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Testing for SARS-CoV-2 seroprevalence: experiences of a tertiary eye centre

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ABSTRACT

Introduction The actual prevalence of a SARS-CoV-2 infection and the individual assessment of being or having been infected may differ. Facing the great uncertainty—especially at the beginning of the pandemic—and the possibility of asymptomatic or mildly symptomatic, subclinical infections, we evaluate the experience of SARS-CoV-2 antibody screening at a tertiary clinical setting.

Methods and analysis All employees of a tertiary eye centre and a research institute of ophthalmology were offered antibody testing in May 2020, using a sequential combination of different validated assays/antigens and point-of-care (POC) testing for a subset (NCT04446338). Before taking blood, a systematic inquiry into past symptoms, known contacts and a subjective self-assessment was documented. The correlations between serostatus, patient contacts and demographic characteristics were analysed. Different tests were compared by Kappa statistics.

Results Among 318 participants, SARS-CoV-2 antibodies were detected in 9 employees. Chemiluminescence assays (chemiluminescence immunoassay and electrochemiluminescence) showed superior specificity and high reproducibility, compared with ELISA and POC results.

In contrast to the low seropositivity (2.8%) of healthcare workers, higher than that of the other departments of the hospital, a large proportion mistakenly assumed that they might have already been infected. Antiviral antibody titres increased and remained on a plateau for at least 3 months.

Conclusions The great demand and acceptance confirmed the benefit of highly sensitive testing methods in the early phase of the pandemic. The coincidence of low seroprevalence and anxious employees may have contributed to internalising the need of hygiene measures.

Key messages

What is already known about this subject?

- A growing number of assays are available that use antibody detection to indicate past SARS-CoV-2 infections or immunisation by vaccination.

What are the new findings?

- The specificity and sensitivity of the antibody tests used differed significantly; if the seroprevalence is assumed to be low, a single, less specific test should not be trusted.
- A high level of insurance during the pandemic is reflected in the large number of staff at a tertiary eye centre who misjudged their symptoms and serostatus.

How might these results change the focus of clinical practice?

- The use of highly sensitive and specific assays allow tracing of earlier Covid-19 infections including immune status.

INTRODUCTION

SARS-CoV-2 is an enveloped RNA virus that can cause a disease spectrum that ranges from asymptomatic and mildly symptomatic infections with subclinical manifestations, despite existing contagiousness and danger of infection, to severe and fatal respiratory infections.[1 2] COVID-19 has swept across the world, overwhelming healthcare systems and raising countless questions, about how best to diagnose patients, treat infections, save lives and contain the pandemic while still providing good quality eye care. Not only is the whistle-blower and ophthalmologist Li Wenliang the prime example of how accurate observation and the risk of SARS-CoV-2 infection due to close physical contact are increased in the field of ophthalmology.[3–5] Ophthalmologists and employees of eye-care centres are exposed to increased risk due to their physical proximity to many patients.[6–9]

Evidence from SARS-CoV-2-infected macaques and from studies with seasonal coronaviruses suggests that the infection is likely to produce an immunity that is protective for a period of time.[10 11] Available test methods allow a high degree of reliability, for example, if high-quality serological methods are combined. Direct detection of the active SARS-CoV-2 virus is possible via a reverse transcriptase PCR (RT-PCR) or direct antigen detection from smear material of the nasopharynx.[12] When rapid antigen tests were not yet available, especially in the early stages of the pandemic,[13 14] capacity problems with a backlog of unprocessed samples and delays in the notification of results by laboratories and health authorities contributed to the uncertainty. A considerable lack of personal

protective equipment—similar to other Western countries[15]—inspired the question as to how strongly the virus could have spread undetected among the staff, similar to the warning example of a South African private hospital (St. Augustine's, Durban, KwaZulu-Natal province) when 80 staff members and 39 patients were infected including a death toll of 15 patients. Finally, a high average age is seen in ophthalmology with a high susceptibility to more severe infections.[16] Although deaths were also seen in younger age groups, the infection-to-fatality ration rises noticeably with the age groups.[17]

In order to counteract the experience of individual infection chains and the concern for colleagues and entrusted patients, a systematic testing for antibodies was planned as part of a scientific investigation. At this time (May 2020), not many serological assays had yet been established for contact tracing, identifying the viral reservoir and epidemiological studies,[18] while the availability of reliable serological assays lagged behind molecular diagnostic tests. The aim of this study was to analyse the perceptions and first-hand experiences in the setting of a tertiary eye centre.

