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Research article

Prevalence of SARS-CoV-2 antibodies at the University hospital Heidelberg and correlation with SARS-CoV-2 incidence

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ABSTRACT

Background: Since the onset of the SARS-CoV-2 pandemic in late 2019, many studies suggest that actual infection rates may far exceed reported cases. Therefore, this study provides a comprehensive overview of anti-SARS-CoV-2 antibody measurements and their significance in epidemiological surveillance, especially regarding the true extent of SARS-CoV-2 infections, allowing more detailed insights into the dynamics of the pandemic.

Methods: Antibodies were measured using the Elecsys® Anti-SARS-CoV-2 assay for the nucleocapsid (N) protein and the SARS-CoV-2 IgG (sCOVG) assay for the spike (S) protein. A total of 25197 specimens from University Hospital Heidelberg were analyzed between May 2020 and December 2023, with 16957 samples measured for both antibodies, 2756 for anti-N only, and 5484 for anti-S only.

For the epidemiological tracing of the SARS-CoV-2 incidence we analyzed changes in the anti-N positivity rate and anti-S positivity rate within our tertiary hospital setting across consecutive quarters.

Results: Anti-N measurements allowed for a retrospective analysis of SARS-CoV-2 epidemiological developments, revealing significant changes ($p < 0.05$) in the anti-N positivity rate following incidence peaks (increase) and low incidence periods (decrease) with only three non-significant transitions.On the other hand, the anti-S positivity rate showed only four significant transitions between consecutive quarters.

Conclusion: The anti-N positivity rate is an effective and straightforward retrospective serological tool for tracking epidemiological trends, while the anti-S rate is influenced by vaccination and epidemiology as well, making it inept for the tracing of epidemiological changes. Although the recent development in SARS-CoV-2 epidemiology with increasing intervals between infection waves already provide an enhanced statistical robustness, we recommend the determination of the anti-N positivity rate in larger study populations.

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1. Introduction

At the end of 2019, SARS-CoV-2 (and COVID-19) was identified as the cause of numerous cases of pneumonia in Wuhan, China [1]. Due to the rapid spread of the virus and its high mutation rate, the epidemic in China quickly evolved into a global pandemic, with almost 800 million confirmed COVID-19 cases [2]. However, since only a small fraction of acute SARS-CoV-2 infections are diagnosed and reported, the estimated number (dark figure) of SARS-CoV-2 infections could be >10 times higher, according to seroprevalence studies [3–6].

Therefore, serological investigations focused on antibody detection to assess B-cell-mediated humoral immune response, might provide a more accurate epidemiological tracking of SARS-CoV-2 infections. By determining various epitope-specific antibodies, both past infections (by measuring anti-Nucleocapsid-specific antibodies) and vaccine effectiveness (by determining anti-Spike antibodies in combination with negative anti-Nucleocapsid-specific antibodies) can be assessed. Epitope-specific IgM and IgG tests are commonly used for serological investigations of SARS-CoV-2. ELISA- or ECLIA-based test systems serve as the gold standard for these investigations, which were also used in this study. IgM antibodies are detectable more rapidly and decline more quickly below the detection threshold compared to IgG antibodies, allowing primarily for the detection of acute or subacute infection [7]. The diagnostic window for detecting IgG antibodies can last up to 14 days, but these antibodies remain detectable for a longer duration [8,9]. For example, spike IgG antibodies can remain detectable for at least one year [10]. The concentration of IgG peaks depends on disease severity, duration of infection, age, genetic factors, and comorbidities, reaching its peak 4–5 weeks after infection recovery, after which antibody titers decline continuously [11].

Accordingly, our group not only performed SARS-CoV-2 PCR testing and genome sequencing in compliance with governmental regulations for molecular genetic surveillance of SARS-CoV-2 (CorSurV), but also tracked the development of SARS-CoV-2 anti-Nucleocapsid (anti-N) and anti-Spike (anti-S) seroprevalence in patients and employees at the University Hospital Heidelberg from May 2020 until the end of 2023.

