in response to a surge in cases exceeding 118,000 across 114 countries, resulting in 4291 fatalities [2]. Following this declaration, global outbreaks ensued, with an estimated 775 million confirmed cases and more than 7 million deaths reported as of July 2024 [3].

On 27 January 2020, the Ludwig-Maximilians-University (LMU) Hospital's Institute of Infectious Diseases and Tropical Medicine diagnosed the first German COVID-19 patient. The crucial revelation of the transmissibility of SARS-CoV-2 by asymptomatic carriers was evidenced through the observed transmission patterns in this case [4]. However, as the fourth anniversary of this event transpired, the current infection risk faced by healthcare workers (HCWs) in close contact with SARS-CoV-2-infected patients, as well as the broader SARS-CoV-2 infection risk among HCWs in general, remains inadequately defined, since most data stems from the pre-omicron phase of the pandemic [5–8]. During the initial pandemic waves, HCWs, grappling with an unfamiliar threat and experiencing acute shortages of critical personal protective equipment (PPE), were among the first victims of nosocomial infection chains [9–12]. Reports from this period indicated that, among other factors, PPE use [13], vaccination status, and exposure location were relevant determinants of risk [14–17]. However, even then, findings underscored the substantial influence of factors such as male sex and Eastern European nationality, suggesting that factors beyond institutional exposure patterns might play a critical role in overall infection risk [18].

In May 2021, the longitudinal cohort named KoCo-Impf (Prospective COVID-19 post-immunization Serological Cohort in Munich—Determination of immune response in vaccinated subjects) was established at the Institute of Infectious Diseases and Tropical Medicine. It predominantly comprises HCWs with high contact risk with the SARS-CoV-2 virus but also non-HCWs categorized as members of the general population [14]. The primary focus of the cohort was to identify the risk factors for infection in HCWs and compare them with the general population of the same cohort. Additionally, KoCo-Impf runs alongside KoCo19, a large longitudinal cohort that focuses on a representative subset of the Munich general population [19].

As pandemic waves progressed, new variants emerged, and vaccine boosters were introduced, the situation became increasingly complex. Therefore, in May 2022 we conducted a follow-up analysis focusing on the impact of the omicron variant.

The Omicron variant, first identified in Botswana and South Africa in November 2021, is the fifth variant to be classified as a Variant of Concern by the WHO. The initial B.1.1.529 lineage has diverged into multiple sub-lineages, with BA.1 initially prevalent but quickly overtaken by BA.2, becoming globally dominant. Omicron's numerous mutations in the spike protein enable it to evade immunity from both prior infections and vaccinations, leading to a higher susceptibility to reinfections and breakthrough infections [20–22]. This ability to evade immune defenses has driven a rapid surge in global COVID-19 cases despite widespread vaccination efforts. Additionally, Omicron's higher

transmissibility but generally less severe course has resulted in more silent and undetected infections [23].

This underscores the strength of our strategy utilizing the detection of antibodies generated after SARS-CoV-2 infection and/or vaccination compared to other methods. By detecting anti-Nucleocapsid (anti-N) antibodies, we can identify undergone natural infections (or vaccinations with nucleocapsid-containing vaccines not commonly used in Europe), while with the anti-Spike (anti-S) antibodies, we can identify both natural infections and vaccinations [24,25].

In this analysis, we present the follow-up data of the KoCo-Impf cohort. Our aim is to describe, determine, and conceptualize the SARS-CoV-2 infection risk for HCWs during the first Omicron wave from May 2022 to July 2022. This study tracks a large cohort of mainly HCWs from various institutions across the greater Munich area, building upon previously reported baseline risk patterns [14]. We present differences between the risk factors of variants before and after Omicron. We explore breakthrough infections and risk factors for reinfection. Additionally, we address why risk assessment for this key population is challenging, investigating why scientific evidence remains limited and, at times, contradictory.

2. Materials and Methods

2.1. The Follow-Up Logistics for the KoCo-Impf

As previously outlined [14], there were differences in the management of participants between the HCWs at the Medical Center of the LMU and the remainder of the cohort (including the participants belonging to the general population). This organizational distinction also extended to the follow-up process, as delineated and compared in Figure 1. For the HCWs at the LMU Medical Center, follow-up information was disseminated through an app and distribution of fliers. Conversely, all other participants were contacted via email. The sampling methods varied further: HCWs of the LMU Medical Center underwent in-person visits from 16 May 2022 to 25 May 2022, during which capillary blood samples (DBS) were obtained on-site by trained personnel. In contrast, all other participants received the DBS kits via mail and self-administered the pricks. Sample returns occurred between 13 May 2022 and 31 July 2022. This standardized protocol has been consistently implemented across all follow-ups of KoCo19 [19].

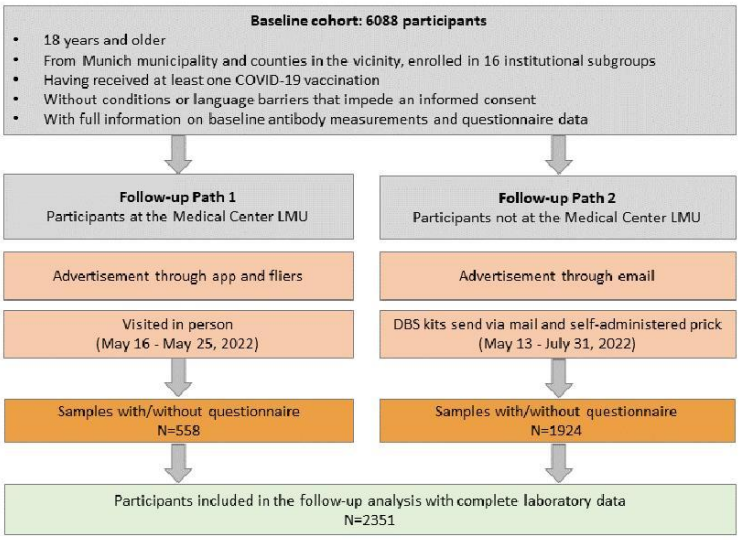

Figure 1. Recruitment paths and criteria for inclusion into the follow-up analysis. Gray boxes: institutional subgroups. Orange boxes: information on advertisement modalities for reaching to participants; modalities of the acquisition of questionnaire data, and capillary blood samples. Green Box: Inclusion criteria for follow-up analysis.

