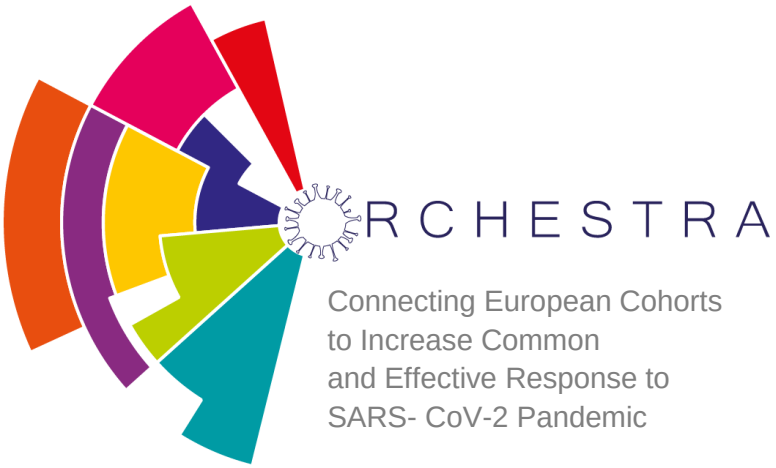

WP3 D3.5

Report on the serological immune responses of non-vaccinated infected, vaccinated, and breakthrough infected individuals

LUDWIG-MAXIMILIANS-UNIVERSITAET MUENCHEN (LMU MUENCHEN)

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Executive summary

Population-based seroprevalence studies aim to measure antibodies generated by silent or symptomatic infections and vaccination in the general population. They have the potential to follow the development of immunity and to assess the number of infections in the population. This Deliverable aims to investigate and compare the serological immune response after SARS-CoV-2 infection or/and vaccination in the general population of Munich and Luxembourg.

Based on newly, explicitly for this purpose developed diagnostic methods, SARS-CoV-2 antibodies generated by silent or symptomatic infections and/or vaccination were measured. In a unique, intra-European collaboration, results from the KoCo19 and CON-VINCE cohorts were compared to estimate the development of SARS-CoV-2 immunity in Europe.

We show that there are differences in the frequency distribution of raw antibody titres in participants from the KoCo19 and CON-VINCE cohorts. These results indicate that despite simultaneous sampling periods, the effects of potential variations in vaccination and infection timings are visibly and detectably evident through the measured lab assays.

Our findings also illustrate the effect of the Omicron variant in the general population. The results show that 96.4% / 99.6% of infected individuals experienced a breakthrough infection after the emergence of the Omicron variant in Munich / Luxembourg, compared to 3.6% / 0.4% infections in naïve subjects, respectively.

Moreover, our intra-European collaborative approach identifies a higher fraction of naïve subjects in the general population of Munich compared to Luxembourg at comparable timepoints. Subsequently, we could also show a higher number of infections in naïve subjects and breakthrough infections in Munich compared to the Luxembourg population.

Our results illustrate the serological immune response of vaccination, as well as silent and symptomatic infections in the general population of Munich and Luxembourg. While the identification of infection based solely on PCR tests might miss a high number of silent infections, our intra-European collaborative approach enables us to invest and compare the true immunity in the population. Moreover, our approach has the potential to identify non-responders to vaccination \or infection and therefore identifies a group that remains highly vulnerable.

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Abbreviations

Anti-N	Anti-Nucleocapsid antibodies
Anti-S	Anti-Spike antibodies
BAU	Binding antibody units
COI	Cut off index
CON-VINCE	COvid-19 National survey for assessing VIral spread by Non-affected CarriErs
COVID-19	Coronavirus 2019 disease
DBS	Dried Blood Spot
FU	Follow-up
KoCo19	Prospective Covid-19 cohort Munich
LIH	Luxembourg Institute of Health
rRT-PCR	Transcription-polymerase chain reaction
SARS-CoV-2	Severe acute respiratory syndrome coronavirus type 2
WHO	World Health Organization

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The serological immune response after SARS-CoV-2 infection or vaccination

Background

The severe acute respiratory syndrome coronavirus type 2 (SARS-CoV-2), which causes the coronavirus 2019 disease (COVID-19), was first reported in December 2019 in Wuhan, China. Due to its highly contagious nature, it quickly spread within China and worldwide [1]. On March 11, 2020, the World Health Organization (WHO) declared it a pandemic after more than 118,000 cases in 114 countries and 4,291 deaths [2]. Within three months, Europe became the epicenter of the pandemic, surpassing the rest of the world in reported cases and deaths, except for China [3].

