



Research paper

Cross-sectional assessment of a history of SARS-CoV-2 infections using IgG

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ABSTRACT

Introduction: Severe acute respiratory syndrome, coronavirus type 2 (SARS-CoV-2) has become a global threat for every healthcare system, and the coronavirus disease 2019 (COVID-19) pandemic has resulted in over 3 million deaths worldwide. SARS-CoV-2 brings symptoms such as fever, cough, shortness of breath, headache, sore throat and loss of taste or smell. Diagnostic of COVID-19 may include specific RT-PCR for viral ribonucleic acid detection, and ELISA testing for virus-derived spike protein or nucleocapsid.

Aim: The aim of this study was to measure the antinucleocapsid level of SARS-CoV-2 IgG to identify the number of asymptomatic cases of COVID-19 after infection in a population of workers from a production company.

Material and methods: Human anti-SARS-CoV-2(N) IgG ELISA kit was used to determine serum IgG level. Study includes 107 individuals (48% female, 52% male) in different ages (18–60 years).

Results and discussion: Of 107 tested individuals in 80 (74.7%) cases SARS-CoV-2(N)-specific IgG antibodies were detected, with an average antibody concentration of the whole study group 4.08 $\mu\text{g/mL}$ ($n = 107$ with the range 0.59–7.91 $\mu\text{g/mL}$; $n = 80$ were included in the study with the sensitivity of the method above 2.344 $\mu\text{g/mL}$). In only 9 cases, SARS-CoV-2 infection was confirmed before using the PCR test. Our data underscore the need for a population study in Poland to test the proportion of asymptomatic IgG positive for SARS-CoV-2 individuals.

Conclusions: This study indicates that within studied sample large proportion of asymptomatic people have undergone SARS-CoV-2 infection and suggests that isolation of only symptomatic patients would not stop the transmission of the virus.

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1. INTRODUCTION

Severe acute respiratory syndrome, coronavirus type 2 (SARS-CoV-2) has become a global threat for every healthcare system. According to Worldometers, coronavirus disease 2019 (COVID-19) cases have been counted to approximately 142,113,000 by the 19th of April 2021; the pandemic has resulted in over 3 million deaths worldwide.¹ SARS-CoV-2 belongs to the family of single-stranded ribonucleic acid (RNA) viruses that infect animals, humans, and causes COVID-19. Clinical presentation varies between patients, but signs and symptoms include cough, fever, shortness of breath, fatigue, myalgias, headache, sore throat, new loss of taste, or smell as well as gastrointestinal symptoms like nausea, vomiting or diarrhea.^{2,3} After 1 week of the onset, some patients develop severe course of the disease, which meet the criteria for the acute respiratory distress syndrome (ARDS).⁴ Diagnostic tests involve a nasopharyngeal swab (or other upper respiratory tract specimens such as a throat or saliva swab⁵), and specific reverse-transcription polymerase chain reaction (RT-PCR) that detects viral RNA. Another group of methods – serological testing – including enzyme-linked immunosorbent assay (ELISA), chemiluminescence enzyme immunoassays, fluorescence immunoassays, and lateral flow immunoassays. These tests are used for rapid detection of the presence of the SARS-CoV-2 virus by detecting virus-derived spike (S) protein or nucleocapsid (N) protein. Alternatively, these assays are used to detect the presence of IgG, IgA, and IgM virus-specific antibodies (both separately or combined), providing evidence not only for a recent infection but also the successful generation of a potentially protective immune response.⁶ Serum is required, as these methods are designed to measure antibodies reflecting the host immune cells response.⁵ The ELISA method examined by Mathuria et. al. showed a sensitivity and specificity for virus-specific IgM detection (77.3% and 100.0%, respectively), and for IgG detection (83.3%, and 95.0%, respectively).⁷ Because of the easy of sample preparation and measurement, rapid results with lower costs, and even point of care potential, serological methods like ELISA and lateral flow assays, have therefore started to become more popular for routine measurements of SARS-CoV-2 specific antibodies in serum.

2. AIM

The aim of this study was to measure the IgG level anti-SARS-CoV-2 N using ELISA test to identify the number of asymptomatic cases of COVID-19 infection in the population of employees of a selected production company in Poland.