2.2. Specimen Collection and Laboratory Analyses

The method of specimen collection transitioned from comprehensive in-person DBS sampling conducted by trained personnel at baseline to partial DBS self-administered pricks during follow-up [14]. For a more comprehensive understanding of the DBS analysis, please refer to [26,27]. The laboratory analysis method remained consistent between baseline and follow-up assessments. Two assays were employed: Ro-RBD-Ig for detecting antibodies post-infection and vaccination, and Ro-N-Ig for detecting antibodies post-infection only. The combination of both assays allows us to distinguish between infection and vaccination. Ro-N-Ig confirms a prior infection but does not provide the exact infection date. The DBS-seropositivity cut-off for Ro-RBD-Ig is 0.115 COI (cut-off index), while for Ro-N-Ig it is 0.105 COI. Both assays have been validated to ensure no cross-reactivity with viral infections occurring prior to the COVID-19 era, as confirmed by the analysis of blood samples from donors preceding the emergence of COVID-19 [24,25].

2.3. Data and Statistical Analysis

The baseline questionnaire data was used to identify risk factors for infection. Therefore, the data descriptions and variable definitions match those in the baseline manuscript [14]. However, the variable "cumulative cases" (the total number of infections up to a given point; see [14] for details) was not included in the models because the follow-up period was similar for the entire cohort, ensuring comparable "time under risk" between baseline and follow-up. The baseline variable "contact with positives" was also excluded as it was outdated for new infections. For individuals recruited on the day of vaccination, their vaccination status prior to recruitment was considered (e.g., non-vaccinated if it was their first shot, vaccinated once if it was their second shot, etc.). For the variable vaccination scheme, the category "1 vaccination" was used as the reference, unlike the baseline analysis where "not vaccinated" was considered the reference category. This change is

because, for the follow-up analysis, all "not vaccinated" participants belong to the "general population" institutional subgroup. Analyses separating the general population from other institutional subgroups were conducted but showed no significant differences. Therefore, all the models presented here include all institutional subgroups, but interpretation requires careful attention.

Additionally, we focus on infections that occur after the completion of the vaccination regimen, specifically breakthrough infections, and reinfection (double infections). Reinfections were identified in participants who tested anti-N positive at baseline and showed a higher anti-N value at follow-up. Since anti-N indicates a prior infection and it is not induced by vaccinations used in Germany, the rise in anti-N between assessments suggests a second infection occurred between baseline and follow-up. To ensure accuracy, we excluded cases where the difference in values could be attributed to the inherent variability of the measurement. To determine this threshold, we compared two measurements of the same sample, calculated the difference in their log10 values, and determined the Standard Deviation (SD), which was 0.1305 COI. Any differences between baseline and follow-up measurements smaller than 2*SD were excluded from the reinfection definition.

The manuscript encompasses five distinct multivariable logistic regression models. One model evaluates the non-responder mechanism, while the other four assess the risk of different types of infection (anti-N seropositivity). Odds ratios (OR), 95% confidence intervals (CI), and *p*-values were calculated. Categorical variables are described with frequencies and percentages.

Model 1 describes the risk factor analysis for participants who contracted the SARS-CoV-2 virus at any time during surveillance (anti-N positive at baseline and/or follow-up vs. anti-N ever negatives). This approach is based on the assumption that risk factors for infection remain constant over time. Consequently, the number of infections increases over time, irrespective of when the infection occurred.

Model 2 focuses solely on new anti-N positives at follow-up vs. anti-N ever negatives, excluding positives at baseline. This approach allows for the examination of risk factors specific to Omicron infection.

Model 3 addresses breakthrough infections vs. anti-N ever negatives, where participants of both groups had at least two vaccinations at baseline (select participants with at least two vaccinations at baseline and exclude anti-N positives at baseline. Anti-N positives at follow-up vs. anti-N ever negatives). The analysis aims to understand the reasons for infection despite complete vaccination coverage. The baseline anti-S level is taken into account to assess potential levels of protection.

Model 4 delves into reinfection and compares individuals who experienced two infections (an increase in anti-N levels at follow-up compared to baseline) vs. subjects with only one infection (anti-N positive only at baseline with no increase in anti-N level at follow-up). The levels of anti-S and anti-N prior to reinfection (i.e., at baseline) are taken into account to assess potential levels of protection. Due to the sparse distribution of anti-N values above a COI value of 4, the nine values exceeding 4 were capped at 4.

The non-response mechanism over the follow-up was studied using a logistic regression coding with 1 for the participants that could be included in the analysis and 0 for the non-responders.

Missing data in the covariates for all five models were addressed through multiple imputations with m = 5 iterations. The response variables were also included in the imputation process to ensure unbiased regression coefficients [28]. Rubin's rules were used to compute the total variance of coefficient estimates over the repeated analyses [29]. Model evaluation was carried out using the Area Under the Receiver Operating Characteristic Curve (AUC) value obtained from ten-fold cross-validation.

All statistical analyses and visualization were performed using the R software (version 4.4.1, R Development Core Team, 2021). The models were estimated using the R package mgcv [30] and the visualization was done using the package APCtools [31].

3. Results

To exclude that the different engagement methods may have led to intrinsic behavioral differences, which could, in turn, influence the point estimates in the models, we reran all the models also excluding the general population and the LMU Medical Center. No significant differences in point estimates were observed with either exclusion. Therefore, only comprehensive models are presented here.

3.1. Non-Responder Mechanism and Follow-Up Cohort Description

The non-responder analysis results are depicted in Figure 2. Estimates for the institutional subgroup Friedenheimer Brücke were omitted from the plot since all members participated in the follow-up. However, the participant information of this institutional subgroup was retained for all other variables.