In December 2020, COVID-19 vaccines were developed and initially administered to high-risk individuals such as healthcare workers, the elderly, and those with underlying medical conditions [4]. Vaccine distribution among the general population started in 2021, aiming for nationwide coverage [5].

The relevance of the relationship between reported cases and testing capacities, as well as testing policies, has become evident as the volume of data continued to grow. Consequently, studies have been conducted to assess the development of SARS-CoV-2-induced antibodies in the population, providing insight into the true rates of infections, vaccination, and immunity [6]. Seroprevalence studies using SARS-CoV-2-induced anti-Nucleocapsid or anti-Spike antibodies have been conducted, but there is heterogeneity in methodologies used, and limited evidence at the national or regional level [7].

To gain a comprehensive understanding of the SARS-CoV-2 immune response in the population, prospective-population based cohort studies like KoCo19 and CON-VINCE were initiated in Munich and Luxembourg, respectively [8,9]. This Deliverable aims to investigate and compare the serological immune response after SARS-CoV-2 infection or vaccination in the general population of Munich and Luxembourg.

Cohorts Description

The KoCo19 Cohort

A more comprehensive description of the KoCo19 cohort until follow-up 4 can be found in [8,10-12]. In short: between April 5th and June 12th, 2020 the Munich cohort of private households was randomly sampled and 5315 participants (14 years and older who gave written informed consent) were recruited. For participants younger than 18 years, informed consent was obtained from the parents as well as the participants themselves.
To date, five follow-ups were done at the following times of the pandemic:

1. Follow-up 1: December 2020, beginning of the second wave in Munich
2. Follow-up 2: March 2021, the peak of the third wave in Munich and the beginning of COVID19-vaccines availability for the general population

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3. Follow-up 3: August 2021, the end of the third wave in Munich and a vaccination rate of 68% in the general population of 14 years or older
4. Follow-up 4: November 2021, the middle of the fourth wave with Delta being the dominant variant in Munich, and
5. Follow-up 5: June 2022, the dominance of the Omicron variant in Munich

The CON-VINCE Cohort

The CON-VINCE cohort is described in more detail in [13] until Follow-up 6. In brief, between April 15th and May 4th, 2020, the CON-VINCE cohort was randomly drawn from a representative panel of Luxembourgish residents. In total, 1865 participants aged 18 years and older gave a written informed consent and were enrolled.

- Follow-up 1: May 2020, 2-week follow-up, the very beginning of the pandemic in Luxembourg, 2.4% weighted cohort seroprevalence
- Follow-up 2: May 2020, 4-week follow-up, precise tracking of the pandemic development, slow linear growth of number of SARS-CoV-2 infections
- Follow-up 3: June 2020, 6-week follow-up, Large Scale Testing programme becomes available in Luxembourg
- Follow-up 4: June 2020, 8-week follow-up, reaching 2.7% weighted cohort seroprevalence
- Follow-up 5: March 2021, the cases are growing steadily, while the two prevalent variants are the Alpha variant (B.1.1.7) and the Beta variant (B.1.351), simultaneously with the vaccination campaign ongoing actively
- Follow-up 6: April-June 2021, Large Scale Testing is still active, isolation and quarantine measures are still in place, and the weighted prevalence in the cohort is 14.8%, while 36.6% of the country population is fully vaccinated