The original framework of CorSurV was established by the Robert Koch Institute (RKI) in 2020, and included testing of symptomatic (or those with suspected SARS-CoV-2 exposure based on medical history) patients, as well as pre-symptomatic and asymptomatic individuals in epidemiologically significant settings, such as outbreak tracing, nosocomial infection prevention, and educational institutions [12].

In addition to detecting previously undiagnosed SARS-CoV-2 infections primarily through the measurement of anti-N, monitoring anti-S levels allowed for the evaluation of vaccination efficacy/success. This enabled risk assessments for lower response rates to booster vaccinations, as well as the potential for more severe long-COVID symptoms and reduced lung function [13,14].

2. Material and methods

2.1. Study protocol

This research was conducted at the University Hospital Heidelberg and the Heidelberg University, Department of Infectious Diseases, Virology.

For the seroprevalence study, our group utilized the Elecsys® Anti-SARS-CoV-2 assay by Roche for the qualitative detection of antibodies targeting the nucleocapsid (N) protein [15] and the SARS-CoV-2 IgG (sCOVG) assay by Siemens for the quantification of antibodies targeting the spike (S) protein [16].

The study protocol received approval by the local ethics committee in accordance with the Declaration of Helsinki (S-280/2024).

2.2. Antibody measurements

The antibody measurements were conducted between May 2020 and December 2023, encompassing a total of 25197 specimen collected from patients and employees of the University Hospital Heidelberg. Since our diagnostics unit primarily analyzed samples from sick (admitted to clinical care) patients, the resulting study population is expected to represent a clinically relevant demographic – which should be more relevant, due to Germany, as of October 2021, basing its pandemic countermeasures on the hospitalization incidence rather than the incidence in the overall population. To ensure a comprehensive overview of anti-SARS-CoV-2 seroprevalence in a tertiary care hospital setting, we did not impose any preliminary exclusions based on age, underlying medical conditions, or immune status. Hence, our specimen pool includes a diverse range of patients, such as uninfected individuals, sub-acute cases, asymptomatic patients, and those in the recovery phase, as well as high-risk groups (primarily immunocompromised individuals) and employees who have completed their vaccination schedules. Additionally, it includes immunocompromised patients undergoing monitoring for delayed antibody induction. Patients with acute SARS-CoV-2 infection were tested using RT-PCR rather than antibody testing, given the appropriate diagnostic window for detecting active infection [17,18]. 16957 samples were assessed for anti-N and anti-S levels, 2756 samples were assessed only for anti-N response, and 5484 were assessed only for anti-S levels. Before 2021, no anti-S test had been available or established in our laboratory. Consequently, the first anti-S measurements began in Q1 2021. Since the vaccination program for employees and high-risk patients (such as immunocompromised individuals) at the University Hospital Heidelberg was also initiated in early 2021, the first vaccination-related measurements were performed in the final weeks of Q1 2021.

For both assays, values ≥1.0 were considered positive. The introduction of the WHO standard NIBCS Code 20–136 allowed the conversion of quantitative titer values of anti-S antibody levels across different assay manufacturers into binding antibody units (BAU/ml). The conversion rate for the anti-SARS-CoV-2 IgG (sCOVG) assay (Siemens) is 21.8, hence values <21.8 BAU/ml were considered

negative.

Anti-S levels were measured 4–8 weeks after the second vaccination (and thus 8–12 weeks after the initial vaccination) to evaluate the antibody response induced by vaccination. The vaccination schedule followed the guidelines issued by the Standing Committee on Vaccination (STIKO) of the RKI [19]. The timing of the antibody measurements was based on recommendations from previous studies on SARS-CoV-2 antibodies [20,21].

To test for significance, a chi-square test with Yates' correction was applied, and p-values <0.05 were considered statistically significant.

3. Results

Out of the 25197 specimens, 9172 were from male patients (54.0±19.66 years), 10077 from female patients (mean age 48.0±18.10 years), and 5948 from individuals with unknown gender and/or age. Another classification was based on patient and employee status. 20622 specimens were from patients (53.0±19.72 years) and 4575 from employees (40.0±13.27 years).

An overview of the antibody measurement results from the study population is provided in Table 1.