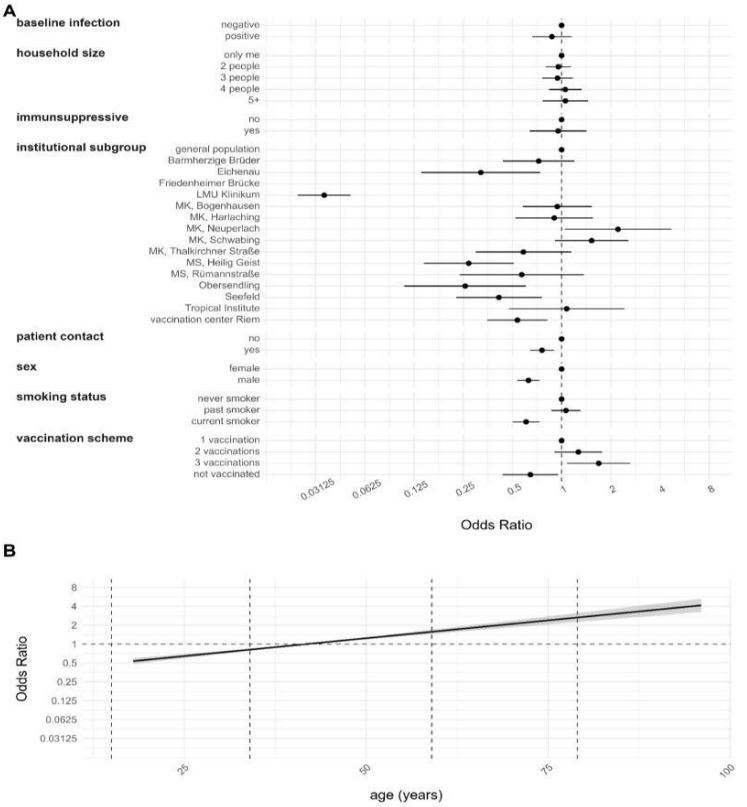

Figure 2. Non-response mechanism at the follow-up using multiple imputation. Results are based on a logistic regression model and are given as ORs with a 95% CI. The outcome was coded with 1 for participants that could be included in the follow-up analysis and 0 for non-responders. The obtained value of the model evaluation unison pooled AUC was 0.8595. (**A**) Estimates for categorical variables. (**B**) Estimates for continuous variables with 95% CI represented by the gray shaded region.

Compared to the general population, institutional subgroups Eichenau, LMU Medical Center (LMU Klinikum), MS Heilig Geist, Obersendling, Seefeld, and the vaccination center Riem were less likely to participate in the follow-up. HCWs with patient contact and male participants were less inclined to participate. Current smokers were less inclined to participate than never smokers. Additionally, unvaccinated individuals were less likely to participate than vaccinated individuals, with vaccinated twice showing no significant difference to only once vaccinated. Participation also statistically significantly increased with increasing age. Interestingly, only the institutional subgroup MK Neuperlach showed significantly higher participation compared to the general population. Covariates such as anti-N sero-positivity at baseline, household size, and intake of immunosuppressive drugs showed either no association or non-significant ones with non-response behavior.

Table 1 describes the follow-up cohort, comprising 2351 participants, focusing also on breakthrough infections and reinfections. Within this diverse cohort, 44.1% (1036/2351) tested anti-N positive. Breakthrough infections were observed in 38.8% (695/1793) of participants having at least two vaccinations and being anti-N negative at baseline. In total, 48.0% (84/175) of the participants being anti-N positive at baseline experienced reinfections. Looking at the demographic patterns, among the participants, 1740 females and 611 males participated, with 42.6% (741/1740) and 48.3% (295/611), respectively, testing anti-N positive. Institutional subgroups displayed variations, showcasing different anti-N positivity rates. Participants with patient contact exhibited a 44.9% (544/1211) positivity rate, while those without contact showed only 40.4% (308/762) sero-positivity. Smoking habits also played a role, with 44.9% (735/1636) of never smokers testing positive, compared to 39.7% (136/343) among current smokers and 44.7% (164/367) among past smokers. All unvaccinated participants belong to the general population subgroup and a significant portion (75.7%, 1779/2351) of the cohort had already received two vaccinations at baseline, with 41.8% (744/1779) testing anti-N positive. The group with intake of immunosuppressive drugs showed a 41.0% (34/83) positivity rate. Household dynamics indicated that larger households (four people or more) exhibited an increased anti-N positivity rate of 53.2% (249/468) or higher, while one-person households showed a 39.3% (262/667) positivity rate.

Table 1. Description of the follow-up cohort included in the analyses with information before imputation. Potential breakthrough infections were identified by selecting participants who had received two or more vaccinations and a negative anti-N result at baseline but tested positive for anti-N antibodies at follow-up. Similarly, potential reinfections were characterized by participants exhibiting a positive anti-N result at baseline and an increased level of anti-N antibodies at follow-up.

Covariate	**Category**	**Number of Participants *n* (%)**	**Qualitative Anti-N *n* (%)**		**Breakthrough Infection *n* (%)**		**Reinfection *n* (%)**	
			Positive	**Negative**	**Yes**	**No**	**Yes**	**No**
Overall cohort		2351 (100.0%)	1036 (44.1%)	1315 (55.9%)	695 (38.8%)	1098 (61.2%)	84 (48.0%)	91 (52.0%)
Sex	Female	1740 (74.0%)	741 (42.6%)	999 (57.4%)	520 (37.3%)	874 (62.7%)	59 (48.8%)	62 (51.2%)
	Male	611 (26.0%)	295 (48.3%)	316 (51.7%)	175 (43.9%)	224 (56.1%)	25 (46.3%)	29 (53.7%)
Institutional subgroup	Barmherzige Brüder	141 (6.0%)	83 (58.9%)	58 (41.1%)	50 (46.3%)	58 (53.7%)	13 (39.4%)	20 (60.6%)
	Eichenau	22 (0.9%)	9 (40.9%)	13 (59.1%)	4 (23.5%)	13 (76.5%)	2 (40.0%)	3 (60.0%)
	Friedenheimer Brücke	34 (1.4%)	14 (41.2%)	20 (58.8%)	12 (37.5%)	20 (62.5%)	1 (100.0%)	0 (0.0%)

