Prevalence of anti-SARS-CoV-2 antibodies in Poznań, Poland, after the first wave of the COVID-19 pandemic

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Abstract: In comparison to other European countries, during the first months of COVID-19 pandemic, Poland reported relatively low number of confirmed cases of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infections. To estimate the scale of pandemic in Poland, a serosurvey of antibodies against SARS-CoV-2 was performed after the first wave of COVID-19 in Europe (March – May 2020). Within this study, we collected samples from July 28 to September 24, 2020 and based on the ELISA results, we found that 1.67% (25/1500, 95%CI 1.13 - 2.45) of the Poznan (Poland) metropolitan area’s population had antibodies against SARS-CoV-2 after the first wave of COVID-19. However, the presence of anti-SARS-CoV-2 IgG antibodies was confirmed with immunoblot in 56% (14/25) samples, what finally resulted in decrease of seroprevalence, i.e. 0.93% (14/1500, 95%CI 0.56 - 1.56). The positive anti-SARS-CoV-2 IgG results were associated with age, occupation related to constant contact with people, travelling abroad, non-compliance with epidemiological recommendations and direct contact with the novel coronavirus. Our findings confirm the low SARS-CoV-2 incidence in Poland and imply that the population had little herd immunity heading into the second and third wave of the pandemic, and therefore herd immunity contributed little to prevent the high numbers of SARS-CoV-2 infections and COVID-19 related deaths in Poland during these subsequent waves.
Keywords: SARS-CoV-2; seroprevalence; antibodies; COVID-19; pandemic; Poland

1. Introduction

The emergence and rapid spread across the globe of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) affected almost every part of our life. The SARS-CoV-2 can cause the coronavirus diseases 2019 (COVID-19), which is manifested by non-specific symptoms, such as fever, cough, fatigue and rapid lack of taste and smell. The symptoms can range from mild to severe illness (1,2). In some cases, the SARS-CoV-2 infection can be asymptomatic (3). Due to the non-specific symptoms or asymptomatic course of novel coronavirus infection, the diagnosis is based on the detection of viral genetic material by the molecular techniques, i.e., reverse transcription and polymerase chain reaction (RT-PCR) or loop-mediated isothermal amplification (LAMP) (4,5). Currently, the RT-PCR is a gold standard in the diagnosis of SARS-CoV-2 infection due to its high sensitivity and specificity, internal controls of the reaction as well as possibility to run many samples at once (6,7).

Based mostly on the RT-PCR results, from January 1 to December 31, 2020 the World Health Organization reported almost 81.5 million confirmed cases of SARS-CoV-2 infections and over 1.8 million deaths related to the virus worldwide (8,9). The most affected countries with highest numbers of new SARS-CoV-2 infections and COVID-19 related deaths among European Union were Italy, Spain, France, Belgium and UK (10,11). At the same time, Poland was hit by “two waves” of the pandemic, i.e. from 10.03.2020 to 20.04.2020 and from 04.10.2020 to 27.12.2020, with 1.3 million confirmed SARS-CoV-2 infections and 28.5 thousand deaths caused by the novel coronavirus (12). Due to the political decisions to test only symptomatic patients (12), nonspecific symptoms of COVID-19 (1,2), and asymptomatic SARS-CoV-2 infections (3), those numbers may be underestimated.

The aims of the present sero-epidemiological study were to estimate the prevalence of SARS-CoV-2 antibodies in the population of the Poznań metropolitan area.
(Poland) after the first wave of the COVID-19 pandemic, to find the risk factors associated with COVID-19 and to compare the immunoassays used in detection of anti-SARS-CoV-2 antibodies.