The test results can be categorized into two distinct sets. The first set includes all anti-N measurements, which were used to retrospectively trace the epidemiologic development of SARS-CoV-2 from Q2 2020 to Q4 2023.

Table 2 presents the results of the anti-N measurements, along with the significance of changes between consecutive quarters. The chi-square test with Yates' correction was employed to evaluate significance, with p-values <0.05 indicating statistical significance.

With the exception of the transitions between Q3 and Q4 2021, Q1 and Q2 2023, as well as Q2 and Q3 2023, all changes in the anti-N positivity rate between consecutive quarters, whether increases or decreases, yielded statistically significant results in the chi-square test with Yates' correction ($p<0.05$). Consequently, shifts in incidence patterns - such as peaks versus low incidence periods - can be effectively traced through retrospective of the relative anti-N positivity rate in tertiary care hospitals evaluations (antibody responses show a time delay of 6–12 weeks compared to the PCR-based/epidemiologic incidence numbers). Further details on the relevance of these findings and their implications are discussed in the corresponding discussion and conclusion sections.

The second set of results tracks the development of the anti-S positivity rate. Since the anti-S test platform was not established until early 2021, statistical evaluation began with the transition between Q1 2021 and Q2 2021. Table 3 presents the results of the anti-S measurements, along with the significance of changes between consecutive quarters. The chi-square test with Yates' correction was employed to evaluate significance, with p-values <0.05 indicating statistical significance.

Compared to the development of the anti-N positivity rate, the anti-S positivity rate exhibited more periods with non-significant changes. Significant shifts were observed during the transitions from Q2 to Q3 2021, Q2 to Q3 2022, and Q3 to Q4 2023. All other periods showed no significant changes.

Table 4 presents a comparison of the anti-N and anti-S positivity rates between patients and employees. The chi-square test with Yates' correction was employed to evaluate significance, with p-values <0.05 indicating statistical significance.

As previously mentioned, the measurements from 2020 were primarily part of the test validation process and are therefore not representative of the general population's antibody positivity rates. When examining the employees' results, it is evident that, apart from Q3 2020 (for anti-N) and Q2 2021 (for anti-S), the number of tests conducted was considerably lower (<150 tests per quarter) compared to the patient group. This is also reflected in the significantly lower chi-square values compared to those in Tables 2 and 3

4. Discussion

Anti-S measurements are primarily used to assess antibody production following vaccination, consistently displaying high positivity rates since their introduction. In contrast, anti-N antibody measurements serve as reliable serological markers for detecting prior SARS-CoV-2 infections [22].

To evaluate the anti-N and anti-S antibody measurement results and their usefulness as tools for epidemiological tracing of SARS-CoV-2 infections, it is essential to first consider the history of SARS-CoV-2 infections in Central Europe.

In 2020 and 2021, the RKI tracked the demographic progression of SARS-CoV-2 infections [23], with the results presented in Fig. 1.

Fig. 1 highlights the highest incidence peaks in early Q2 2020, mid to late Q4 2020, early to mid Q1 2021, early to mid Q2 2021, mid Q3 2021, and the second half of Q4 2021. Considering a six-week delay between infection and a robust antibody response, we would expect increased antibody positivity rates in late Q2 2020, early Q1 through late Q2 2021, and early Q4 2021 through mid Q1 2022. However, since anti-N antibody testing in 2020 was primarily part of the test validation process, the positivity rates from that period are not representative of the general population and will not be included in this evaluation. Consequently, it is not possible to correlate the incidence peaks from Q2 and Q4 2020, and the absence of a baseline anti-N positivity rate in Q4 2020 prevents the statistical evaluation of Q1 2021.

Therefore, we began the evaluation by using Q1 2021 as the baseline for the anti-N antibody positivity rate in the consecutive quarter. With Q1 2021 showing a prolonged incidence peak extending into mid-quarter, we expected - and indeed observed - a significant increase in the anti-N positivity rate in early Q2 2021.