METHODS

Subjects

Testing for SARS-CoV-2 antibodies was offered to all employees of the joint facility of the University Eye Hospital and the Institute for Ophthalmic Research via email in May 2020. In advance, the works council, board and task force were informed and gave their consent. The prospective study followed the tenets of the Declaration of Helsinki and was approved by the Ethics Review Board of Tübingen University (NCT04446338).

Patients and the public were involved in the design, conduct and dissemination plans of our research. The design contained no exclusion criteria, but included a combination of a pseudonymised survey and the performance of multiple assays. Prior to the interview and blood sampling, informed consent was obtained after detailed explanation of the voluntary nature of participation, the nature of the study and the lack of immediate consequences of the personal results (later risk of infection and transmission, retention of hygiene measures).

Questionnaire

All participants were asked whether a previous test (RT-PCR smear results) had been performed. In addition, information about known symptoms of SARS-CoV-2 during the last 3 months was collected. For fever, participants were asked to recall duration and maximum measured body temperature during the recent months (time of the pandemic). Participants were also asked whether they noticed any influenza-like or cold-like symptoms in the past 24 months in order to assess corona-related viral infections. Contact with persons with proven SARS-CoV-2 infectivity was recorded, including the intensity of the contact and the environment (professional vs private). A visual analogue scale was used to assess the likelihood of a past SARS-CoV-2 infection in the subjective assessment of the individual participant (the measured steps/distances from 1 to 10 corresponded to the range 'excluded' to 'certain').

Assays

Blood was collected in three tubes containing lithium heparin, one of which was sent to the central laboratory for immediate testing. The other samples were prepared with identical preanalytics (centrifugation radius 157 mm, centrifugal force 2.680×g, 7 min, 18°C) and frozen at −80°C until further analysis. As recommended by the Centers for Disease Control and Prevention and International Federation for Clinical Chemistry and Laboratory Medicine, a sequential combination of different validated assays (and antigens) was used.[19] One test used the technology of electrochemiluminescence. During the electrochemical reaction, the ruthenium complex emits light signals that are measured to quantify the analyte in order to detect antibodies to the nukleocapsid(N-)proteins (Elecys) (Roche Diagnostics, Germany). The other system uses acridinium ester technology-based chemiluminescence immunoassay to detect antibodies to the S1 RBD antigen (ADVIA Centaur) (Siemens Healthcare Diagnostics, Germany). Two conventional ELISAs were also used, measuring antibodies to the spike protein (S1-domain (EI 2606–9601 G), Euroimmun, Lübeck Germany; S1 RBD (Ref E111), Mediagnost, Germany). The laboratory analysis was performed in a certified central laboratory according to defined standard operation procedures in compliance with Good Clinical Practice requirements.

A subset of 120 samples was also analysed with point-of-care (POC) testing. The lateral flow chamber of the POC Rapid Test (E-UNCOV-40) (Wuhan Science, China) was photo-documented after a time of 10 min and read by two readers. The result of the tests was treated strictly confidential and communicated to each employee in a personal email. Two study physicians were available at all times to answer questions.

Statistical analysis

Descriptive statistics were applied to summarise respondent characteristics using count and percentage, means and SD. The reported symptoms were summarised as sum counts in the following four groups (respiratory symptoms including dry cough, throat problems, hoarseness, rhinitis, shortness of breath; aches/pains including muscle aches, headache, chills, disease feeling; abdominal problems including diarrhoea, abdominal pain, loss of appetite; illness feeling including fever, muscle aches, chills, loss of appetite loss). These groups were determined based on exploratory factor analysis, although they did not represent distinct characteristics. Descriptive analysis of groups in terms of patterns has also been facing the possible inconsistent use of terms and the probable response bias. Due to little variation or few respondents, groups were analysed (subjective assessment of suspected

history 1–6 vs 7–10, respiratory symptoms 0–4 vs 5–6, aches/pains: 0–4 vs 5, abdominal symptoms 0–1 vs 2–3). These categories were determined by assessing a point in the scales which represented a difference in proportion of COVID-19-positive respondents.