After joining the ORCHESTRA project, a sub-cohort of the CON-VINCE cohort was invited to take part in additional analyses with the baseline visit taking place in December 2021 - January 2022. This sub-cohort is called ORCHESTRA Luxembourg and comprises 1220 participants aged 18 and older. The CON-VINCE Follow-up 7 is thus considered the initial ORCHESTRA Luxembourg visit (Baseline). To date, 2 follow-ups were done at the following times of the pandemic:

- CON-VINCE Follow-up 8/ORCHESTRA Luxembourg Follow-up 1: May-June 2022, the number of cases increasing rapidly due to the dominance of Omicron variants (BA.2, BA.5), whereas 78.8% of eligible population is vaccinated
- CON-VINCE Follow-up 9/ORCHESTRA Luxembourg Follow-up 2: December 2022 - January 2023, the rate of new infections and re-infections is low, a mix of Omicron variants is prevalent, the vaccination rate of eligible population reached 79.0%

Within the framework of the ORCHESTRA project, the KoCo19 and ORCHESTRA Luxembourg studies aligned their follow-up assessments to facilitate intra-European analysis. Specifically, KoCo19 Follow-up 4 and 5 coincided with CON-VINCE Follow-up 7 and 8. Following this, CON-VINCE conducted an additional follow-up (Follow-up 9). Furthermore, after data collection, the samples from the CON-VINCE cohort were transported to Munich and analyzed in the KoCo19 laboratory. The analyses presented in this report concentrate on these parallel follow-up assessments.

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Specimen Collection and Laboratory Analyses

Sampling and lab procedures are detailed in [8,10-12]. For the parallel follow-ups self-sampling kits for capillary blood (dry blood spot; DBS) were used. More details on DBS analysis can be found in [14,15]. For the KoCo19, if self-DBS collection was not possible, participants were invited to provide serum and DBS at the study center. After sampling, the CON-VINCE DBS cards were shipped to Munich and analyzed in the Munich lab. The Elecsys® Anti-SARS-CoV-2 anti-N (Roche) assay (referred to as Ro-N-Ig) was used for antibody detection post-infection [14]. We also employed the Elecsys® Anti-SARS-CoV-2 anti-S (Roche) assay (referred to as Ro-RBD-Ig). This differentiation was crucial for discerning infection-induced antibodies (anti-S and anti-N present) from those solely due to vaccination (only anti-S present). The DBS anti-N measurements had a positivity cutoff of 0.105, while for anti-S, the cutoff was set at 0.115.

Comparing the representative COVID-19 cohorts in Munich (KoCo19) and in Luxembourg (CON-VINCE): the serological immune response to the Delta and Omicron virus variants

Within the framework of the ORCHESTRA project, the KoCo19 and ORCHESTRA Luxembourg studies aligned their follow-up assessments to facilitate intra-European analysis. The analyses presented in this report concentrate on these parallel follow-up assessments.

Figure 1 shows the detected antibody titre (raw value distributions) generated by SARS-CoV-2 vaccines and infections in the participants of the KoCo19 and CON-VINCE cohorts.

The DBS Ro-N-Ig distributions in the CON-VINCE cohort exhibit remarkable similarity between baseline and follow-up (FU). The sole discrepancy is a second peak in the FU within the 10-100 COI (cut off index) range, suggesting potential re-infections (attributed to the absence of very high values before). Conversely, the KoCo-19 cohort displays bimodality from the baseline measurement. In the FU measurement, the second peak around 0.8 COI becomes dominant, indicating a surge in infections between the rounds. A comparable peak to CON-VINCE is evident in the 10-100 COI range, signifying re-infections. When comparing cohorts, the DBS Ro-N-Ig baseline distributions of CON-VINCE and KoCo19 almost overlap. However, in the FU measurement, the KoCo19 distribution is bimodal in the range of 0.105-0.8, with the second peak exhibiting larger values compared to CON-VINCE. This discrepancy may be attributed to more recent infections between the rounds in the KoCo19 cohort.

