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Dynamics of humoral response to coronavirus antigens among employees of COVID-dedicated hospital: an observational study.

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Short title: Humoral response to SARS-CoV2 antigens among hospital staff

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Key words: antibodies, occupational exposure, occupational safety and health, pandemic COVID-19, SARS-CoV-2.
What’s new?

During the COVID-19 pandemic healthcare workers are facing a challenge of working in special conditions. One of the widely discussed topics is occupational safety. We aimed to investigate if working in a COVID-19-dedicated hospital is reflected in results of anti-coronavirus antibodies panels. We analyzed the results of about 2500 tests taken by more than 1500 employees and found that about one in four persons had positive or equivocal result. Moreover, we detected gradual increases in the levels of antibodies among workers with negative results. Further analysis revealed that the rate of change of antibodies levels was higher among employees having direct contact with COVID-19 patients; the highest rate of change was found among medical staff participating in high-risk procedures. It is possible that healthcare workers have regular contact with coronavirus antigens and the immune response depends on the intensity of the exposure.
Abstract

Introduction: A high priority for study during the pandemic of SARS-CoV-2 is the safety at work of hospital staff taking care of COVID-19 patients. The authors decided to analyze the results of opportunistic screening for anti-SARS-CoV-2 antibodies among employees of a COVID-19-dedicated centre.

Objectives: The authors aimed to investigate if potential exposition to SARS-CoV-2 antigens is reflected in the results of serological studies.

Patients and methods: Every employee who took at least one test between 21st April and 20th July was included in the study. Every test assessed levels of immunoglobulin G (IgG) and immunoglobulin M+A (IgM+IgA). Employees were divided in terms of having contact with COVID-19 patients and participating in aerosol-generating procedures.

Results: Results of 2455 tests taken by 1572 persons were analyzed. 357 (22.7%) participants had at least one positive or equivocal result during the observation period. Linear mixed models revealed gradual increases in mean levels of both IgG and IgM+IgA antibodies among employees with all negative results. The rate of change was higher among persons having direct contact with COVID-19 patients and the highest rate of change was observed among person participating in aerosol-generating procedures.

Conclusions: We detected developing humoral immune response to a new set of antigens of coronavirus among studied group. It is possible that employees of COVID-19-dedicated centres have regular contact with non-infectious amounts of SARS-CoV-2 or its antigens.
Introduction

The first wave of the COVID-19 pandemic in Poland began in March 2020 and approached its maximum of about 350 daily cases, 25 daily deaths and 3000 hospitalized patients in April and May [1]. During the wave, COVID-19 patients in Poland were treated in hospitals transformed into COVID-19-dedicated centres; the Central Clinical Hospital of the Ministry of Interior and Administration in Warsaw was one of the largest centres, hosting over 850 beds in 27 wards and clinics [2]. The transformation included work reorganization, implementing PPE-usage training system, creating procedures targeted on ensuring work safety (especially epidemiological safety) [3]. As real time polymerase chain reaction (rt-PCR) test is considered to detect active infection, it was used to check if an employee with symptoms of respiratory tract infection has COVID-19.

Additionally, starting from the 21st April 2020, a system of opportunistic screening for anti-SARS-Cov2 antibodies among hospital staff was implemented. In contrary to rt-PCR tests which detect active infections, the serological studies check if person had contact with viral antigens. Little is known about the anti-SARS-CoV-2 seroprevalence in Poland (especially during the first wave of the pandemic) and the dynamics of levels of antibodies among healthcare workers has not yet been studied. The aim of the study was to investigate if potential exposition to SARS-CoV-2 antigens is reflected in the results of performed tests, including its time- and type of job-dependence.

Materials and methods

Design and setting

We designed and conducted an observational study. Every employee of the Central Clinical Hospital of the Ministry of Interior and Administration in Warsaw who did at least one test for levels of anti-SARS-CoV-2 antibodies between 21st April 2020 and 20th July 2020
was enrolled. There were no exclusion criteria. All employees of the hospital were followed-up regarding having COVID-19 by the end of July 2020. Every employee with symptoms of respiratory tract infection was obligatorily tested for COVID-19 with real time polymerase chain reaction (rt-PCR) of nasopharyngeal swab. In case of a positive result, employees who have had relevant contact with infected person (as described by World Health Organization guidelines [4]) were also tested.