Because both ELISA tests produced skewed data, Spearman's rank correlation, which is robust to outliers, was applied to analyse the relationship between the two tests. Fisher's exact test was used to determine statistical significance of contingency tables due to the very small numbers of positive cases. Kappa statistic was used to examine the agreement of the tests' categorisation of positive, borderline and negative, where a value of 0 is interpreted as chance agreement between the measures and 1 perfect agreement. We used the rules of thumb suggested by <0.2 poor, 0.21–0.4 fair, 0.41–0.6 moderate, 0.61–0.8 substantial agreement.[20]

Statistical analysis was performed using SPSS (IBM, SPSS Statistics, V.19; SPSS) and STATA V.13 (StataCorp, 2013, Stata Statistical Software: Release 13, College Station, Texas, USA: StataCorp LP.)

RESULTS

Out of 450 employees, 318 (70.7%) participated in the trial, including all different occupational groups of the centre (table 1). The majority of participants (62%) had direct contact to patients within the department. Eighty-six employees (27.1%) reported at least one contact to a person with active SARS-CoV-2 infection (from the vicinity to patients=10, colleagues=48, private environment=21), of which 28 reported short and 13 as close contact (contact for at least 15 min).

Although only a small group of participants had previously been tested by swab (5.7%), an abundance of symptom complaints during the first 3 months of the pandemic were reported. Most of the symptoms represented influenza-like symptoms (table 2; fever: 47 (mean duration: 3.8 days, mean temperature: 38.5°C); respiratory symptoms: 150 with dry cough: 81, sore throat: 99, hoarse voice: 39, rhinitis: 38, pneumonia: 3; muscle aches: 55, headache: 143; chills: 21). In the subjective assessment, only 15% of the respondents ruled out with certainty to have gone through a SARS-CoV-2 infection. There was no association of this subjective association with age (p=0.655), gender (p=0.872), profession (p=0.367) or educational level (p=0.304).

Testing results

Both luminescence assays, Elecys and ADVIA Centaur, resulted in the same nine employees exhibiting specific SARS-CoV-2 antibodies, corresponding to a seroprevalence of 2.8% (95% CI 1.5 to 5.3) (figure 1).

In the first run, the EUROIMMUN ELISA identified 12 participants with positive findings and 5 borderline values. A repeat measurement after a new blood sample (at least 7 days apart) led to the assumption of two additional positive reactions. The Mediagnost ELISA confirmed also 9 persons, but 16 borderline values.

Table 1 Participants' characteristics and questionnaire results

N	318
Age in years | mean (SD)	41.0 (12.8)
Female gender | n (%)	230 (72.3)
Facility of the department	
University Eye Hospital | n (%)	247 (77.7)
Institute for Ophthalmic Research | n (%)	71 (22.3)
Professions | n (%)	
Administration*	14 (4.4)
Maintenance†	15 (4.7)
Medical assistance personal‡	46 (14.5)
Medical technical assistance§	10 (3.1)
Nursing staff¶ | n (%)	46 (14.5)
Surgical nursing staff	14 (4.4)
Orthoptists**	7 (2.2)
Service††	34 (10.7)
Physicians	37 (11.6)
Physicians in training	19 (6.0)
Medical-technical laboratory assistance‡‡	7 (2.2)
PhD students	21 (6.6)
Research management§§	5 (1.6)
Scientists	43 (13.5)
Level of education | mean (95% CI)	5.2 (5.0 to 5.5)
Contact to patients | n (%)	198 (62.2)
Previously tested (SARS-CoV-2 swab) | n (%)	18 (5.7)
Thereof detection of virus/positive | n (%)	1 (0.3)
Subjective assessment scale: suspected history of infection mean (95% CI)	3.3 (3.1 to 3.6)

*Including controlling (n=3), facility management, IT personnel (n=3), postman.
†Including cleaning staff (n=4), electrician (n=1).
‡Including medical students (n=5), student assistants (n=14), study coordinators (4), technicians (4), trainees (n=2).
§Including photographer (n=5).
¶Including nurse trainees (n=2) and physiotherapists.
**Including optometrist and optometry student.
††Including doorman (n=4), registry (n=12), secretary (n=9), student (n=9).
‡‡Including pharmaceutical technical assistance (n=1).
§§Including research assistance.