Analyzing baseline DBS Ro-RBD-Ig values reveals bimodality in both cohorts within the 10-1000 BAU (binding antibody units) range. The peak heights differ, with KoCo19 displaying a high left peak and a low right peak, while CON-VINCE exhibits the opposite pattern. Since Ro-RBS-Ig can arise from infection or vaccination, it appears to stem from distinct vaccination schemes, as the anti-N distributions are similar at baseline. In FU measurements, the distribution shapes are very similar, with KoCo19 presenting a peak more shifted to the right. This may be influenced by the previously described anti-N peak at FU (range 0.8 COI) and, consequently, by infections.

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Remarkably, despite simultaneous rounds, the effects of potential variations in vaccination and infection timings are visibly and detectably evident through the measured lab assays.

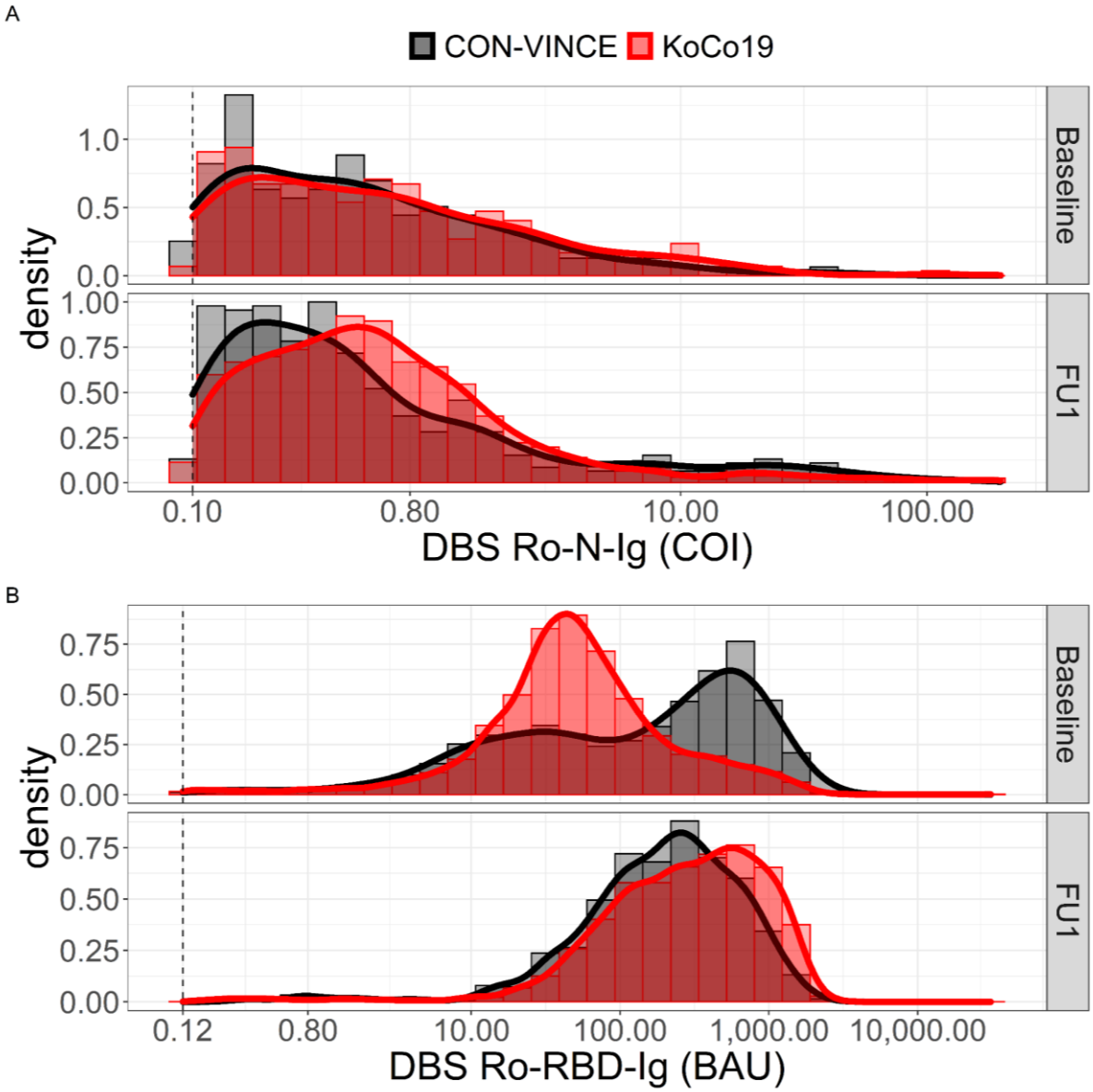

Figure 1: Frequency distribution of detected antibody titre generated by SARS-CoV-2 vaccines and infections from participants of the KoCo19 and CON-VINCE cohorts in the two aligned sampling periods. The dashed vertical lines denote the cutoff values for result classification (for DBS Ro-N-Ig and Ro-RBS-Ig 0.105 and 0.115, respectively).

Based on these results, an analysis on the determinants of antibody response is being conducted to further analyze the differences in the raw value distribution. Figure 2 describes the development of the serological immune response of participants of the KoCo19 and CON-VINCE cohorts from Baseline to Follow-up. Anti-N and anti-RBD values are depicted in the x- and y-axis, respectively.

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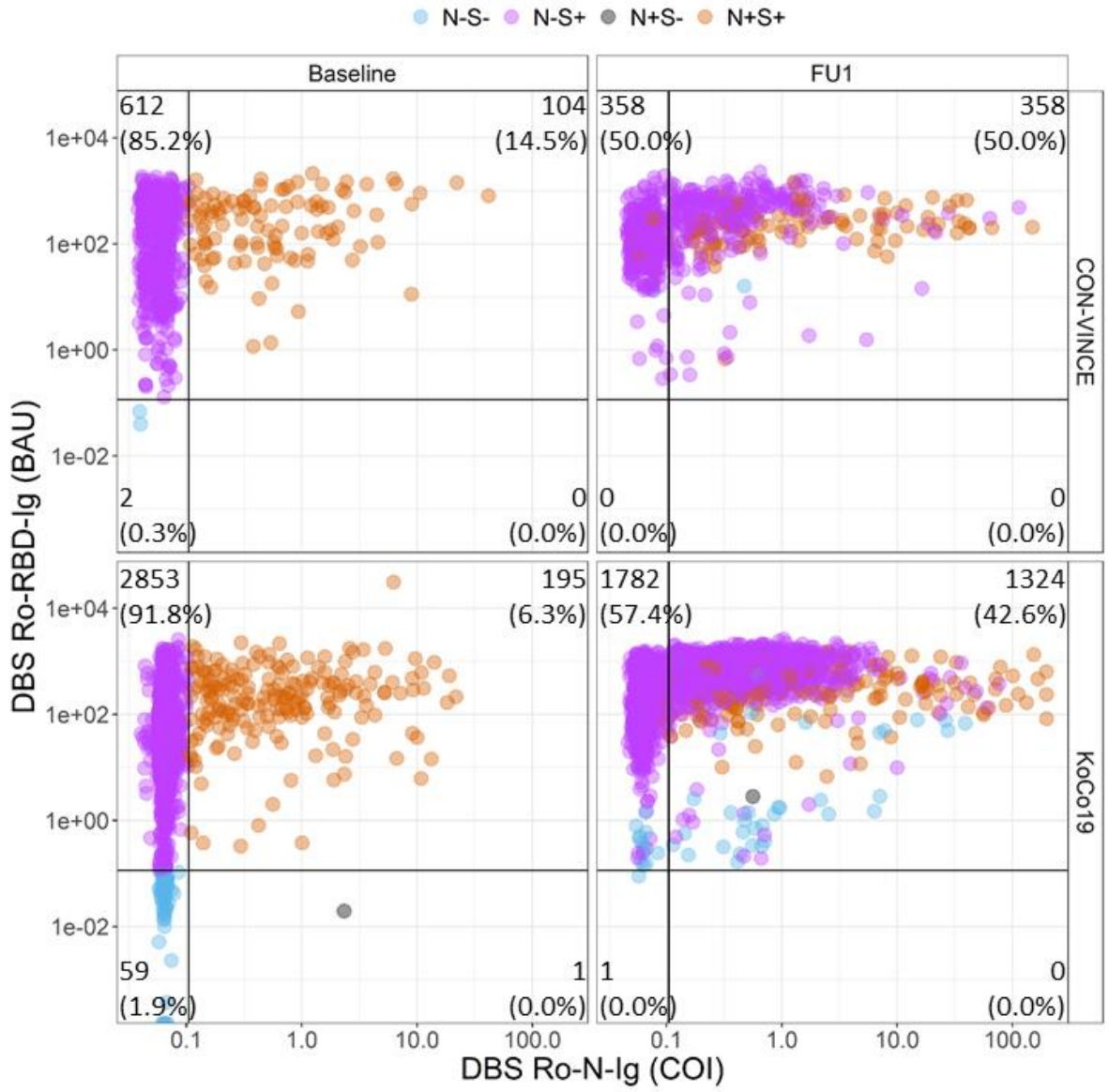