The study protocol was approved by The Ethics Committee of The Central Clinical Hospital of the Ministry of Interior and Administration, Warsaw, Poland (Number 209/2020).

**System of screening for anti-SARS-CoV-2 antibodies**

A system of opportunistic screening for anti-SARS-CoV-2 antibodies was started on 21\textsuperscript{st} April 2020. Every employee had an opportunity to test levels of immunoglobulin G (IgG) as well as combined immunoglobulin M (IgM) and immunoglobulin A (IgA) in serum by the use of Vircell’s assay “COVID-19 ELISA” (G1032[5] and MA1032[6]). The test was recommended to be repeated about every 2 weeks. All samples were assayed and validated in compliance with the protocol provided by the manufacturer. The cut-off values for equivocal and positive results were 6 and 8 for the IgM+IgA assay and 4 and 6 for the IgG assay, respectively. All persons having equivocal and positive results were tested for COVID-19 with rt-PCR of nasopharyngeal swab.

The specificity declared by the manufacturer is 98% and 99% for the IgG and IgM+IgA assays, respectively. The sensitivity depends on time from onset of the disease and amounts to 83% and 87% for IgG and IgM+IgA assays performed 5 days after positive rt-PCR result, respectively. The within-run precision coefficient of variation is declared to not exceed 5% and between-run precision coefficient of variation is declared to not exceed 9%. The IgG assay is declared not to cross react with parainfluenza virus, influenza A and B viruses,
adenovirus, respiratory syncytial virus, Mycoplasma pneumoniae, Chlamydia pneumoniae, Coxiella burnetii and Legionella pneumophila. The manufacturer declares no cross reactivity of IgM+IgA assay with influenza A and B viruses and Legionella pneumophila, while partial cross reactivity of IgM+IgA assay with adenovirus, Mycoplasma pneumoniae, Chlamydia pneumoniae, Coxiella burnetii and respiratory syncytial virus was found.

**Study population and data collection**

We obtained the results of all antibodies tests performed between 21st April and 20th July 2020 from the Diagnostic Laboratory providing 13 weeks of observation. We considered data regarding sex, type and place of job, rt-PCR results (where applicable). We assessed all working positions in terms of having direct contact with COVID-19 patients and participating in aerosol-generating procedures.

**Statistical analysis**

We performed statistical analysis using SAS® software, version 9.4 (SAS Institute Inc., Cary, North Carolina, USA). Quantitative variables were described using median and quartile values, whereas qualitative variables were expressed as number (percent). We decided to distinguish group of persons with at least one positive or equivocal result (nonnegative result) of either IgM+IgA or IgG levels. The group of Ig-nonnegative employees was compared with the group of Ig-negative employees by the use of chi square tests. We used linear mixed models for repeated measures over time by type of job to analyse changes in levels of IgM+IgA and IgG antibodies among Ig-negative group and impact of direct contact of COVID-19 patients with fixed effects of time, type of job and interaction between time and type of job (SAS Proc Mixed). This approach allowed participants to have different schedules and numbers of measurements. Analysis of results of all participants (both Ig-
negative and Ig-nonnegative) would require calculating nonlinear mixed model; we were unable to perform such analysis due to insufficient data quality. Time distribution of nonnegative immunoglobulin tests and rt-PCR positive results was also studied.

Results

One thousand five hundred seventy-two employees took the opportunity to test levels of anti-SARS-CoV-2 antibodies. About sixty-three percent of employees have had direct contact with COVID-19 patients, both in the studied group and among all employees. Thirty persons were diagnosed with COVID-19 from the beginning of the pandemic. One in five tested persons had nonnegative (positive or equivocal) IgG or IgM+IgA during observation. As described above, every such person was tested for COVID-19 with rt-PCR of nasopharyngeal swab; two tests were positive. The employees were a personal care aide and medical secretary; they presented no symptoms and epidemiologic investigation did not identify infection source. Both persons had maximum detectable levels of IgM+IgA (40 units), whereas IgG amounted to 25.5 and 5.8 units. 17 employees tested levels of antibodies after COVID-19; median (first – third quartile) levels of IgM+IgA and IgG were 12.0 (6.7 – 17.3) and 31.6 (20.9 – 40), respectively.