Interestingly, the semiquantitative values of the two ELISA tests were only weakly correlated (Spearman's rho=0.286, p<0.0001): The borderline cases did not overlap. Seven positive cases were in agreement, but only two borderline cases in accordance to the Mediagnost test were found to be positive following the EUROIMMUN test. All six borderline cases from Euroimmun were negative

Table 2 Relationship between subjective scale, symptoms and serostatus*

		Anti-SARS-CoV-2 negative		Anti-SARS-CoV-2 positive		P value (Fisher's test)	Raw total		P value (rank-sum test)
	Total N	n	%	n	%		Median	IQR	
Total		294	97.03%	9	2.97%		0.12	0.05–0.18	
Subjective assessment scale 1–6	277	272	98.91%	3	1.09%	<0.001	0.12	0.05–0.18	0.0562
Subjective assessment scale 7–10	28	22	78.57%	6	21.43%		0.155	0.06–0.37	
Respiratory symptoms 0–4	304	297	98.34%	5	1.66%	<0.001	0.12	0.05–0.18	0.2246
Respiratory symptoms 5–6	13	9	69.23%	4	30.77%		0.15	0.05–1.90	
Aches/pains 0–4	30 9	301	98.05%	6	1.95%	0.001	0.12	0.05–0.18	0.4205
Aches/pains 5	9	6	66.67%	3	33.33%		0.15	0.05–3.70	
Abdominal symptoms 0–1	290	282	97.92%	6	2.08%	0.037	0.12	0.05–0.18	0.6149
Abdominal symptoms 2–3	28	25	89.29%	3	10.71%		0.145	0.05–0.21	
Illness 0–2	300	294	98.66%	4	1.34%	<0.001	0.12	0.05–0.18	0.2613
Illness3-4	16	11	68.75%	5	31.25%		0.14	0.05–6.85	

*Semiquantitative results of ADVIA Centaur test binding to S1 RBD antigen are shown.

in Mediagnost. The poor to fair agreement between the two ELISA tests (Kappa 0.373 SE 0.042, p value<0.001) underlined the need of sequential testing when the prevalence in the cohort is low. All four tests produced results with extremely right-skewed distributions, which were to be expected. The POC rapid test also uncovered 8 of the positive subjects when applied in a subset of 118 participants. However, six tests were found not to be evaluable (blurry staining, lack of control band).

When comparing the different occupational groups, there were no obvious differences between doctors, nurses and other professions in test positivity (table 3). The majority of infections assumed by the healthcare works were not confirmed. When comparing reported symptoms with serological results, a cluster of general illnesses complaints performed the best when compared with testing results, while abdominal symptoms performed worse. Loss of smell and taste was not explicitly reported by the persons affected. By offering a measurement to the relatives/contact persons of employees who tested positive, a total of five additional people with immunity were identified.

DISCUSSION

Overall, the validated assays described here can be instrumental for the detection of SARS-CoV-2-specific antibodies for diagnostic, sero-epidemiological and vaccine evaluation studies. The strategy with several independent sequential tests was essential.[21] Moreover, especially facing a low prevalence, as was prevalent at the beginning of the pandemic, the use of highly specific tests is even more important (because more informative)

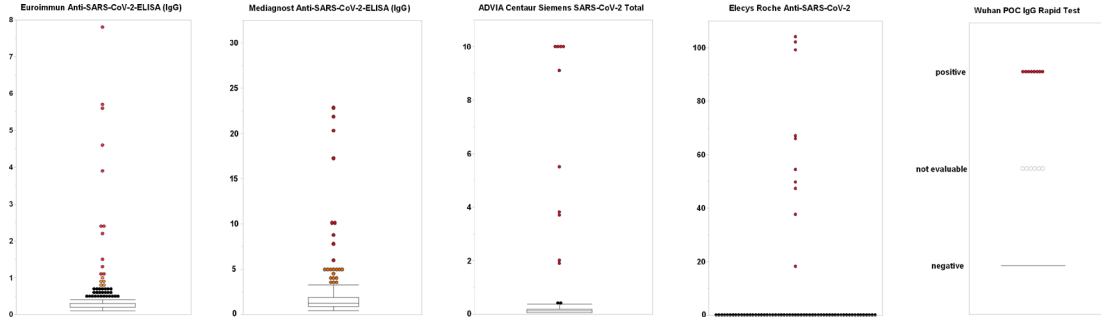

Figure 1 Box plots showing the readouts of the different antibody tests applied (whiskers indicating 95% CI, Euroimmun ELISA distinguishes between negative (ratio <0.8), positive (ratio ≥1.1, shown in red), borderline (ratio ≥0.8 and <1.1, shown in orange), Mediagnost ELISA distinguishes between negative (ratio <3), positive (ratio ≥5, shown in red), borderline (ratio ≥3 and <5-fold negative control (NC) (optical densitiy/NC), outlier shown in orange), Siemens and Roche Assays distinguishes between negative (COI<1.0) and positive (COI≥1.0, shown in red), Wuhan point-of-care only tested in a subset of n=120).