Figure 2: Scatterplot of the serological immune response of participants of the KoCo19 and CON-VINCE cohorts. The Ro-N-Ig measurement from DBS is abbreviated with “N”, Ro-RBD-Ig-quant from the same DBS is abbreviated with “S”. Positivity is represented with “+”, negative, below cutoff with “-”. The color code is defined by the status of the respective subject in the Baseline. Blue dots represent N-S-, orange dots represent N+S+, pink dots are N-S+ and gray dots are N+S-, considering the left column as reference for color-coding. **(A)** Evolution from Baseline to follow-up in the CON-VINCE cohort. Left: Baseline sampled between December 2021 and January 2022; right: follow-up sampled in between May and June 2022. **(B)** Evolution from Baseline to follow-up in the KoCo19 cohort. Left: Baseline sampled in November 2021; right: follow-up sampled in June 2022.

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From Delta to Omicron: Comparison of the Serological Immune Response over Time

For the KoCo19 participants, it can be observed that 1.9% of the participants were negative in anti-S and anti-N at baseline, indicating no serological immune response induced by infection or vaccination (SARS-CoV-2 naïve subjects). At this point, up to November 2021, the vaccination campaign was already under way for quite a long time among the Munich population. Therefore, 91.8% of the samples show a result pattern expected in vaccinated individuals, namely anti-S positive and anti-N negative. Moreover, 6.3% of samples show a pattern expected of infected individuals with both values positive, while one individual was only positive in anti-N, but negative in anti-S. This pattern is representing a group being either an anti-S non or late-responder after infection, or a false positive value for anti-N, or a false negative for anti-S. It is likely to be individuals shortly after acute infection which are not yet positive in anti-S but already show a beginning anti-N reactivity.
This is also supported by the fact that this individual was found to be anti-N and anti-S positive in the follow-up. The amount of participants still naïve to both SARS-CoV-2 vaccination and infection decreased to one individual. With the advent of Omicron, a massive horizontal shift of the data points can be observed: the proportion of anti-N positives increased dramatically to 42.6%, with 86.4% of newly positives (96.4% breakthrough infections, 3.6% infections in naïve subjects).
In parallel, the anti-N+/anti-S- individual migrated to the double positive fraction, supporting that this subject was only at the beginning of his seroconversion and developed his anti-S titre after baseline. The proposition of only vaccinated subjects (anti-S positive but anti-N negative) decreased to 57.4%.

Similar trends can be observed for the participants of the CON-VINCE cohort. Only 0.3% of the participants were negative in anti-S and anti-N at Baseline, indicating naïve subjects. Most participants, 85.2%, show anti-S positive and anti-N negative results, a pattern expected in vaccinated but uninfected individuals. 14.5% of participants show results positive in both values.
Similar to the results shown in the KoCo19 participants, after the emergence of Omicron in the Luxembourg population, a massive horizontal shift can be observed with 50.0% of participants seroconverting to anti-N positive results, with 74.9% of newly positives (99.6% of these breakthrough infections, 0.4% infections in naïve subjects - one individual).