The occupancy structures of studied group and all employees were similar (about 63% of employees were working with direct contact with COVID-19 patients); however, percentage of nurses and midwives was higher in studied group (33.1% vs. 27.8%), whereas percentage of physicians was lower (11.5 vs. 16.1). The comparison between studied group and all employees is presented in Table 1.

Two thousand four hundred fifty-five samples were tested. A majority of employees (68.3%) tested their levels of antibodies only once; 14% performed three or more measurements. The percentage of nonnegative samples (either IgM+IgA or IgG) amounted to
18.4%. Sixty-four from 277 IgM+IgA-nonnegative results were higher than double the upper normal range. Fifty-two from 259 IgG-nonnegative results were higher than double the upper normal range. Results of all tests are summarized in Table 2.

The group of employees with nonnegative results of antibodies tests was analyzed separately. The structure of this group differed from the group with negative results—254 out of 357 (71.1%) had contact with COVID-19 patients as opposed to 732 out of 1215 persons (60.2%) \( (P < 0.001) \). The groups did not differ in terms of percentage of personnel participating in aerosol-generating procedures (22.1% vs. 20.8%, \( P = 0.60 \)). Occupation structure of Ig-negative and Ig-nonnegative employees having direct contact with COVID-19 patients did not differ \( (P = 0.90) \).

Time distribution of Ig-nonnegative samples and rt-PCR positive tests is presented in Figure 1. Screening for anti-SARS-CoV-2 antibodies started on 21st April 2020 (“week 0”). About 30 to 50 Ig-nonnegative samples were detected every week by the end of tenth week of observation. Number of Ig-nonnegative samples in the last two weeks of observation was smaller and amounted to about 20. The weekly percentage of nonnegative results was constant during weeks 0 to 6. Starting with 7th week, the percentage multiplied and had temporary fall in week 9th and 10th.

Linear mixed models for repeated measures over time found that mean level of IgM+IgA antibodies and IgG antibodies among Ig-negative employees increased by 0.66 \( (P < 0.001) \) and 0.31 \( (P < 0.001) \) monthly, respectively (Figure 2a and b). Female employees had higher initial level of IgM+IgA by 0.15 \( (P = 0.045) \), while monthly rate of change did not differ between sexes. Further analysis revealed interaction between time and type of job: monthly increase of IgM+IgA antibodies was higher by 0.09 among personnel having direct contact with COVID-19 patients \( (P = 0.045, \text{Figure } 2c) \). Difference in monthly
increase of IgG antibodies was insignificant \((P = 0.17)\). We did not detect differences in levels of antibodies at the beginning of the observation. We further divided group of personnel having direct contact with COVID-19 patients in terms of participating in aerosol-generating procedures. The linear mixed model found difference of 0.06 in monthly rate of change of IgM+IgA antibodies between personnel participating and not participating in aerosol-generating procedures and personnel without contact with COVID-19 patients \((P = 0.06)\). Employees participating in aerosol-generating procedures had the highest monthly rate of change of IgM+IgA antibodies (Figure 2d). Covariance structure analysis revealed that the model fully explained differences in individual rates of change.

**Discussion**

The main objective of the study was to investigate dynamics of levels of anti-SARS-CoV-2 antibodies amongst employees of the large COVID-dedicated hospital. We found that during 13 weeks of observation 357 out of 1572 (22.7%) persons who took part in screening had nonnegative (positive or equivocal) levels of IgM+IgA or IgG antibodies. Amongst these 357 persons only 17 were diagnosed with COVID before and in 2 cases a rt-PCR test was positive. We also found gradual increases in mean levels of antibodies of both classes amongst remaining 1215 employees (increase in IgM+IgA levels was steeper). The results suggest presence of developing immune response to a new set of antigens among studied group. It is possible that employees of COVID-19-dedicated centres have regular contact with non-infectious amounts of SARS-Cov2 or its antigens (small amounts of SARS-CoV-2 were detected in social and storage areas as well as in ventilation systems[7–9]), which is reflected in results of serological studies. Of note, as testing was voluntary, with no formal requirement of serial testing, about one third of participants were tested twice or more; however, the statistical analysis accounted for this issue.
Further analysis revealed that groups of employees with nonnegative (positive or equivocal) and negative results of serological test differed significantly. Namely, percentage of persons having direct contact with COVID-19 patients was higher amongst the group with nonnegative antibodies levels. The rate of increase in IgM+IgA level was also higher among persons having contact with COVID-19 patients. The highest rate of increase of IgM+IgA level was observed among employees participating in aerosol-generating procedures. Therefore, it seems that the groups differ also in terms of exposure to the viral antigens; the difference cannot be explained by its presence in social, storage areas and ventilation systems, because these are common-used areas. The effectiveness of personal protective equipment (PPE) does not reach 100%[10, 11] and doffing procedures are also imperfect[12, 13], which may lead to additional exposure to SARS-CoV-2 antigens among employees working in “red zones”. Moreover, working in PPE is not obligatory with recovalescents, who are known to produce viral antigens for weeks after diagnosis[14].