3. MATERIAL AND METHODS

3.1. Characteristics of research group

Previously 107 individuals (48% female, 52% male), of different ages (18–60 years), and all were Caucasian, and were

workers of a company in Olsztyn (Poland, Warmia and Mazury province) recruited from 20 to 24 of March 2021. The company employs 115 people (42% female, 58% male), aged 18–64 years old. Employees work in two spaces: office (27%) and production facility (73%). Work in the office space takes 8 hours a day, while work in the production space is organized in two shifts (both 8 h). Workers in both zones have limited contact with each other. The company has restricted rules required by sanitary institutions: wearing masks, disinfecting, and social distancing. None of the workers had been vaccinated prior to the testing.

Previously 107 participants took part in the study, but 27 patients were rejected due to the sensitivity of the method (where IgG concentration was less than 2.344 $\mu\text{g/mL}$). Therefore, the final results of 80 participants were used for statistical analyses (percentage of IgG-positive subjects was equal to 74.7%).

3.2. Questionnaire

All individuals were asked to answer the following questions including typical epidemiological history and clinical characteristics: sex; age; information about previous SARS-CoV-2 virus infection (based on a positive PCR test) and/or 10–17 days of quarantine; symptoms (in confirmed and nonconfirmed individuals: type of symptoms and duration); disinfection of hands, and social distancing.

3.3. Biological material

Peripheral blood samples (5 mL) were collected from each patient by medical staff, in adherence to the infection prevention and control guidelines of World Health Organization (WHO) and used personal protective equipment such as gown, gloves, eye protection and N95 mask while collecting the specimen. All blood samples were immediately transported to the laboratory and for direct analysis.

3.4. Determination of human anti-SARS-CoV-2 N IgG concentration

The peripheral blood was collected from the research group, and after serum isolation (clotting at room temperature for at least 30 minutes and centrifuging for 20 minutes at 2500 rpm) was immediately used for analysis, which was performed in duplicate using Human anti-SARS-CoV-2 N IgG ELISA Kit according to the manufacturer's instruction (FineTest, Wuhan, China). Test range: 3.906 – 250 ng/mL, sensitivity: 2.344 ng/mL, species: human.

All reagents and samples were kept at room temperature for 20 minutes before use, and then every step of the ELISA test was carried out in an incubator at 37°C with gentle shaking (250 rpm) on a microplate shaker (SkyLine ELMI Shaker DTS-4, Riga, Latvia). Test samples were diluted before analysis with sample dilution buffer from the kit (ratio 1 : 50). Plates were washed 2 times, and ELISA test was performed as follows: 50 μL of standard curve, control (blank) and diluted test samples were pipetted into the precoated microtiter strips, and incubated for 30 minutes at 37°C. Then plate was washed 3 times with wash buffer, and 50 μL HRP-labeled an-

tibody working solution was added into each well. After 30 minutes of incubation, plate washing was performed 5 times while leaving the Wash Buffer in the wells for 1–2 minutes each time. Then 50 μL of TMB substrate was added into each well, and incubation was carried out in the dark for 10 minutes. The reaction was stopped with 50 μL stop solution. Absorbance was measured at a wavelength of $\lambda = 450 \text{ nm}$ using microplate reader (BiogenetAsys UVM 340, Cambridge, UK).

3.5. Statistical analysis

Results of human anti-SARS-CoV-2 N IgG concentrations are presented as a mean \pm confidence interval (CI). Statistical analysis was conducted with GraphPad Prism 6 software (GraphPad Software Inc., San Diego, CA, USA).

4. RESULTS

4.1. Human anti-SARS-CoV-2 N IgG concentration

Of the 107 tested individuals, in 80 (74.7%) cases SARS-CoV-2-N-specific IgG antibodies were detected, with an average antibody concentration of 4.08 $\mu\text{g/mL}$ (0.59–7.91 $\mu\text{g/mL}$). In 9 (11.3%) cases out of these 80 individuals, the SARS-CoV-2 infection was confirmed by PCR test and by doctors (1–6 months before the anti-SARS-CoV-2 N IgG test: between October and December 2020, and February 2021). In 98 of all tested (91.5%) there was no confirmation, no symptoms (or weak symptoms), and no quarantine. The mean level of anti-SARS-CoV-2 N IgG in the PCR-confirmed group (within 1–6 months) was 3.94 $\mu\text{g/mL}$ (0.77–6.04 $\mu\text{g/mL}$), and in the non-confirmed group 3.99 $\mu\text{g/mL}$ (0.59–7.89 $\mu\text{g/mL}$). In 27 individuals, no specific IgG was detected so for statistics only 80 individuals were taken.