From Delta to Omicron: Comparison of the Serological Immune Response in the Population

While 1.9% of the KoCo19 population was still anti-N and anti-S negative in November 2021, therefore not immunized by vaccination or infection, this applies only to 0.3% of the CON-VINCE population at a comparable time point, indicating a lower group immunity in the KoCo19 compared to the CON-VINCE population. At the same time, the fraction of anti-S positive/anti-N negative individuals was 91.8% in KoCo19 but 85.2% in CON-VINCE participants, respectively, indicating a higher vaccination rate in the KoCo19 cohort compared to the CON-VINCE cohort. Moreover, 6.3% and 14.5% of the samples collected within KoCo19 and CON-VINCE were anti-N positive, visualizing a higher fraction of the CON-VINCE population that underwent an infection compared to the KoCo19 population at a comparable time point.

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When comparing the serological immune response in the KoCo19 and CON-VINCE population after the emergence of Omicron, it can be observed that the number with anti-N positive individuals drastically increased simultaneously to 42.6% in KoCo19 and 50% in CON-VINCE. While 57.4% of the cohort population remain anti-S positive but anti-N negative in KoCo19, this fraction is lower in CON-VINCE, with 50% of the population being immunized but naïve to infection. The fraction of double negative samples disappeared in both the cohorts (with only one case in the KoCo19).

Discussion

In this report, we present the serological immune response in the general population of Munich and Luxembourg. To assess the development of antibodies induced by SARS-CoV-2 infection and vaccination, the members of the representative, population-based KoCo19 and CON-VINCE cohorts were asked multiple times to donate blood for epidemic surveillance purposes. In a unique, intra-European collaboration, results from the KoCo19 and CON-VINCE cohorts were compared to estimate the development of SARS-CoV-2 immunity in Europe.

The findings presented here show that there are differences in the frequency distribution of raw antibody titres in participants from the KoCo19 and CON-VINCE cohorts. These results indicate that despite simultaneous rounds, the effects of potential variations in vaccination and infection timings are visibly and detectably evident through the measured lab assays.

Our results also show the evolution of antibody titres from November 2021 to June 2022. Our findings illustrate the effect of the Omicron variant in the general population with a major increase of anti-N seropositivity from baseline to follow-up. Based on this, our research enables us to visualize and therefore assess the serological immune response of breakthrough infections and infections in naïve subjects. Based on this, we could show that 96.4% / 99.6% of infected individuals experienced a breakthrough infection after the emergence of the Omicron variant in Munich / Luxembourg, compared to 3.6% / 0.4% infections in naïve subjects, respectively.

Moreover, our intra-European collaborative approach identifies a higher fraction of naïve subjects in the general population of Munich compared to Luxembourg at baseline. Subsequently, we could also show a higher number of breakthrough infections and infections in naïve subjects in Munich compared to the Luxembourg population.

Outlook

Our results illustrate the serological immune response of vaccination, as well as silent and symptomatic infections in the general population of Munich and Luxembourg. While the identification of infection based on PCR tests might miss a high number of silent infections, our intra-European collaborative approach enables us to invest and compare the true immunity in the population. Moreover, our approach has the potential to illustrate non-responders to vaccination and infection and therefore identifies a group that remains highly vulnerable.

Major strengths of our analysis are its population-based approach, the high number of participants, as well as the thorough validation of the assays used. Moreover, the intra-European collaboration enables us to use homogeneous testing and therefore to compare the results from two cohorts, representative

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for the population in Munich and Luxembourg. Based on this collaborative research, the identification of breakthrough infections in population-based cohorts was further investigated *in Deliverable 3.6 (submitted in November 2023).* The results presented in both Deliverables will be published in peer-reviewed journals..

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