2. Materials and Methods

2.1. Study design and participants

We randomly selected and invited 1500 adult (over 18 years old) study participants from approximately 15,000 volunteers living in the Poznań metropolitan area, Poland, who answered the online epidemiological survey. The web-based survey was opened for 5 days, i.e. 18-23.07.2020 and broadly advertised in local and national newspapers, radio, TV, web portals as well as social media. Demographic data, including age, gender and occupation of each participant, were collected. Additionally, we asked volunteers about their current health status, potential flu-like symptoms in the last nine months and behavior during the COVID-19 pandemic, namely compliance with epidemiological recommendations. To estimate the severity of an individual's flu-like symptoms, we developed a scoring system based on subjects' self-assessment answers. If the patient did not show any symptoms, a value of 0 was assigned. Each symptom, such as fever, cough, runny nose, fatigue, muscle and joint pain, sore throat, headache, diarrhea and loss of smell or taste, was rated as 1, and hospitalization due to the flu-like symptoms was rated as 5. Additionally, the study participants were asked to compare flu-like symptoms in the last nine months before the serological test to flu-like symptoms in the previous years. Symptoms of flu-like illness in the last nine months milder than in the past were graded as 0, the same as 3 and more severe as 5. Based on the total sum of values of individual responses, study participants were divided into four groups: “asymptomatic” (0 points), “mild” (1–5 points), “moderate” (6–14 points) or “severe” (15–24 points). A similar classification approach was implemented to assess individuals' compliance with epidemiological recommendations. If the study participant did not follow any recommendations, a value of 0 was assigned. Each of the preventive measures, such
as face mask use, disinfection and social distance, remote work, avoiding contact with other people, and avoiding the use of public transport, was rated as 1. Depending on the total sum of values of individual responses, study participants were divided into three groups: “non-compliant” (0 points), “at some point” (1–2 points) and “in general” (3–4 points) compliant with epidemiological recommendations.

2.2. Laboratory analysis

The blood samples were collected from individuals from July 28, 2020 to September 24, 2020 (Figure S1) at the Wielkopolskie Centrum Medycyny Podróży, Poznan, and transferred to the IBCH PAS for analysis of anti-SARS-CoV-2 antibodies presence. In case of ELISA positive results for the presence of anti-SARS-CoV-2 IgA or borderline results for the anti-SARS-CoV-2 IgG, the study participants were asked to come again within 2–3 weeks and a blood collection as well as an ELISA analysis were repeated. For five study participants with anti-SARS-CoV-2 IgG-positive results, we did the follow-up analysis at 10 weeks after the first test.

The presence of IgA and IgG antibodies against SARS-CoV-2 was determined using anti-SARS-CoV-2 IgA ELISA (EuroImmun) or anti-SARS-CoV-2 IgG ELISA (EuroImmun) assays, respectively. The chosen immunoassays recognized specific anti-SARS-CoV-2 antibodies against the spike (S) protein and according to Beavis and colleagues (13) demonstrates good and excellent specificity for IgA and IgG antibodies, respectively. All anti-SARS-CoV-2 IgG positive-samples were confirmed with quantitative anti-SARS-CoV-2 IgG immunoblot (Polycheck), which uses the S protein and phosphorylated nucleocapsid protein (PNC) as antigens.

2.3. Statistical analysis

The categorical variables were presented as counts and percentages, and the seroprevalence estimates were presented together with 95% CI. The 95% CI of the seroprevalence was calculated using the hybrid Wilson/Brown method. The
differences between groups were analyzed with Mann-Whitney or Kruskal-Wallis tests. All statistical analyses were performed with the GraphPad Prism 9 software.

2.4. Ethics approval

The study was approved by the Bioethics Committee at the Poznan University of Medical Sciences, Poznan, Poland (Resolution No. 470/20 from 17th June 2019). In addition, written informed consent was obtained from 1500 study participants before the blood collection.

3. Results

3.1. Characteristics of study participants

The study group included 1500 adults without any flu-like symptoms at the sampling time and consisted of 896 (59.7%) female and 604 (40.3%) male subjects at mean age 38.7 ± 12.7 years old (Table 1). Within the volunteers, 964 (64.2%) did not report any chronic diseases, while 536 (35.8%) study participants were treated due to the chronic diseases, such as hypertension, asthma, Crohn disease, rheumatoid arthritis, diabetes, depression. (Table 2). In the last 9 months before the serological tests, 1240 (82.7%) study participants reported flu-like symptoms, i.e., fever, cough, fatigue, muscle and joint pain, etc. (Table 2), mostly with mild (454/1240, 36.6%) and moderate (720/1240, 58.1%) severity. In addition, 356 (23.7%) of volunteers with flu-like symptoms in the 9 last months before blood collection or with potential contact with SARS-CoV-2 infected person, were tested for the presence of SARS-CoV-2, and 7 (1.9%) got the positive result (Table 1). Most of study participants (96.9%) followed the epidemiological recommendations, i.e., wearing mask covering nose and mouth, keeping social distance and often hand disinfection. Only 40 (2.7%) individuals had known contact with SARS-CoV-2 infected person, while majority, i.e., 1085 (72.3%) study participants hadn’t known about such situations (Table 3).