Incidence rates in Q2 were considerably lower than in Q1 2021, corresponding with a notable decrease in the anti-N positivity rate. Q3 showed similar infection dynamics to Q2 2021, leading to a non-significant change in the transition from Q3 to Q4 2021. However, the incidence surged to new peak levels in Q4 2021, resulting in a significant increase in the anti-N positivity rate by Q1 2022.

The anti-S antibody testing platform was diagnostically established in 2021, making Q1 2021 the first baseline quarter. While Q1 and Q2 2021 mainly focused on measuring antibody levels in employees and high-risk patients to assess vaccination success, the anti-S

Table 1
Results of antibody measurements.

Time	N/						N -								N +								Total
	S -	%	S +	%	Total	%	S/	%	S -	%	S +	%	Total	%	S/	%	S -	%	S +	%	Total	%	
Q2 2020							956	100.0					956	78.0	270	100.0					270	22.0	1226
Q3 2020							211	97.7	3	1.4	2	0.9	216	49.0	225	100.0					225	51.0	441
Q4 2020							101	100.0					101	82.8	21	100.0					21	17.2	122
Q1 2021	6	54.5	5	45.5	11	0.3	568	16.2	479	13.7	2452	70.1	3499	94.2	66	32.0	5	2.4	135	65.5	206	5.5	3716
Q2 2021	19	0.5	3860	99.5	3879	56.3	68	2.8	1052	43.1	1321	54.1	2441	35.5	20	3.5	43	7.6	501	88.8	564	8.2	6884
Q3 2021	47	10.0	425	90.0	472	20.4	36	2.3	576	36.4	972	61.4	1584	68.4	11	4.2	19	7.3	231	88.5	261	11.3	2317
Q4 2021	23	13.5	148	86.5	171	6.9	40	2.0	541	27.6	1379	70.4	1960	78.9	15	4.2	18	5.1	321	90.7	354	14.2	2485
Q1 2022	9	11.5	69	88.5	78	2.6	36	1.6	507	21.9	1773	76.6	2316	77.5	9	1.5	41	6.9	545	91.6	595	19.9	2989
Q2 2022	5	8.3	55	91.7	60	3.7	14	1.3	181	17.0	869	81.7	1064	64.8	22	4.3	37	7.2	458	88.6	517	31.5	1641
Q3 2022	8	5.9	128	94.1	136	12.5	13	2.6	64	12.9	420	84.5	497	45.7	5	1.1	20	4.4	430	94.5	455	41.8	1088
Q4 2022	19	9.4	183	90.6	202	24.8	3	1.1	37	13.1	243	85.9	283	34.7	7	2.1	11	3.3	313	94.6	331	40.6	816
Q1 2023	17	7.4	213	92.6	230	40.9	6	5.3	11	9.6	97	85.1	114	20.2	16	7.3	9	4.1	194	88.6	219	38.9	563
Q2 2023	5	10.0	45	90.0	50	16.6	2	2.5	8	9.9	71	87.7	81	26.8	6	3.5	5	2.9	160	93.6	171	56.6	302
Q3 2023	1	0.8	121	99.2	122	32.1	4	3.8	9	8.7	91	87.5	104	27.4	2	1.3	7	4.5	145	94.2	154	40.5	380
Q4 2023			73	100.0	73	32.2	1	2.8	2	5.6	33	91.7	36	15.9	2	1.7			116	98.3	118	52.0	227
Total	159	2.9	5325	97.1	5484	21.8	2059	13.5	3470	22.8	9723	63.7	15252	60.5	697	15.6	215	4.8	3549	79.6	4461	17.7	25197

/antibody measurement not performed.
+ positive antibody measurement (COI ≥1.0).
- negative antibody measurement (COI <1.0).

Table 2
Development of the anti-N positivity rate.