Table 3 Serostatus* and subjective assessment in different occupational groups

		Anti-SARS-CoV-2 negative		Anti-SARS-CoV-2 positive		P value (Fisher's test)	Raw data total		P value (Kruskal-Wallis)
	Total N	n	%	n	%		Median	IQR	
Total	318	294	97.03%	9	2.97%		0.12	0.05–0.18	
Nurses and trainees	45	43	95.56%	2	4.44%	0.099	0.11	0.05–0.26	0.5638
Surgical nurses	14	14	100%	0	0%		0.13	0.05–0.20	
Physicians and trainees	56	51	92.73%	4	7.27%		0.145	0.05–0.21	
All others	203	199	98.51%	3	1.49%		0.12	0.05–0.18	
		Subjective scale 1–6		Subjective scale 7–10			Subjective assessment scale continuous		
Total		277	90.82%	28	9.18%				
Nurse and trainees	43	38	88.37%	5	11.63%	0.827	3	2–5	0.4542
Surgical nurse	12	12	100%	0	0%		3	1.5–4	
Physicians and trainees	56	51	91.07%	5	8.93%		2	2–3	
All others	194	176	90.72%	18	9.28%		3	2–5	

*Results of ADVIA Centaur test binding to S1 RBD antigen are shown.

than combining independent assays to avoid false positive reports.[22 23] However, these have only been (further) developed over time and were not initially available.

Although individual test results have to be interpreted with caution, surveillance in a tertiary eye care centre and large eye research institute can reduce the anxiety and provide clarity regarding the actual number of (unreported) SARS-CoV-2 infections. Symptomless infections were described early in the pandemic[24 25] and the spread of the virus was able to go partially unnoticed, showing large regional variability also based on early testing policies and capabilities. Interestingly, under special conditions, rates of up to 81% (cruise-ship) asymptomatic infected persons were found.[26] There is evidence that infections without symptoms show a lower immune response and also spread viruses for shorter periods of time ('shedding').[27] Interestingly, anosmia and disgeusia as early symptoms and dry eye symptoms were less frequently reported in our cohort, among the employees with suspected infection alone, just as among those actually affected.[28 29] Undiscovered cases can complicate the containment measures, especially in sensitive environments such as eye hospitals. Other sample screenings showed much higher figures than this current research (up to six times the positive cases).[30] These findings are, however, in accordance with previous studies in asymptomatic healthcare workers, but probably dependent on the regional differences in infections and undiagnosed cases.[31] Our data are not surprising given observations that transmission is taking place more frequently in households than at working space,[32] in particular if protective measures have been installed.

A few weeks later (June 2020), 81 of 3215 (2.5%) employees of the remaining departments (University Hospital Tuebingen) were found to show antibodies based on the ADVIA Centaur test. However, since this testing was less systematic and comprehensive than that in the eye clinic (participation rate 71% vs 37%), it must be assumed that symptomatic employees and high-risk areas (emergency room, intensive care unit, infection wards) were over-represented. If corresponding areas were excluded, the proportion would correspond to 1.1% and would be even lower for complete area-wide testing. Therefore, the delta confirms an increased risk of infection in ophthalmology healthcare workers compared with other medical specialties. Healthcare workers, in particular medical support staff, exhibited the highest risk for testing positive or severe disease including death due to COVID-19 in recent reports.[33]

Population immunity is typically estimated through cross-sectional surveys of representative samples measuring humoral immunity. Surveys performed in countries affected early during the COVID-19 epidemic, such as Spain and Italy, suggest that nationwide prevalence of antibodies varied between 1% and 10%, with peaks around 10%–15% in heavily affected urban areas.[34] This is consistent with earlier predictions made by mathematical models, using death counts reported in national

statistics and estimates of the infection fatality ratio, that is, the probability of death given infection.[35]