3.2. Anti-SARS-CoV-2 seroprevalence
In total, 25 of 1500 (1.67%) collected samples were found to be positive for anti-SARS-CoV-2 IgG antibodies by the ELISA (Table 1). Simultaneously, 60 of 1500 (4.0%) collected samples were found to be positive for anti-SARS-CoV-2 IgA antibodies, but in any of those cases, we did not observe IgA to IgG seroconversion (data not shown). There were no significant differences in seroprevalence between the weeks when the blood was collected (data not shown). We did not observe the significant differences in the seroprevalence within gender (Table 1). The differences in the anti-SARS-CoV-2 antibodies presence were found among group at different age (Table 1) and with different health status (Table 2). Based on the ELISA results, the highest seropositivity was found among people over 65 years old, and study participants with respiratory system chronic diseases (Table 2).

The antibodies against the novel coronavirus were found in only 3 (42.9%) and 8 (2.3%) study participant with positive and negative PCR-based test result, respectively. We found the anti-SARS-CoV-2 antibodies in 14 samples from volunteers previously not tested with PCR or antigen tests (Table 1). Additionally, we found that among the tested study participants, 8/25 (35%) of SARS-CoV-2 infections followed an asymptomatic course (Table 2).

### Table 1. Prevalence of anti-SARS-CoV-2 IgG antibodies in Poznań metropolitan area.

| Category        | Participants | ELISA | Immunoblot |
|-----------------|--------------|-------|------------|
|                 |              | Positive results | Seroprevalence (95% CI) | Positive results | Seroprevalence (95% CI) |
| Overall         | 1500         | 25     | 1.67%     | (1.13 – 2.45) | 14     | 0.93%     | (0.56 – 1.56) |
| Gender          |              |        |           |               |        |           |               |
| Female          | 896          | 14     | 1.56%     | (0.93 – 2.60) | 8      | 0.89%     | (0.45 – 1.75) |
| Male            | 604          | 11     | 1.82%     | (1.02 – 3.23) | 6      | 0.99%     | (0.46 – 2.15) |
| Age             |              |        |           |               |        |           |               |
| 18 – 33         | 623          | 10     | 1.61%     | (0.87 – 2.92) | 5      | 0.80%     | (0.34 – 1.87) |
| 34 – 49         | 606          | 11     | 1.82%     | (1.02 – 3.22) | 7      | 1.16%     | (0.56 – 2.37) |
| 50 – 65         | 194          | 2      | 1.03%     | (0.18 – 3.68) | 0      | 0.00%     | (0.00 – 1.94) |
| 65+             | 77           | 2      | 2.60%     | (0.46 – 8.99) | 2      | 2.60%     | (0.46 – 8.99) |

**Test for the SARS-CoV-2 presence**
Table 2. Anti-SARS-CoV-2 seroprevalence related to health status and severity of last flu-like illness.

| Category                  | Participants | ELISA Positive results | Seroprevalence (95%CI) | Immunoblot Positive results | Seroprevalence (95%CI) |
|---------------------------|--------------|------------------------|------------------------|-----------------------------|------------------------|
| Chronic diseases          |              |                        |                        |                             |                        |
| None                      | 964          | 16                     | 1.66%                  | (1.02 – 2.70)               | 11                     | 1.14%                  | (0.64 – 2.03)           |
| CS-CDs                    | 185          | 1                      | 0.54%                  | (0.03 – 3.00)               | 1                      | 0.54%                  |                             |
| RS-CDs                    | 78           | 3                      | 3.85%                  | (1.05 – 10.71)              | 0                      | 0.00%                  |                             |
| I-CDs                     | 8            | 0                      | 0.00%                  | (0.00 – 32.44)              | 0                      | 0.00%                  |                             |
| CKD                       | 1            | 0                      | 0.00%                  | (0.00 – 94.87)              | 0                      | 0.00%                  | (0.00 – 94.87)           |
| DT-CDs                    | 23           | 0                      | 0.00%                  | (0.00 – 14.31)              | 0                      | 0.00%                  | (0.00 – 14.31)           |
| A-CDs                     | 120          | 4                      | 2.33%                  | (0.90 – 5.76)               | 1                      | 0.59%                  | (0.03 – 3.26)            |
| NeoD                      | 5            | 0                      | 0.00%                  | (0.00 – 43.45)              | 0                      | 0.00%                  | (0.00 – 43.45)           |
| MetD                      | 50           | 1                      | 2.00%                  | (0.10 – 10.50)              | 1                      | 0.00%                  | (0.10 – 10.50)           |
| MentD                     | 29           | 0                      | 0.00%                  | (0.00 – 11.70)              | 0                      | 0.00%                  | (0.00 – 11.70)           |
| Severity of flu-like illness in the last 9 months before serological tests |              |                        |                        |                             |                        |
| No symptoms               | 260          | 8                      | 3.08%                  | (1.57 – 5.95)               | 5                      | 1.92%                  | (0.82 – 4.42)            |
| Mild                      | 454          | 5                      | 1.10%                  | (0.42 – 2.30)               | 4                      | 0.88%                  | (0.34 – 2.24)            |
| Moderate                  | 720          | 12                     | 1.67%                  | (0.96 – 2.89)               | 5                      | 0.69%                  | (0.30 – 1.62)            |
| Severe                    | 66           | 0                      | 0.00%                  | (0.00 – 5.50)               | 0                      | 0.00%                  | (0.00 – 5.50)            |