4.2. Symptoms

Our study showed that the vast majority of workers did not have symptoms (or scanty symptoms that may not have been considered a symptom of COVID-19 at the time) but still have specific IgG. The detailed characteristic of the symptoms in all groups is presented in Table 1.

5. DISCUSSION

Previously 107 participants took part in the study, but 27 patients were rejected due to the sensitivity of the method (IgG concentration less than 2.344 $\mu\text{g/mL}$). The results of 80 participants were used for statistical analyses (percentage of IgG-positive subjects was equal to 74.7%). Therefore, we found that asymptomatic patients had undergone COVID-19 infection based on IgG antibodies.

IgG antibodies are indicators of immune memory that even after a gradual decline remain present in recovered patients.⁸ Interestingly, in our results, the level of antibodies in individuals that recovered from a proven COVID-19 infection remained high until even 6 months after infection

(6.04 $\mu\text{g/mL}$). Nevertheless, in 1 case the IgG level dropped from 0.79 ng/mL to below 10 ng/mL at 6 months after infection. Long-term studies showed the kinetics of IgG specific for N and S antigens. Isho et al. confirmed that serum and saliva SARS-CoV-2 IgG are maintained in the majority of COVID-19 patients for at least 3 months post-symptom onset.⁹ Moreover, S protein-specific memory B cells were more abundant at 6 months than at 1 month post symptom onset, and IgG to the S protein was relatively stable for over 6 months.¹⁰

This study did not determine the level of IgM, because the company conducts strict restrictions on the health of employees (temperature is measured daily, antigen tests are selectively performed, and the employees are required to stay at home or contact a doctor if their health deteriorate or show COVID-19-like symptoms). IgM is detectable after 3–6 days, while IgG is detectable after 8 days.¹¹ However, detecting specific antibodies among newly infected patients (in the early stage of the putative development of disease) might be difficult as most patients experience IgG seroconversion on average at least 20 days after the onset of the first symptoms.¹²

Our study indicates that there may be many asymptomatic people who have had SARS-CoV-2 infection. Similar to other studies,^{13,14} our data support that an indoor and crowded environment favors SARS-CoV-2 infections.

Moreover, it supports the opinion that measures like isolation of only symptomatic patients would not stop the transmission of the virus.¹³ In the study of Johansson et al.,¹⁵ asymptomatic individuals were responsible for more than half of all transmissions. Additionally, the super spreaders are not responsible for COVID-19 outbreaks, but these are rather the type and conditions of the events.¹⁶ According to WHO symptoms of SARS-CoV-2 infection could be divided into the most common (fever, dry cough, tiredness) and less common (e.g.: sore throat, diarrhea).¹⁷ Some of the symptoms were linked to a subsequent SARS-CoV-2 infection at a later time point. When noting not specific to COVID-19

Table 1. Symptoms noted in the last 6 months in tested groups.

Symptoms/group	Positive PCR (positive IgG), 9 cases <i>n</i> (%)	Non-confirmed infection (positive IgG), 71 cases <i>n</i> (%)	Negative IgG 27 cases, <i>n</i> (%)
Loss of smell	9(100)	3(4.2)	0(0)
Fever	6(67)	3(6)	1(3.7)
Tiredness	6(67)	12(17)	9(33.3)
Pain in the chest	6(67)	0(0)	0(0)
Muscle pain	5(56)	2(4)	0(0)
Headache	6(67)	14(19.7)	0(0)
Loss of taste	6(67)	2(4)	0(0)
Dry cough	2(22)	0(0)	0(0)
Breathing difficulties	2(22)	0(0)	0(0)
Sore throat	2(22)	6(11)	0(0)