Time	N +	%	N -	%	Total	χ2 value	p value
Q2 2020	270	22.0	956	78.0	1226		
Q3 2020	225	51.0	216	49.0	441	129.2	<0.00001
Q4 2020	21	17.2	101	82.8	122	43.0	<0.00001
Q1 2021	206	5.6	3499	94.4	3705	26.7	<0.00001
Q2 2021	564	18.8	2441	81.2	3005	283.7	<0.00001
Q3 2021	261	14.2	1584	85.9	1845	17.0	0.00004
Q4 2021	354	15.3	1960	84.7	2314	1.0	0.32
Q1 2022	595	20.4	2316	79.6	2911	22.6	<0.00001
Q2 2022	517	32.7	1064	67.3	1581	82.0	<0.00001
Q3 2022	455	47.8	497	52.2	952	56.6	<0.00001
Q4 2022	331	53.9	283	46.1	614	5.3	0.021
Q1 2023	219	65.8	114	34.2	333	12.0	0.00054
Q2 2023	171	67.9	81	32.1	252	0.2	0.66
Q3 2023	154	59.7	104	40.3	258	3.3	0.068
Q4 2023	118	76.6	36	23.4	154	11.6	0.00067
Total	4461	22.6	15252	77.4	19713		

Table 3
Development of the anti-S positivity rate.

Time	S +	%	S -	%	Total	χ2 value	p value
Q2 2020							
Q3 2020	2	40.0	3	60.0	5		
Q4 2020							
Q1 2021	2592	84.1	490	15.9	3082		
Q2 2021	5682	83.6	1114	16.4	6796	0.3	0.56
Q3 2021	1628	71.7	642	28.3	2270	153.3	<0.00001
Q4 2021	1848	76.0	582	24.0	2430	11.2	0.00082
Q1 2022	2387	81.1	557	18.9	2944	19.9	<0.00001
Q2 2022	1382	86.1	223	13.9	1605	18.1	0.000021
Q3 2022	978	91.4	92	8.6	1070	16.8	0.000041
Q4 2022	739	91.7	67	8.3	806	0.02	0.89
Q1 2023	504	93.2	37	6.8	541	0.8	0.37
Q2 2023	276	93.9	18	6.1	294	0.06	0.80
Q3 2023	357	95.5	17	4.5	374	0.5	0.46
Q4 2023	222	99.1	2	0.9	224	4.9	0.026
Total	18597	82.9	3844	17.1	22441		

positivity rate in the subsequent quarters followed a similar trend to the anti-N positivity rate, with a decrease in Q3 and increases in Q4 2021 and Q1 2022.

From Q4 2021 to Q2 2022, the emergence of the Omicron variant replaced all other circulating variants, driving incidence numbers to unprecedented levels, at least in terms of PCR-confirmed SARS-CoV-2 infections. However, since Omicron was found to cause milder disease compared to previous variants of concern (VOCs), the RKI halted individual infection tracing in 2022. This led to a significant drop in the number of PCR tests performed, reducing the usefulness of PCR testing for epidemiological surveillance [24]. Instead, wastewater surveillance, which tracks SARS-CoV-2 viral loads in sewage, became a key method for monitoring viral spread [25], as illustrated in Fig. 2.

Fig. 2 also shows the incidence peak for Q4 2021, allowing for a comparison of the peak heights between the two figures. In 2022, the incidence peaks are observed primarily in mid to late Q1, early Q3, and at lower levels in Q3 to Q4 and late Q4. In 2023, the incidence peaks in mid to late Q1 and mid to late Q4.

Regarding the anti-N positivity rate, we anticipated (and observed) a significant increase from Q1 to Q2 2022, with a further significant rise in Q3 due to the incidence peak in early Q3 2022. The smaller peak in Q3 to Q4 2022 led to only a slight, yet significant, increase in the anti-N positivity rate in Q4 2022. The prolonged incidence peak in late Q4 2022 and early Q1 2023 resulted in a significant increase in the antibody positivity rate in Q1 2023. However, despite the higher and longer incidence peak in Q1, there was no significant increase in the positivity rate for Q2 2023. The incidence in Q2 2023 was notably lower compared to previous periods, leading to a non-significant reduction in the antibody positivity rate in Q3. Conversely, the increased incidence in Q3 and early Q4 2023 resulted in a significant rise in the positivity rate for Q4. It seems plausible that Q1 2024 will show a significant increase in the anti-N positivity rate due to the high incidence peak observed in mid to late Q4 2023.