	General population	504 (21.4%)	232 (46.0%)	272 (54.0%)	50 (39.1%)	78 (60.9%)	17 (53.1%)	15 (46.9%)
	Medical Center of LMU	527 (22.4%)	200 (38.0%)	327 (62.0%)	175 (35.2%)	322 (64.8%)	8 (32.0%)	17 (68.0%)
	MK, Bogenhausen	193 (8.2%)	87 (45.1%)	106 (54.9%)	64 (38.6%)	102 (61.4%)	10 (55.6%)	8 (44.4%)
	MK, Harlaching	124 (5.3%)	58 (46.8%)	66 (53.2%)	46 (41.8%)	64 (58.2%)	4 (33.3%)	8 (66.7%)
	MK, Neuperlach	102 (4.3%)	34 (33.3%)	68 (66.7%)	30 (30.9%)	67 (69.1%)	3 (75.0%)	1 (25.0%)
	MK, Schwabing	248 (10.5%)	78 (31.5%)	170 (68.5%)	66 (28.6%)	165 (71.4%)	7 (58.3%)	5 (41.7%)
	MK, Thalkirchner St.	51 (2.2%)	20 (39.2%)	31 (60.8%)	16 (34.8%)	30 (65.2%)	1 (50.0%)	1 (50.0%)
	MS, Heilig Geist	32 (1.4%)	23 (71.9%)	9 (28.1%)	14 (60.9%)	9 (39.1%)	4 (66.7%)	2 (33.3%)
	MS, Rümannstraße	27 (1.1%)	12 (44.4%)	15 (55.6%)	10 (41.7%)	14 (58.3%)	0 (0.0%)	2 (100.0%)
	Obersendling	15 (0.6%)	8 (53.3%)	7 (46.7%)	7 (50.0%)	7 (50.0%)	0 (0.0%)	1 (100.0%)
	Seefeld	57 (2.4%)	22 (38.6%)	35 (61.4%)	18 (34.0%)	35 (66.0%)	3 (75.0%)	1 (25.0%)
	Tropical Institute	39 (1.7%)	20 (51.3%)	19 (48.7%)	16 (47.1%)	18 (52.9%)	1 (50.0%)	1 (50.0%)
	Vaccination center Riem	235 (10.0%)	136 (57.9%)	99 (42.1%)	117 (54.9%)	96 (45.1%)	10 (62.5%)	6 (37.5%)
Contact with patients	Yes	1211 (51.5%)	544 (44.9%)	667 (55.1%)	431 (39.9%)	648 (60.1%)	43 (44.3%)	54 (55.7%)
	No	762 (32.4%)	308 (40.4%)	454 (59.6%)	178 (35.2%)	328 (64.8%)	23 (51.1%)	22 (48.9%)
	Unknown *	378 (16.1%)	184 (48.7%)	194 (51.3%)	86 (41.3%)	122 (58.7%)	18 (54.5%)	15 (45.5%)
Smoking status	Never smoker	1636 (69.6%)	735 (44.9%)	901 (55.1%)	500 (40.0%)	750 (60.0%)	60 (50.0%)	60 (50.0%)
	Current smoker	343 (14.6%)	136 (39.7%)	207 (60.3%)	91 (34.1%)	176 (65.9%)	9 (36.0%)	16 (64.0%)
	Past smoker	367 (15.6%)	164 (44.7%)	203 (55.3%)	103 (38.0%)	168 (62.0%)	15 (50.0%)	15 (50.0%)
	Unknown *	5 (0.2%)	1 (20.0%)	4 (80.0%)	1 (20.0%)	4 (80.0%)	-	-
Vaccination scheme	No vaccination **	242 (10.3%)	119 (49.2%)	123 (50.8%)	-	-	13 (54.2%)	11 (45.8%)
	One vaccination	226 (9.6%)	139 (61.5%)	87 (38.5%)	-	-	33 (54.1%)	28 (45.9%)
	Two vaccinations	1779 (75.7%)	744 (41.8%)	1035 (58.2%)	665 (39.3%)	1028 (60.7%)	36 (41.9%)	50 (58.1%)
	Three vaccinations	104 (4.4%)	34 (32.7%)	70 (67.3%)	30 (30.0%)	70 (70.0%)	2 (50.0%)	2 (50.0%)
Household size	One person	667 (28.4%)	262 (39.3%)	405 (60.7%)	163 (33.3%)	327 (66.7%)	23 (43.4%)	30 (56.6%)
	2 people	803 (34.2%)	333 (41.5%)	470 (58.5%)	219 (35.9%)	391 (64.1%)	27 (47.4%)	30 (52.6%)
	3 people	367 (15.6%)	169 (46.0%)	198 (54.0%)	121 (41.3%)	172 (58.7%)	12 (50.0%)	12 (50.0%)

	4 people	349 (14.8%)	176 (50.4%)	173 (49.6%)	119 (44.1%)	151 (55.9%)	15 (50.0%)	15 (50.0%)
	5+ people	119 (5.1%)	73 (61.3%)	46 (38.7%)	54 (58.1%)	39 (41.9%)	5 (62.5%)	3 (37.5%)
	Unknown *	46 (2.0%)	23 (50.0%)	23 (50.0%)	19 (51.4%)	18 (48.6%)	2 (66.7%)	1 (33.3%)
Intake of immunosupp. drugs	Yes	83 (3.5%)	34 (41.0%)	49 (59.0%)	20 (31.7%)	43 (68.3%)	2 (40.0%)	3 (60.0%)
	No	2252 (95.8%)	996 (44.2%)	1256 (55.8%)	672 (39.0%)	1049 (61.0%)	81 (48.2%)	87 (51.8%)
	Unknown *	16 (0.7%)	6 (37.5%)	10 (62.5%)	3 (33.3%)	6 (66.7%)	1 (50.0%)	1 (50.0%)

* The values for the "unknown" category of the corresponding variables have been imputed for the modeling process; ** These participants were vaccinated on the day of baseline blood sampling and thus considered as "not vaccinated".

3.2. Development of the Antibodies over Time: Group Characterization and Vanishing Effect of Vaccination

Examining the progression of SARS-CoV-2-related antibodies over time provides crucial insights into the cohort's evolution. This assessment involves plotting anti-N and anti-S antibodies on the x- and y-axes, respectively (see Figure 3A). The left side represents the baseline status, while the right side represents the follow-up status. The color scheme corresponds to the baseline result, with naive participants in blue (negative in both), solely vaccinated individuals in pink (anti-S positive but anti-N negative), those vaccinated and/or infected in orange (positive in both), and in gray individuals infected but with anti-S non-responder or late-responder status after infection, or a false positive value for anti-N. Observing the follow-up values, we notice a shift; no participants remain naive (quadrant bottom left of the follow-up plot is empty), and there is a shift to the right in the anti-N values of the infected and vaccinated group (orange dots), signifying potential reinfections. Additionally, we observe an increase in the number of solely vaccinated participants who became infected (shift to the right of pink dots), indicating breakthrough infections.

Reinfections were identified by comparing positive anti-N values of participants at baseline and the values at follow-up (see Figure 3B top). For some participants, anti-N levels decreased, indicating a natural decline in antibody levels (denoted in black). Conversely, for others, an increase signaled a reinfection (denoted in red). In this case, the baseline values of participants who experienced reinfection varied across the entire range, suggesting that Omicron reinfections may not be dependent on this variable. A similar pattern can be found by looking at the anti-S baseline values . However, it is advisable to include these variables in subsequent models to further elucidate their potential role in protection.

To further analyze breakthrough infections, we focused on at least double vaccinated participants with a negative anti-N value at baseline and examined the change in anti-S levels in the follow-up (see Figure 3B bottom). Participants showing a positive anti-N at follow-up are marked in red. Participants showed a clear anti-S increase, which could be attributed to an additional vaccination or an infection. Also, for breakthrough infections, the baseline values varied across the entire anti-S range.