Our findings suggest that even incidental contact with viral antigens leads to production of specific antibodies. During the epidemic, more and more people have contact with viral antigens, even without getting infected (e.g. too short/small exposition to evoke infection, inhalation of inactive virions or antigens only). Therefore, we can expect growing percentages of persons tested positive for the antibodies. Unfortunately, the seroprevalence among healthcare professionals in Poland has not yet been studied. Moreover, we did not find a study on longitudinal changes in seroprevalence among general population in Poland, but only a series of cross-sectional studies. What can be observed is that percentage of persons having IgG antibodies have increased from about 4.3% measured in July and August (group of police officers [15]) to about 18% in December and 25% in February (project “West Pomeranian Monitoring and Prevention Program of Coronavirus SARS-CoV-2 and COVID-19 Disease” [“Zachodniopomorski Program Monitorowania i Prewencji Epidemii
Coronawirusa SARS-CoV-2 i Choroby COVID-19”], unpublished data [16, 17]). When it comes to seroprevalence of anti-SARS-CoV-2 antibodies among healthcare workers as well as risk factors of elevated levels of immunoglobulins (taking care of COVID-19 patients, participating in aerosol-generating procedures) our findings are consistent with recent meta-analysis [18].

It is noteworthy that weekly number of tests dropped 2-3 times starting from 7th week. We are of the opinion that this may be result of two factors. The first is the start of holiday season (including 4-day-long weekend in 7th week). The second is change in general approach to the pandemic – its first wave was over, and people tended to forget about coronavirus. Considering that number of nonnegative results remained high despite decline in weekly number of tests, we can suspect that if we continued to perform the same number of tests, the number of observed nonnegative results would be multiplied.

Our observational study has several limitations. The relation between levels of anti-SARS-CoV-2 antibodies and immunity to COVID-19 remains unknown. The system of screening of antibodies was opportunistic, therefore the study may suffer from selection bias, despite the presented comparison of occupational structure. We cannot rule out that we detected cross-reactions with other viruses and bacteria. However, declared specificity is high and other pathogens are common for decades, therefore mean levels of antibodies should remain stable, for example. We did not detect differences between rate of change of IgG antibodies between groups having and not having contact with COVID-19 patients. The possible explanation is slower response of this class of antibodies leading to smaller effect size and insufficient power of the study.

Our study revealed that healthcare workers employed in the COVID-19-dedicated centre are developing humoral immune response to SARS-CoV-2, even without getting infected. Taking into account the close association between presence of anti-SARS-CoV-2
antibodies and substantially reduced risk of infection and severe COVID-19 [19], we can expect that the seropositive employees are protected from severe COVID-19. On the other hand, contact with coronavirus antigens within the community may contribute to achieving herd community in a analogous way. However, the hypotheses are yet to be tested. Our findings also confirm efficacy of providing health care in PPE in preventing getting infected with SARS-CoV-2.

**Contribution statement:** RR conceived the concept of the study. RR and PS contributed to the design of the research. RR, KH, MZ, AS and TK were involved in data collection. RR performed the statistical analysis. All authors discussed the results. RR and PS wrote the manuscript. All authors edited and approved the final version of the manuscript. AZ, ZK and WW supervised the project.