Careful interpretation of test results

Facing the problems of molecular testing and false positive rates of rapid antigen tests,[14 36] serological diagnostics offers important information. Several reports described a high conversion rate, showing antiviral immunoglobulin G (IgG) and immunoglobulin M (IgM) within 19 days after symptom onset.[37] Titres plateaued within 6 days after seroconversion and allow the detection of infections over very large time windows. Meanwhile, accurate data on assay performance are available from head-to-head evaluations[38 39]: Test specificity ranges from 84.3% to 100.0%, underlining the importance of seropositivity threshold determination. Thus, the expected prevalence has a decisive influence on the suitability of the individual diagnostic test. Among over 200 antibody tests in the market, 54 tests of anti-SARS-CoV-2 IgG currently hold the Food and Drug Administration (FDA) authorisation (online supplemental table).[38] In the meantime, there have been several large systematic studies, enabling meaningful meta-analyses.[18 40] Binding to the S1 subunit of the S protein antigen is optimal for large-scale serological assays and more sensitive by capturing antibodies binding to the receptor-binding domain (RBD) as well as non-RBD.[41] In contrast, the full length S trimer, which harbours the S2 subunit in addition to S1, can lead to cross-reaction with circulating antibodies secondary to other coronaviruses.[42] Distinguishing pre-existing and de novo immunity is critical for the specific detection of SARS-CoV-2 infections.

Regardless of the antibody status, T cell immunity may exist, so sero-diagnosis may be incomplete.[43] Regardless of the strength of the infection, infected-maintained B and T cell phenotypes consistent with activation and cellular exhaustion throughout the first 2 months of infection. Additionally, follow-up samples from non-hospitalised patients showed that activation markers and cellular exhaustion increased over time.[10] In our cohort, there was the example of a nurse who went through a generalised COVID-19 infection, but in contrast to her husband had no evidence of any antibodies. In contrast, only one ophthalmologist was identified who had apparently experienced an asymptomatic infection.[44]

Limitations

The seroprevalence of the cohort was far too small to speculate on age-related susceptibility among eye specialists.[16] The study was also not suitable to conclusively assess the test performance, but which has been well characterised in the meantime.[45] Data collection occurred early during the pandemic, which may explain the low number of unreported cases compared with later studies.[30 46] Therefore, there are few conclusions on epidemiology and transmission dynamics.[47] So far, only a follow-up control was performed for those who tested positive and their relatives, so the dynamics of the second wave cannot yet be assessed.

Without any deep serological profiling, no conclusion can be made with regard to the binding epitopes of the individual antibodies and their anti-infective efficacy.[48] Neutralising antibodies may be of importance here.[45 49] Although the antibodies in saliva and tear fluid are likely to accompany the serological response,[46] our study does not provide any data here, but is limited to first-hand experience on a sober basis. There were few asymptomatic or mildly affected employees; nevertheless, the sensitive assays showed clear antibody levels.[49] The temporal relationships must be considered for the interpretation because the sensitivity depends on the time interval to the symptoms: While antigen tests and molecular virus detection indicate the risk of infection (viral shedding highest 2 days before and first 5 days of symptoms[50]), neutralisation tests showed a dependence on a minimum interval before effective antibody levels.[45]

Outlook

Follow-up tests in our cohort showed no decrease in titre, in contrast to other cohorts of healthcare professionals.[51] The persistence and decay of antibody responses is difficult to interpret in individual cases.[46 52] Long-term experience must be awaited, especially since there seem to be differences to seasonal coronaviruses.[53 54] After all, stable antibody levels were found after half a year,[55] especially secondary to more severe infections.[56 57] Fortunately, recent reports also point to a stable humoral response after vaccination.[58] Nevertheless, reinfections might occur and can also rarely be more severe, based not only on a mutated, more virulent virus, but also on a mechanism called antibody-dependent enhancement[59]; this means that persons with very severe infections may have ineffective antibodies, which might make them more prone to severe reinfections.

In summary, the survey showed that a threat was seen during the early pandemic. The lack of personal protective equipment in particular may have contributed to the perception of occupational risks.[60] The serological testing may offer clarity including better awareness of potential infectiousness: Knowing the lack of immunity, personal protective measures and disinfection might be better followed in contact with patients.[15 60 61] The rate at which people in the population are likely to become infected depends on which venues they visit and how that changes over time.[62] Immunological characterisation can illustrate the need of serial vaccination beyond the measurement of active infections and helps to project the future landscape.[63 64]

Especially in rectified discussions about mortality and the extent of political measures, it is important to know the actual extent of the infection.[65] Modelling is limited by a large tail risk accompanying extremes and large fluctuations. The personal information helped to counter the misinformation.[66 67] Serological testing was relevant to reduce fears and uncertainties and provides an added

value in the confirmation of the ongoing vaccination strategies.

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