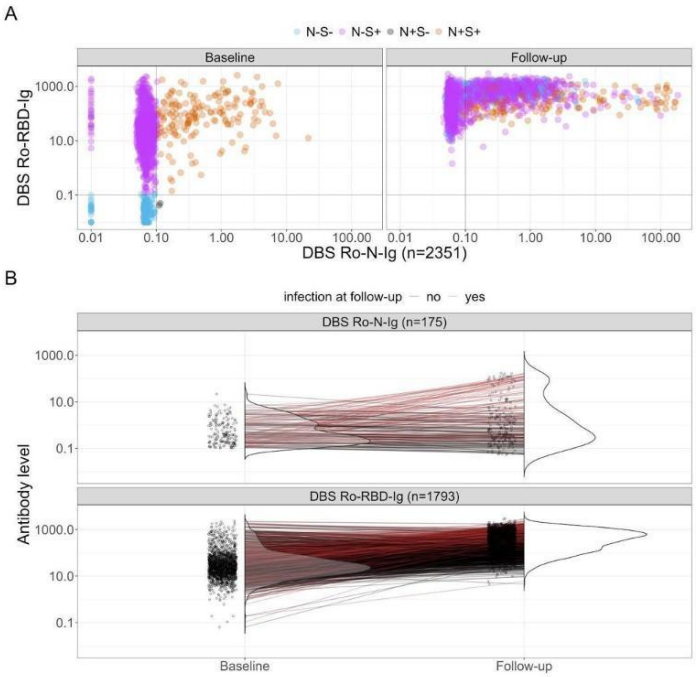

Figure 3. Analysis of anti-N and anti-S antibodies over time at baseline and follow-up. (**A**) Scatterplot displaying raw values of anti-N and anti-S antibodies (n = 2351). The Ro-N-Ig measurement is abbreviated with "N", while Ro-RBD-Ig is represented as "S". Positivity is indicated with "+", negativity is denoted with "-". The color code is determined by the subjects' status at baseline. (**B**) Top: Assessment of individuals who were anti-N positive at baseline, with a focus on the identification of reinfections (n = 84, depicted in red). Bottom: Examination of participants with at least two vaccinations and were anti-N negative but anti-S positive at baseline (n = 1793). Breakthrough infections are identified with a positive anti-N result at follow-up and are denoted in red (n = 695).

3.3. Risk Factor Analysis for the anti-N Sero-Positivity during Different Observation Periods

The results of the risk factor analysis for individuals who tested sero-positive in anti-N either at baseline and/or follow-up (referred to as ever positives, Model 1) are presented in Figure 4. The analysis revealed that individuals living in households with more than four residents were more susceptible to infection compared to those living alone. Similar tendencies were observed for household sizes of two and three, although these were not statistically significant. Among the institutional subgroups, only five centers (Barmherzige Brüder, MK Bogenhausen, MK Harlaching, MS Heilig Geist and vaccination center Riem) exhibited an increased risk for infection compared to the general population.

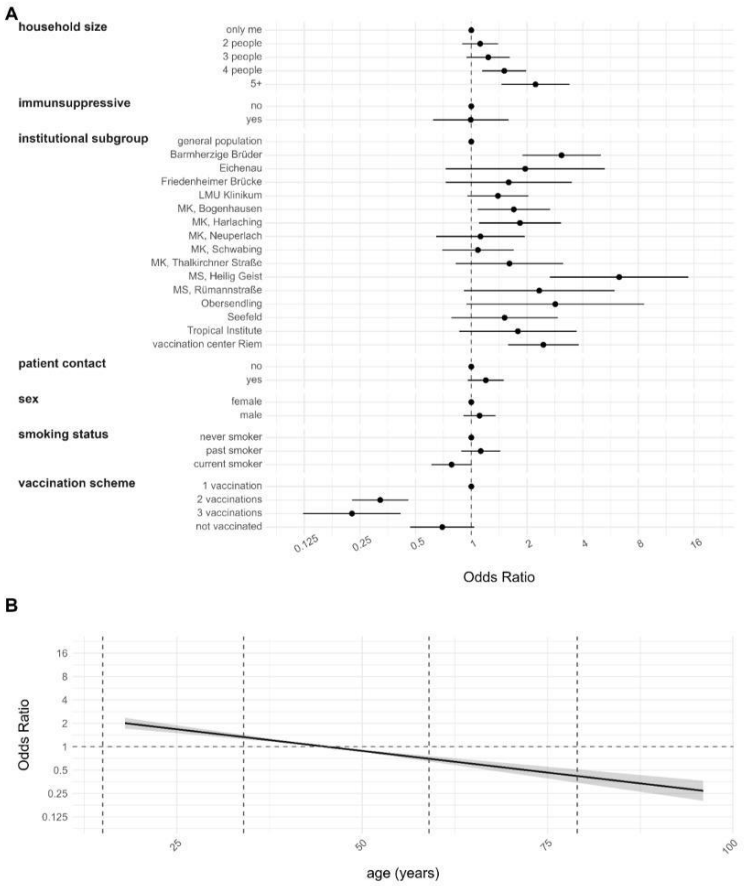

Figure 4. Risk factor analysis for infection at any time point in the study period (n = 2351, Model 1). A person with a prior infection was identified as being anti-N positive either at baseline, follow-up, or both (ever positive definition). Findings are derived from multiple imputations. The obtained value of the model evaluation unison pooled AUC was 0.6485. (**A**) Estimates for categorical variables. (**B**) Estimates for continuous variables with 95% CI represented by the gray shaded region.

Participants who received more than one vaccination were less likely to contract SARS-CoV-2 compared to those who received only a single vaccination. No statistically significant difference in infection risk was observed between participants who were not vaccinated and those vaccinated only once. Younger participants demonstrated an increased risk, whereas individuals older than 50 years exhibited a lower risk of infection. Other factors such as the intake of immunosuppressive drugs, patient contact, sex, and smoking status did not show a significant influence on the risk of infection.

When exclusively examining new anti-N-seropositive cases during follow-up, with a specific focus on Omicron infections, the identified risk factors remained unchanged (Supplemental Figure S1, Model 2, n = 2176). However, when considering the institutional subgroup variable, only the institutions MS Heilig Geist and vaccination center Riem

remained statistically significant. Although the effects related to vaccination remained statistically significant, they were observed to be less pronounced.

3.4. Risk Factor Analyses for Infection after Complete Vaccination and Reinfection

Model 3 aims at identifying the risk factors for infection among individuals who have completed their vaccination regimen, comparing those who have been double or more vaccinated and subsequently infected with those who have received only vaccination and were not subsequently infected (n = 1793). The findings are illustrated in Figure 5. The only covariates that indicated an elevated risk for breakthrough infection were household size, institution subgroup and age above 50 years.