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| Characteristic                          | Hospital | Total | Studied group |                  |                  |
|----------------------------------------|----------|-------|---------------|----------------|-----------------|
|                                        |          |       |               | Employees with  | Employees with   |
|                                        |          |       |               | negative tests  | non-negative     |
|                                        |          |       |               |                 | tests b          |
| Number of employees                    | 3177     | 1572  | 1215          | 357            |                 |
| Female sex                             | 2435 (76.6)| 1284 (81.7)| 983 (80.9)| 301 (84.3)     |                 |
| COVID-19\(^a\)                         | 30 (0.9) | 19 (1.2)| 0 (0)         | 19 (5.3)       |                 |
| Type of job:                           |          |       |               |                 |                 |
| With contact with COVID-19 patients    | 1996 (62.8)| 986 (62.7)| 732 (60.2)| 254 (71.1)     |                 |
|  Physician                             | 510 (16.1)| 181 (11.5)| 138 (11.4)| 43 (12.0)      |                 |
| Nurse or midwife                       | 882 (27.8)| 521 (33.1)| 386 (31.8)| 135 (37.8)     |                 |
| Other medical staff                    | 224 (7.1) | 107 (6.8)| 79 (6.5)     | 28 (7.8)       |                 |
| Support and technical staff            | 380 (12.0)| 177 (11.3)| 129 (10.6)| 48 (13.5)      |                 |
| Without contact with COVID-19 patients | 1181 (37.2)| 586 (37.3)| 483 (39.8)| 103 (28.9)     |                 |
|  Medical staff                         | 548 (17.2)| 252 (16.0)| 207 (17.0)| 45 (12.6)      |                 |
|  Non-medical staff                     | 633 (19.9)| 334 (21.3)| 276 (22.7)| 58 (16.3)      |                 |
| Type of job:                           |          |       |               |                 |                 |
|  Aerosol-generating procedures         |          |       |               | 332 (21.1)     | 253 (20.8)      | 79 (22.1)       |
|  Non-aerosol-generating procedures     |          |       |               | 1240 (78.9)    | 962 (79.2)      | 278 (77.9)      |

All values are presented as N (%).

\(^a\) persons with positive result of rt-PCR; \(^b\) nonnegative result = positive or equivocal result

Abbreviations: COVID, coronavirus disease; rt-PCR, real time polymerase chain reaction
| Characteristic                  | N (%) or median (Q1 – Q3), range |
|--------------------------------|----------------------------------|
| Total number of tests          | 2455                             |
| Immunoglobulin M and A         | 2.5 (1.6 – 4.0); 0 – 40.0        |
| Immunoglobulin G               | 1.9 (1.4 – 2.8); 0 – 44.8        |

Number of test repetitions per person

|                   |                   |
|--------------------|-------------------|
| One                | 1074 (68.3) a     |
| Two                | 271 (17.2) a      |
| Three and more     | 227 (14.4) a      |

Persons with non-negative result c

|                   |                   |
|--------------------|-------------------|
| Positive IgM+IgA   | 104 (6.6) a       |
| Equivocal IgM+IgA  | 107 (7.4) a       |
| Positive IgG       | 74 (4.7) a        |
| Equivocal IgG      | 136 (8.7) a       |

Tests with non-negative result b

|                   |                   |
|--------------------|-------------------|
| Positive IgM+IgA   | 152 (6.2) b       |
| Equivocal IgM+IgA  | 125 (5.1) b       |
| Positive IgG       | 103 (4.2) b       |
| Equivocal IgG      | 156 (6.4) b       |

a percentage of all persons; b percentage of all tests; c non-negative result = positive or equivocal result

Abbreviations: IgA, immunoglobulin A; IgM, immunoglobulin M; IgG, immunoglobulin G; Q1, first quartile; Q3, third quartile.
Figure 1. Time distribution of immunoglobulin-nonnegative (positive or equivocal) samples and real time polymerase chain reaction positive tests (COVID-19 cases). The percentage of nonnegative samples multiplied starting with 7th week. Numbers of nonnegative samples remained high by the 9th week of observation. Of note, the holiday season started from 7th-9th week of observation.
Figure 2. Changes over time of the levels of immunoglobulin M and A together and immunoglobulin G among employees with nonnegative tests (positive or equivocal) modelled by linear mixed models. We observed gradual increases in levels of both immunoglobulin M and A (A) and immunoglobulin G (B). Rates of changes of immunoglobulin M and A levels differed significantly between groups with and without contact with COVID-19 patients (C) as well as participating and not in aerosol-generating procedures (D).