CDs – chronic diseases; CS-CDs – circulatory system CDs, i.e. hypertension; RS-CDs – respiratory system CDs, i.e. asthma; I-CDs – infectious CDs, i.e. HIV/AIDS; CKDs – chronic kidney disease; DT-CDs – digestive track CDs, i.e. Crohn disease; A-CDs – autoimmune chronic diseases, i.e. allergies; NeoD – neoplastic diseases, i.e. cancer; MetD – metabolic diseases, i.e. diabetes; MentD – mental disorders, i.e. depression

3.3. SARS-CoV-2 infection risk factors

One of the highest anti-SARS-CoV-2 IgG seroprevalence was found among study participants who travelled abroad in the last 9 months before serological tests (Table 3). Others were individuals who did not follow epidemiological recommendations and persons who had direct contact with SARS-CoV-2 (Table 3). Based on the ELISA
results, we did not find significant differences in the anti-SARS-CoV-2 antibodies presence between individuals working at different positions.

### Table 3. SARS-CoV-2 infection risk factors.

| Category                                                      | ELISA Positive results | Seroprevalence (95%CI) | Immunoblot Positive results | Seroprevalence (95%CI) |
|---------------------------------------------------------------|-------------------------|-------------------------|------------------------------|-------------------------|
| **Occupation related to constant contact with people (i.e., physicians, nurses, shop assistants, civil servants)** |                         |                         |                              |                         |
| Yes                                                          | 749                     | 11                      | 1.47%                        | 9                       | 1.20%                    |
|                                                              |                         |                         | (0.82 – 2.61)                |                         | (0.63 – 2.27)            |
| No                                                           | 751                     | 14                      | 1.86%                        | 5                       | 0.67%                    |
|                                                              |                         |                         | (1.11 – 3.11)                |                         | (0.29 – 1.55)            |
| **Travelling abroad**                                        |                         |                         |                              |                         |
| Yes                                                          | 574                     | 16                      | 2.93%                        | 8                       | 1.46%                    |
|                                                              |                         |                         | (1.81 – 4.70)                |                         | (0.74 – 2.86)            |
| No                                                           | 953                     | 9                       | 0.94%                        | 6                       | 0.63%                    |
|                                                              |                         |                         | (0.50 – 1.78)                |                         | (0.29 – 1.37)            |
| **Compliance with epidemiological recommendations (i.e. remote work, wearing a mask covering nose and mouth, avoiding contact with other people, avoiding the use of public transport)** |                         |                         |                              |                         |
| No                                                           | 46                      | 1                       | 2.17%                        | 1                       | 2.17%                    |
|                                                              |                         |                         | (0.11 – 11.33)               |                         | (0.11 – 11.34)           |
| At some point                                                | 405                     | 6                       | 1.48%                        | 4                       | 0.99%                    |
|                                                              |                         |                         | (0.66 – 3.19)                |                         | (0.39 – 2.51)            |
| Yes, in general                                              | 1049                    | 18                      | 1.72%                        | 9                       | 0.86%                    |
|                                                              |                         |                         | (1.09 – 2.70)                |                         | (0.45 – 1.62)            |
| **Known contact with SARS-CoV-2 infected person**            |                         |                         |                              |                         |
| Yes                                                          | 40                      | 2                       | 5.00%                        | 2                       | 5.00%                    |
|                                                              |                         |                         | (0.85 – 15.79)               |                         | (0.89 – 16.50)           |
| No                                                           | 375                     | 7                       | 1.87%                        | 5                       | 1.33%                    |
|                                                              |                         |                         | (0.91 – 3.80)                |                         | (0.57 – 3.08)            |
| Not known                                                    | 1065                    | 16                      | 1.47%                        | 7                       | 0.65%                    |
|                                                              |                         |                         | (0.91 – 2.38)                |                         | (0.31 – 1.33)            |