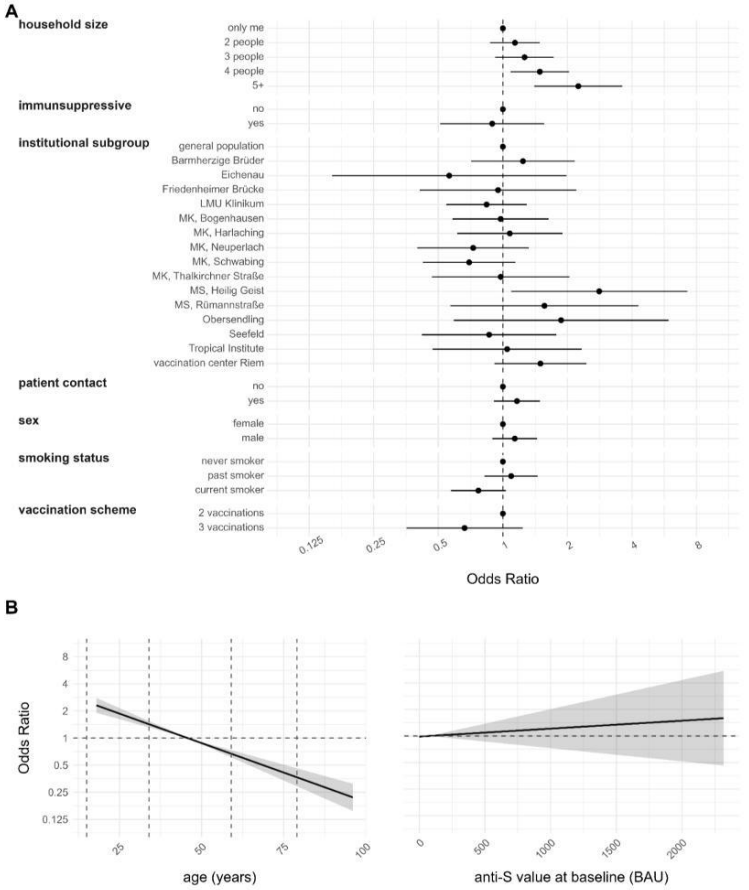

Figure 5. Risk factor analysis for breakthrough infection (n = 1793, Model 3). A person with a breakthrough infection was identified as having at least two vaccinations at baseline and being anti-N positive only at follow-up (anti-N negative at baseline but positive at follow-up). AS comparison only anti-N ever negatives with at least two vaccinations at baseline were selected. Findings are derived from multiple imputations. The obtained value of the model evaluation unison pooled AUC

was 0.6302. (**A**) Estimates for categorical variables. (**B**) Estimates for continuous variables with 95% CI represented by the gray shaded region.

Consistent with our previous analyses, only households with four or more occupants exhibited a significantly increased risk compared to individuals living alone. Similar trends were observed for households with two and three members, although these were not statistically significant. The institutional subgroup MS Heilig Geist exhibited a higher risk compared to the general population. Interestingly, having received three vaccinations did not show any significant difference compared to having received only two. The anti-S value at baseline was included in the analysis to study a potential protective effect but the variable did not show any statistical significance.

The risk of reinfection, comparing individuals infected only once to those with double infections, was analyzed in Model 4 (n = 175). The variable 'institutional subgroup' was excluded from the analysis due to insufficient individuals categorized for each institution. The model's findings are depicted in Supplemental Figure S2. Among the variables examined, only the anti-N value prior to the second infection exhibited a statistically significant association with reinfection. Specifically, a lower baseline value indicated a protective effect compared to a higher one.

4. Discussion

In this investigation, we examine the factors associated with COVID-19 infections within a study group inclusive of both, the general population and HCWs, who encounter elevated exposure risks to the SARS-CoV-2 virus. We employed capillary blood specimens to ascertain the presence of SARS-CoV-2 antibodies, serving as indicators of prior infections encompassing symptomatic and asymptomatic instances, alongside vaccination records. As a follow-up of the cohort presented previously [14], our focus shifts to discerning differences in risk factors among virus variants, instances of infection post-completion of the vaccination regimen, and factors contributing to reinfection.

The variable "institutional subgroup" has proven to be highly significant already in the baseline analyses, emerging as the most influential factor [14]. This significance persists in this subsequent follow-up analysis of all the models. This demonstrates the considerable variation in institutional structures and, consequently, in the rates of new infections across different institutions. Similarly, protective measures should be tailored to the specific contexts of each institution, moving beyond generalized approaches such as the use of PPE. Rather, a nuanced understanding of infection transmission dynamics within each institution is imperative. In addition, the variance in SARS-CoV-2 transmission risks observed among distinct institutions in our study may not solely be indicative of differences in risk-associated behaviors, procedural implementations, or adherence to PPE guidelines. Such disparities may be rooted in the fundamental characteristics of SARS-CoV-2 dissemination, which is typified as a series of hyperlocal events [32]. Both interpretations highlight the intricacies of transmission dynamics, proposing that a confluence of broader contextual factors alongside stochastic elements substantially influences the likelihood of institutional SARS-CoV-2 outbreaks.

Since the institutional subgroup was one of the strongest variables in the analyses, all models were also run separately for the general population only and all other institutional subgroups were combined to assess the impact of this variable on the other observed effects. No relevant differences were found, except in Model 1, where patient contact was significant only for the general population (OR for patient contact: (i) general population 2.69 [1.07–6.76], (ii) all other institutions 1.20 [0.96–1.50],). This might indicate that HCWs are better able to protect themselves from possible infections compared to individuals from the general population not being classified as HCWs but carrying out activities involving patients. For all the risk factor analyses presented here, generalized linear mixed effects models (GLMM) with institutional subgroup as random intercept would also have been appropriate, as this accounts for similar behavior among individuals from the same

institution. However, it was crucial to include the coefficients of the different institutional subgroups to allow for direct comparison between hospitals. The possible power loss was manageable, and the coefficients remain interpretable. The risk profiles of institutional subgroups varied across baseline, follow-up, and Omicron-only cases, reflecting fluctuations in infection rates relative to the general population over time. Given the ongoing nature of the pandemic, this aspect warrants careful consideration and interpretation alongside the evolving waves and timelines of the pandemic. A time-to-event analysis, such as Cox regression, would not adequately address this feature and is therefore unsuitable for this analysis. This analysis can also serve as a sensitivity assessment for case numbers.