Therefore, our findings suggest that the anti-N positivity rate provides a robust method for evaluating epidemiological incidence trends using serological measurements of anti-N levels. Even in cases where changes in the anti-N positivity rate were not statistically significant, the expected patterns of increase or decrease could still be reliably identified.

From mid-2021 to the end of 2023, the anti-S positivity rate in the general population consistently increased, primarily driven by

Table 4
Comparison of antibody positivity rate in patients and employees.

Time	Patients						Employees						Significance N		Significance S	
	N +	%	Total	S +	%	Total	N +	%	Total	S +	%	Total	χ2 value	p value	χ2 value	p value
Q2 2020	270	22.0	1226													
Q3 2020	218	89.3	244				7	3.6	197	2	40.0	5	317.6	<0.00001		
Q4 2020	10	11.6	86				11	30.6	36				5.1	0.023		
Q1 2021	176	4.8	3653	2568	84.4	3044	30	57.7	52	24	63.2	38	263.0	<0.00001	22.3	<0.00001
Q2 2021	502	17.2	2920	1796	62.3	2885	62	72.9	85	3886	99.4	3911	164.7	<0.00001	1665.4	<0.00001
Q3 2021	255	14.0	1815	1515	70.3	2154	6	20.0	30	113	97.4	116	0.4	0.51	38.5	<0.00001
Q4 2021	342	15.0	2287	1806	75.7	2386	12	44.4	27	42	95.5	44	15.7	0.000074	8.2	0.0042
Q1 2022	582	20.2	2879	2345	80.8	2902	13	40.6	32	42	100.0	42	6.9	0.0086	6.8	0.0092
Q2 2022	511	32.7	1563	1367	86.0	1589	6	33.3	18	15	93.8	16	0.04	0.85	0.3	0.60
Q3 2022	443	47.7	928	953	91.2	1045	12	50.0	24	25	100.0	25	0.0001	0.99	0.3	0.59
Q4 2022	320	53.7	596	723	91.5	790	11	61.1	18	16	100.0	16	0.1	0.70	0.004	0.95
Q1 2023	216	66.9	323	499	93.1	536	3	30.0	10	5	100.0	5	4.3	0.037	0.02	0.90
Q2 2023	169	67.6	250	275	93.9	293	2	100.0	2	1	100.0	1	0.3	0.56	1.15	0.28
Q3 2023	119	71.3	167	269	94.4	285	35	38.5	91	88	98.9	89	25.0	<0.00001	2.2	0.14
Q4 2023	110	76.4	144	212	99.1	214	8	80.0	10	10	100.0	10	0.02	0.90	0.9	0.34
Total	4243	76.4	19081	14328	79.1	18123	218	34.5	632	4269	98.9	4318				

N/S + number of positive antibody measurements (anti-N or anti-S).
% antibody positivity rate.
Total sum of positive and negative antibody measurements.