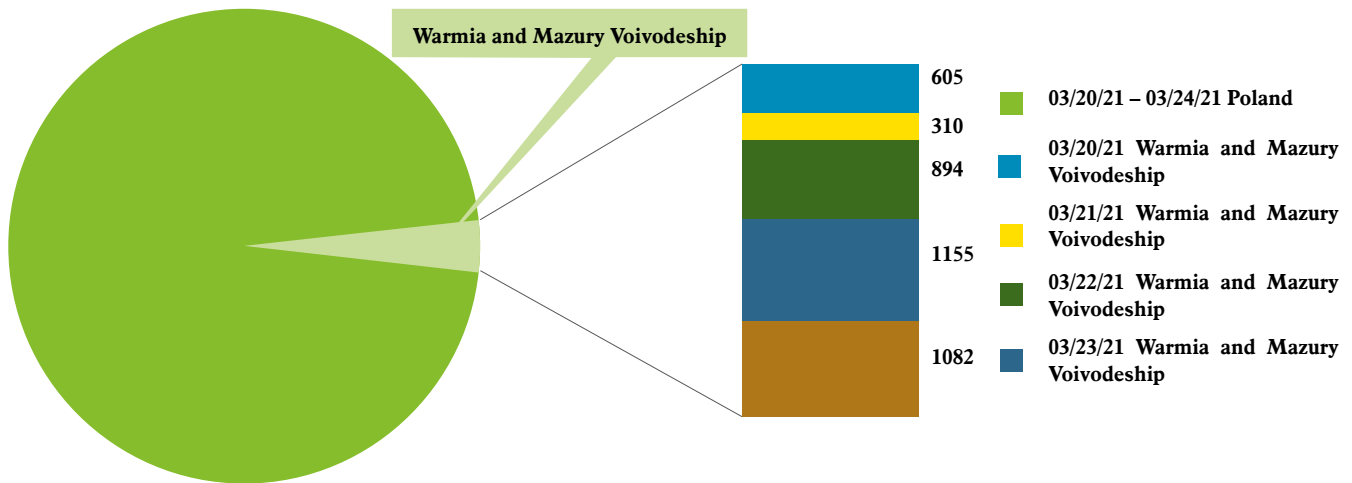


Figure 1. COVID-19 cases in Poland from 03/20/21 until 03/24/21 based on government's official coronavirus infection report.¹⁸

at that time symptoms, there was no chance to confirm the infection with a PCR test. In the positive tested group (proven COVID-19 infection by a positive PCR test) there were clinical symptoms recorded including loss of smell, headache, fever over 38°C, fatigue, chest pain, loss of speech, and loss of taste. Tiredness and headache were also noted in the other workers (not proven with PCR test). In some cases, only a few symptoms appeared at the same time. In 27 cases there was no anti-SARS-CoV-2 N IgG detected, while some claimed tiredness within this group (33.3%), and fever (1 individual).

We also collected information on the number of COVID infections in Poland and in the region (Warmia and Mazury Voivodeship) where employees of the examined company in Olsztyn work and live. The data on the chart (Figure 1) comes from the government's official coronavirus infection report.¹⁸

During the 4 days of our research, over 117 200 cases were confirmed in Poland. In the Warmia and Mazury Voivodeship, over 4000 covid infections were recorded, which accounted for approximately 3% of all infections in Poland at that time.

Interestingly, Ochal et al. published the study conducted from Shelter for the Homeless named after Sabina Kucznierów in Olsztyn. Study confirmed that in the period starting from December 2020 to March 2021, 50% of the whole group of analyzed individuals ($n = 82$) became infected. According to authors, prevention against the SARS-CoV transmission was implemented in shelter. Obligatory rules were distance (minimum of 2 m between beds), masks covering nose and mouth, and testing residents. Only one resident needed to be hospitalized.¹⁹

Limitations of our study include the relatively small number of participants – the single site-based design. Nevertheless, the number of people after infection based on a positive IgG test is surprisingly high in this population. Our data underscore the need for a population study in Poland to test the proportion of asymptomatic IgG positive for SARS-CoV-2 individuals.

6. CONCLUSIONS

This study indicates that within studied sample large proportion of asymptomatic people have undergone SARS-CoV-2 infection in and suggests that isolation of only symptomatic patients would not stop the transmission of the virus.

Conflict of interest

Authors declare no conflict of interests.

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Ethics

The study was conducted according to the guidelines of the Declaration of Helsinki and approved by the Bioethics Commission at the University of Warmia and Mazury (no. 11/2021). An informed consent was obtained from all participants. The raw data did not contain any personal identifying information that can be linked to particular individuals and was anonymized before its use.

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