Numerous publications have undertaken analyses of infection risks among HCWs. For instance, part of our data has contributed to the examination of determinants of anti-S immune response at 6, 9, and 12 months post-COVID-19 vaccination within a multicentric European cohort as part of the ORCHESTRA project [33–35]. While relative risks were adjusted for country and, in some cases, institutional subgroup, the robustness of these findings may still be influenced by the strength of the institutional subgroup effect, which might just be a proxy for local outbreaks. Consequently, analyses involving multicentric cohorts offer expanded and arguably more representative population samples but may yield less reliable results compared to those from single-center analyses. Similarly, the analysis of larger hospitals is contingent upon the specific departments to which HCWs are assigned. Therefore, comprehensive investigations into infection transmission mechanisms across different departments and institutions are warranted.

Examining the non-responder mechanism, it was observed that non-vaccinated and younger participants demonstrated less inclination to engage in the follow-up analysis. This phenomenon could stem from the perception that these groups do not perceive themselves to be at risk and therefore lack interest in monitoring new infection rates. Conversely, it may also be the case that these individuals, being aware of their heightened exposure to potential infections, already consider themselves at elevated risk and hence do not require further quantification from the study. Previous research has already reported lower non-responder rates of younger healthcare workers [36]. Another possibility could be that at the vaccination center Riem, we recruited younger participants who had recently been vaccinated. It is plausible that these young participants had moved out of Munich and therefore could not participate in the follow-up.

The primary focus of the KoCo-Impf cohort is to identify the risk factors for infection in HCWs and compare them also with the general population. In addition to the general population of KoCo-Impf itself, for this comparison, recruitment occurred concurrently with the third and fourth follow-up of KoCo19 in Munich, a prospective and Munich-representative COVID-19 cohort, although comparing the two cohorts poses substantial challenges [19]. Notably, the variables of sex and age exhibited similar patterns of missing data compared to the KoCo19 cohort [19,36], indicating that despite the focus on HCWs, this study can provide insights applicable to the broader population. Intriguingly, prior infection status at recruitment did not exhibit statistical significance in terms of missing data, a contrast to findings in KoCo19 [19,36]. This discrepancy may be attributed to several factors. Firstly, the level of interest in infection dynamics might differ between the general population and HCWs, with the latter, perceiving a heightened risk, displaying sustained interest even after a previous infection. Secondly, it could be influenced by the different timing of follow-up assessments. During the KoCo-Impf follow-up, the emergence of the Omicron variant and the understanding that previous infections might not confer immunity against subsequent infections became increasingly pertinent. Consequently, risk perceptions evolved over time, aligning with findings from other studies [37–39]. Although comparing the two cohorts posed challenges and required careful evaluation, it was confirmed in both cohorts that HCWs had a higher risk of infection. Sex, age, household size, and intake of immune-suppressing drugs were not found to be significant risk factors for infection in either cohort, but being a current smoker was [14,19]. The lower

number of detected cases in the KoCo-Impf, however, indicates a more complex scenario. Disparities in vaccination timing, behavioral adaptations, and methodological challenges in comparing the representative KoCo19 with the convenience sample of HCWs could potentially influence, or even bias, the assessment of exposure and infection risk.

In the follow-up analysis, risk factors for infection—including household size, current smoking status, and institutional subgroup—showed changes compared to baseline (see Figures 2 and 4 of [14]). Household sizes of four or more exhibited statistically significant increases in infection risk during the follow-up, a pattern not observed at baseline. This shift may be attributed to the predominance of Omicron infections, which are more closely linked to contact intensity [40]. Larger household sizes correspond to higher probabilities of virus exposure, consistent with findings that the Omicron variant is considerably more contagious than previous variants [20–22,40]. During lockdown, the HCWs had possibly the most external contacts due to their job. With the lifting of lockdowns, the impact of having more individuals in the households became evident. The confirmation that this effect primarily stems from Omicron infections, rather than merely a larger sample size, is supported by risk factor analysis focusing solely on Omicron cases (see Supplemental Figure S1). Households with smaller household sizes showed similar effects but were not significant. This might just be due to a too-small sample size. Interestingly, current smokers exhibited a lower risk of infection in the baseline analysis, a trend that persisted in the follow-up but did not reach statistical significance. This change may be attributable to fluctuations in sample size. However, this effect was previously discussed in the baseline paper [14] and has been observed in other independent cohorts [41–45] as well as in the RisCoin cohort [46]. The estimates for the institutional subgroups remained highly significant in the follow-up analysis, although their magnitude diminished (see Figure 2 of [14] compared to Figure 4). This may indicate a leveling of infection risk over time between the general population and the other institutions. During the Omicron period, only two institutional subgroups remained statistically significantly different from the general population (see Figure 4 and Supplemental Figure S1). This suggests that the risk of institutional subgroups was more similar to the general population in the Omicron period than in the pre-Omicron period.

Another distinctive feature associated with the Omicron variant is its impact on the SARS-CoV-2 infection risk among HCWs compared to the general population. Previous studies [9,10,12], including our baseline analysis [14], highlighted a significantly elevated infection risk for HCWs, particularly those in patient-facing roles [46,47], during the first waves of the pandemic. However, in this follow-up analysis, an increased risk is not evident when analyzing Omicron infections exclusively (refer to Supplemental Figure S1). Factors such as enhanced personal infection protection practices in healthcare settings and the Omicron variant's notably higher reproduction rate facilitating its widespread dissemination across traditionally low-risk environments may have led to an equalization of risk across populations. This phenomenon aligns with the outcomes of additional research [40,48], indicating a 'socialization' of infection risks at least since the emergence of the Delta variant. However, other studies still found a higher proportion of infected HCWs compared to the general population during the first Omicron wave [49]. Additionally, the Omicron infections result in a decreased hospitalization rate, leading to fewer infectious individuals in the hospitals. This inevitably reduces the difference in infection pressure between hospitals and the general community.

When comparing Omicron to non-Omicron infections, the most notable difference is observed in the vaccination status variable. The direction of effects remains consistent, with participants who received two or three vaccinations demonstrating a protective effect compared to those vaccinated only once. However, the magnitude of these effects notably decreases when examining Omicron infections. This reduction in effectiveness is attributed to the waning protection of vaccinations against Omicron variant infections [20,21,50].

For breakthrough infections, there has been no identified correlate of protection based on the anti-S baseline value. This observation does not necessarily indicate the absence of a protective threshold. Rather, it suggests that the value fluctuates depending on the viral load to which an individual is exposed relative to the contagiousness of the current SARS-CoV-2 variant. Exposure levels can vary significantly. It is conceivable that an individual with assumed low protection (characterized by a low anti-S level) may encounter a low viral load, thereby preventing infection as the immune system can intercept the infection before symptoms manifest. Conversely, it is possible that an individual considered with high protection (characterized by a high anti-S level) may encounter such a high viral load that protection is rendered ineffective. Although this scenario may result in non-significance in risk factor analysis, it underscores the presence of probably relevant biological meaningful values. The same argument can be brought with the neutralization capacity of the exposed subject, making the identification of a correlate of protection even more challenging.