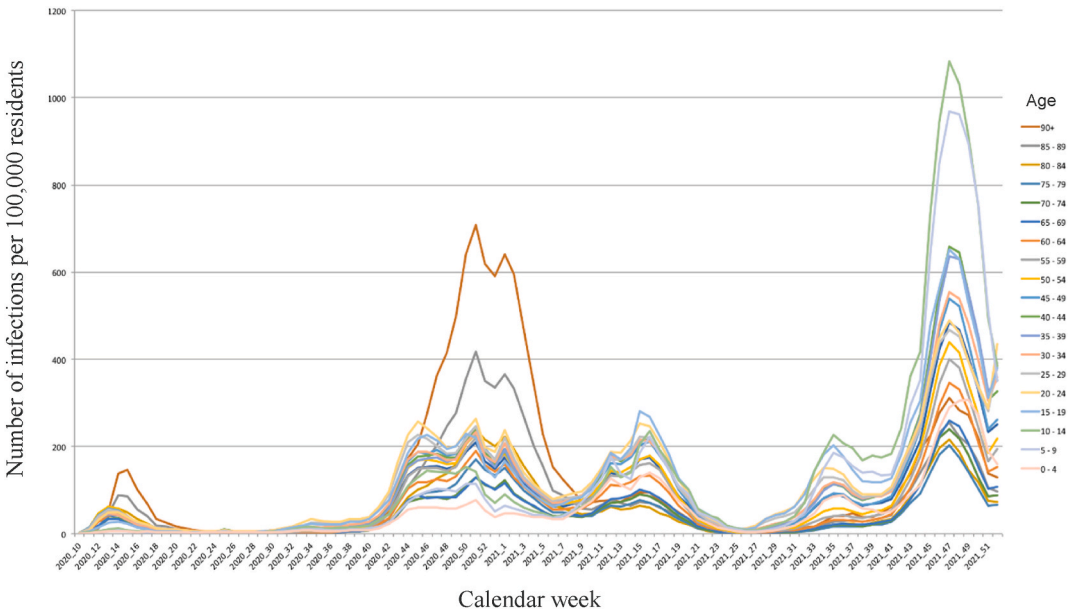

Fig. 1. SARS-CoV-2 infections per week in 2020 and 2021 [23].

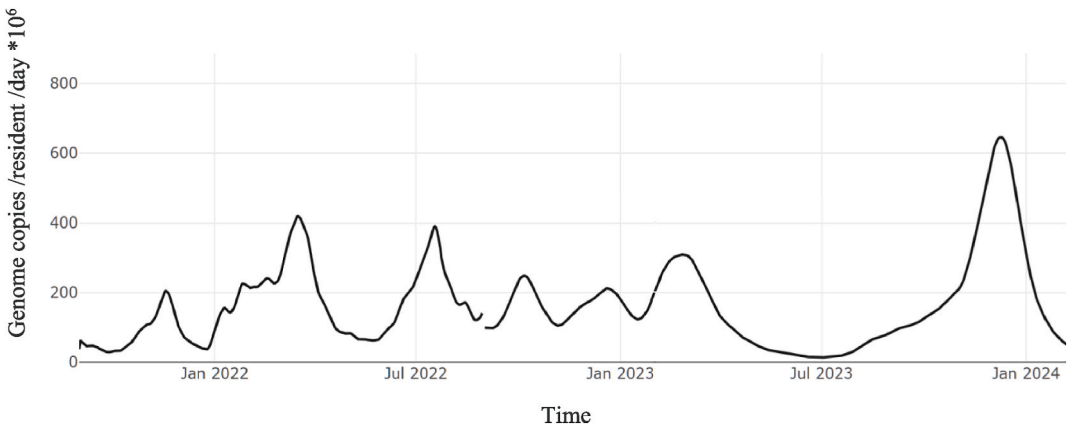

Fig. 2. Austrian waste water surveillance data [26].

federal vaccination programs and ongoing incidence peaks. The increased positivity rates were statistically significant until mid-2022. However, from the second half of 2022 onward, most increases in positivity rates (except for Q4 2023) became statistically insignificant. The main issue affecting the significance of the anti-S (and anti-N) positivity rate is likely the already high baseline positivity rate, as already noted by the RKI [23].

In comparing patient and employee measurement results, a major issue is the significant disparity in the number of tests conducted between the two groups, with an overall ratio of approximately 1:30 for anti-N and 1:4 for anti-S, favoring the patient group. This discrepancy arises from differing testing strategies: anti-N was included in the initial admission screening for patients, while for employees, tests were conducted after recovery from SARS-CoV-2-like illnesses. This led to higher anti-N positivity rates in the employee group until the end of 2023, with only two outliers in Q1 and Q3 2023. The significance of these results largely depended on the number of tests conducted in each timeframe and should therefore be taken with a pinch of salt.

No significant difference in the anti-S positivity rate was detected from Q4 2021 until the end of 2023. The variation observed from Q1 through Q3 2021 can be attributed to the vaccination strategy implemented at the University Hospital Heidelberg. In Q1 2021 high-risk patients were vaccinated before employees, which explains the initial differences (higher patient numbers in Q1). By Q2 and Q3 2021, anti-S seroprevalence was significantly higher in the employee group. This could be due to either a higher rate of antibody induction (immunocompetent individuals) or the targeted testing of vaccinated individuals, while anti-S in patients was also assessed in non-vaccinated individuals as part of the admission screening. Most likely, it is a combination of both factors, as immunocompromised patients often required additional vaccinations to produce adequate antibody levels [27].