Other studies have examined in vitro neutralization levels to identify correlates of protection, revealing a non-linear relationship. While this approach offers a possible solution to the issue, it was not feasible in our case due to the use of DBS sampling and a much larger sample size [51]. Similar to our analysis, other studies have investigated antibody responses, finding that higher anti-S levels were associated with a reduced risk of reinfection, while no association was found for anti-N levels. This discrepancy may be attributed to differences in sample composition, as all donors in those studies were vaccinated prior to sampling, potentially leading to a distinct antibody response [52].

In a comprehensive multicenter analysis of breakthrough infections [53], which incorporated an earlier subset of our data, significant correlations were observed between infection risk and the number of booster doses received. In our analysis, this was not the case. This divergent finding could be attributed to several factors: the impact of pre-Omicron variant infections, a shorter observational timeframe, considerable variability among study centers with notably high rates of breakthrough infections in Northern Italy, enhanced statistical robustness stemming from a larger sample size under investigation, and different approaches in case definition.

Regarding reinfections, no demographic factor despite age exhibited a statistically significant association, suggesting that reinfection could potentially affect any individual or that the specific variable under scrutiny remains unknown. This observation may be attributed to the diminished protective effect of prior pre-Omicron infections against Omicron SARS-CoV-2 infections, a phenomenon documented in numerous studies following the emergence of this variant [54]. Nonetheless, the inconsistency in the definition of reinfection across the literature complicates direct comparisons. In our analysis, reinfections correlate with an increase in the anti-N baseline value. This may seem counterintuitive, as one might anticipate greater protection with higher antibody levels [55]. However, elevated anti-N values can also reflect the behavior of the participant. Higher values could indicate increased exposure to the virus through more frequent contacts. Alternatively, individuals exhibiting elevated anti-N values following a SARS-CoV-2 infection may represent a subset more susceptible to severe COVID-19 outcomes [56,57]. This subgroup could inherently possess risk factors not considered in this analysis predisposing them to infection initially.

The cohort was recruited between June and December 2021, allowing participants a maximum of 22 months to contract the infection prior to recruitment. It is possible that some participants who were negative at baseline had been infected earlier but had reverted to seronegative status, thus excluding them from the reinfection analysis. However, a drop in seronegative status between study rounds can be ruled out, as discussed in Kroidl et al. [58].

The pattern of younger participants facing a heightened risk persisted across all facets of our analysis, encompassing the general infection risk (Model 1), the risk specific to Omicron (Model 2), and the risks associated with breakthrough infections and reinfections

(Models 3 and 4). This observation is consistent with the results of other investigations [53]. However, some studies have identified a more nuanced relationship between age and these risks [50], while others have noted an elevated risk among older populations [59]. Notably, within our framework, the influence of age appears to be more behavioral than biological.

The analysis presented primarily focuses on (re)infections and breakthrough infections within the KoCo-Impf study cohort. We have demonstrated that the differences among institutional subgroups are a fundamental factor. The capacity to identify institutional disparities in our study was enabled by the strategic timing of follow-up evaluations, conducted at analogous time points across all 15 institutions. Such disparities are likely to be overlooked in studies that focus on single institutions or in meta-analyses that include follow-ups conducted at varying times. This highlights the critical importance of uniform temporal alignment in observational research to capture nuanced differences between institutions effectively. Operationally, this insight underscores the imperative for the development and implementation of localized outbreak management and rapid response mechanisms, tailored to the different needs of each institution in space and time. This approach should form the basis for better safeguarding this essential sector of our society.

Finally, if 'every good regulator of a system must be a model of that system' [60], our results imply that the outbreak management for HCWs in the era of the Omicron variant should extend the scope of strategies beyond the healthcare facilities. The healthcare environment was a primary risk factor at the pandemic's outset. However, our findings indicate the importance of considering the wider environmental risks HCWs face within their personal households and social circles. Like interconnected vessels, risks from these private spheres inevitably impact workplace safety. Effective management must therefore prioritize understanding and influencing the behavioral risk patterns among specific demographics, for instance, younger HCWs. Additionally, it involves recognizing and responding to the significant variances across specific healthcare facilities over time, and implementing outbreak response mechanisms that are swift and hyper-local in their adaptation. As a foundation for such efforts, research must consistently integrate additional behavioral, institutional, and biological determinants of risk alongside those identified in this study.

5. Conclusions

HCWs constitute a distinctive sector of our society. The fluctuating nature of risk factors for infection highlights the need for adaptable preventive measures over time. Notably, the institutional subgroup emerged as the most influential variable in all risk factor analyses, emphasizing the importance of comprehending infection patterns within specific hospitals and departments as well as elderly and nursing homes. Furthermore, behavioral aspects are crucial for understanding the differences in infection rates. It is also important to remember that outbreaks can occur randomly as part of a stochastic process.

A higher seroprevalence in a specific institution might not necessarily indicate ineffective local infection control guidelines but reflect an earlier introduction of the virus into that institution by chance, causing subsequent local outbreak waves. Nevertheless, tailored standard operating procedures, specific to the institutional environment, can still make a significant difference by optimizing outbreak preparedness, early warning, and rapid response within the healthcare setting.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/v16101556/s1, Figure S1: Risk factor analysis for only Omicron-related infections (n = 2176, Model 2). A person with a prior infection was identified as being anti-N positive only at follow-up. Individuals who were anti-N positive at baseline were excluded. Findings are derived from multiple imputations. The obtained value of the model evaluation unison pooled AUC was 0.6400. (**A**) Estimates for categorical variables. (**B**) Estimates for continuous

variables with 95% CI represented by the gray shaded region. Figure S2: Risk factor analysis for reinfections ($n = 175$, Model 4). A person with a prior infection was identified as being anti-N positive at follow-up. Only individuals who were anti-N positive at baseline were included. Findings are derived from multiple imputations. The obtained value of the model evaluation unison pooled AUC was 0.6803. (**A**) Estimates for categorical variables. (**B**) Estimates for continuous variables with 95% CI represented by the gray shaded region.

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Data Availability Statement: Data are subject to data protection regulations and can be made available upon reasonable request to the corresponding author. To facilitate reproducibility and reuse, the code used to perform the analyses and generate the figures was made available in an open-source GitHub repository (https://gitlab.lrz.de/TROP.noemi.castelletti/kocoimpf_followup) (accessed on 22 July 2024)).

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