5. Conclusion

Our results indicate that the anti-N positivity rate is a reliable method for evaluating epidemiological trends through straightforward retrospective analyses of serological data. Even nonsignificant changes in this rate helped identify expected increases or decreases, facilitating the assessment of SARS-CoV-2 incidence in the preceding quarters.

Since our measurements were performed in a tertiary care hospital setting, our anti-N positivity rate wasn't influenced as much by fluctuating test numbers due to changes in public health policy as well as due to increased/decreased public interest in serological SARS-CoV-2 results compared to the outpatient sector.

In contrast, the anti-S positivity rate, likely influenced by vaccination, is less suitable for tracking epidemiological progression due to numerous nonsignificant changes between quarters. It is better suited for evaluating individual antibody responses post-vaccination.

Factors contributing to nonsignificant results in the anti-N positivity rate include persistently high positivity since Q4 2022 and decreased public testing interest following the rise of the Omicron variant, which has altered cell targeting and reduced complications. To improve statistical power, serological measurements should be conducted in larger populations. A recent positive development is the longer intervals between infection waves, allowing for better tracking of incidence fluctuations as reflected in changes to the anti-N positivity rate.

5.1. Outlook/Future Scope

Currently, it appears that SARS-CoV-2 is establishing a pattern similar to influenza, with less frequent incidence peaks, as seen in 2023 and 2024 [26]. This trend should enable a more accurate and statistically significant assessment of the epidemiological situation based on anti-N seroprevalence levels. Consequently, our group plans to continue the anti-N seroprevalence project by analyzing data from 2024 to determine if the less frequent incidence peaks facilitate more robust epidemiological tracing of SARS-CoV-2.

Limitations of the study

Since our measurements were conducted in a tertiary care hospital setting, our study was less affected by fluctuations in test numbers related to changes in public health policy and varying public interest in serological SARS-CoV-2 results compared to outpatient settings. However, we cannot entirely rule out the possibility of population bias, as we did not implement any preliminary exclusions based on age, underlying medical conditions, or immune status.

Because we focused primarily on patients with clinically relevant conditions (admittance to the hospital) for the anti-N positivity rate, our measurements reflect seropositivity in individuals with more severe COVID-19 symptoms (or other illnesses) compared to the general population. Consequently, while our analysis is not representative of the general population, it provides valuable insights within a clinically relevant context, especially with the hospital-based incidence rate for determining necessary countermeasures in Germany, which was established in October 2021. Additionally, our non-quantitative correlation aligns well with the broader epidemiological trends observed in the general population.

At the onset of the SARS-CoV-2 pandemic, more severe disease courses generated high public interest. However, the currently circulating variants are associated with milder disease courses, leading to reduced public interest in SARS-CoV-2 and a subsequent decline in testing numbers, particularly since the end of 2022. This decrease in testing has led to less significant results [28,29].

CRediT authorship contribution statement

C. Bundschuh: Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **N. Weidner:** Writing – review & editing, Formal analysis. **T.F.M. Scholz:** Writing – review & editing, Formal analysis. **S. Parthé:** Writing – review & editing, Formal analysis. **L. Jost:** Writing – review & editing, Formal analysis, Conceptualization. **E. Gößnitzer:** Writing – review & editing, Formal analysis, Conceptualization. **H.G. Kräusslich:** Writing – review & editing, Validation, Resources, Methodology, Formal analysis.

Ethics and consent

The study protocol received approval by the Heidelberg University's ethics committee in accordance with the Declaration of Helsinki (S-280/2024) on May 21, 2024.

Since this an anonymized, retrospective survey with approximately 26,000 values, obtaining the consent of the respective participants is a disproportionately high effort.

Since the (final) evaluation of the data is carried out only in an anonymized form (only the examination results, age, and gender of the participants are analyzed), the personal rights of the participants are not affected.

Therefore, the informed consent of the participants has been waived.

Data availability

Will be made available on request.

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Declaration of competing interest

None declared.

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