3.4. Comparison of ELISA and immunoblot methods of anti-SARS-CoV-2 antibodies detection

The presence of anti-SARS-CoV-2 IgG antibodies was confirmed with immunoblot in 14 of 25 samples (56%) (Table 1). For most analyzed parameters, the trend in the anti-SARS-CoV-2 seropositivity was not changed, i.e. based on the immunoblot, study participant over 65 years old, individuals who did not follow the epidemiological recommendations and people with direct contact with SARS-CoV-2 were characterized with highest seroprevalence (Table 1, 2, 3). At the same time, the
immunoblot analysis revealed that there are significant differences in the seroprevalence between individuals whose occupation was related with constant contact with other people, e.g., physicians, nurses, shop assistants, civil servants (Table 3). The ELISA false positive results were found mostly among study participants between 18 and 33 years old (Table 1), as well as among volunteers with autoimmunological and respiratory system chronic diseases (Table 2).

3.5. Anti-SARS-CoV-2 antibodies level

With quantitative immunoblots, we were able not only to confirm the ELISA results, but also to analyze the levels of anti-SARS-CoV-2 phosphorylated nucleoprotein (PNC) and anti-SARS-CoV-2 spike (S) antibodies. We found different levels of analyzed antibodies among study participants, as shown in Figure 1a. In addition, the anti-SARS-CoV-2 PNC and anti-SARS-CoV-2 S antibodies level differed within the same samples. Due to the low number of positive individuals, we did not correlate the antibodies level with previously mentioned parameters, i.e., age, gender.

With five study participants we performed follow-up study and analyzed the level of anti-SARS-CoV-2 antibodies at 10 weeks after the first blood collection. As shown in Figure 1b, we did not observe any significant differences, but in 4 of 5 samples we reported slight decrease in the antibody level. For one sample, we found an increase in anti-SARS-CoV-2 S antibodies level (Figure 1b).
Figure 1. Anti-SARS-CoV-2 antibodies levels among volunteers. (a) The total levels of anti-SARS-CoV-2 phosphorylated nucleoprotein (PNC) and anti-SARS-CoV-2 spike (S) antibodies among study participants (N=14). (b) The changes in the anti-SARS-CoV-2 PNC (left) and anti-SARS-CoV-2 S (right) antibodies levels within 10 weeks in five study participants.

4. Discussion

For many months, the reported novel coronavirus infection cases and COVID-19-related mortality in Poland were among the lowest in Europe (8,9,11,12). For example, on 1 October 2020, there were 2,469.99 confirmed SARS-CoV-2 cases and 67.20 COVID-19-related deaths per 1 million of citizens, but at the same time, in Spain and Germany there were 16,652.99 and 3,527.39 confirmed infections and 683.84 and 113.49 deaths per 1 million of citizens, respectively (8,9). Those differences may be explained by the early implementation of public health measures in Poland, such as the closing of primary schools and the so-called deep lockdown in March and April 2020, just after the first confirmed SARS-CoV-2 infections. In addition, the discrepancies in the novel coronavirus cases between Poland and other comparable European Union members may result from the number of performed diagnostic tests. In example, at the beginning of October 2020, an average of 22,125 tests were performed per day in Poland. In comparison, there were 679,134 and 1,123,823 tests performed daily in Spain and Germany, respectively (14). Finally, the differences in pandemic scale between Poland and other countries may be due to the political decisions to test only symptomatic patients (12) and the lack of free tests for the presence of novel coronavirus for general population. This excludes individuals with asymptomatic SARS-CoV-2 infection from official statistics and does not prevent emergence of new epidemic foci, since the asymptomatic SARS-CoV-2 infected persons still can infect others (3,15,16). And according to our results, as well as data presented by others, the asymptomatic rate can range from 20% to up to 80% (17,18). All above mentioned reasons may cause that the official numbers of SARS-CoV-2 infections are underestimated.
The estimation of scale of COVID-19 pandemic, as well as objective comparison of different populations, can be done by the sero-epidemiological studies. Our data demonstrates low (0.93%) seroprevalence of anti-SARS-CoV-2 antibodies in general population of Poznan metropolitan area. Poznan is one of the biggest cities in Poland with almost 1 million inhabitants and therefore may represent the situation in other Polish metropolitan and big cities, which in total represents approximately 30% of the society. However, it should be noted that the seroprevalence can differ in smaller cities and villages, due to among others the lower access to health care system and diagnostic centers, as shown by others (19,20). Based on our results of anti-SARS-CoV-2 seroprevalence, i.e. 0.93%, demographic data, i.e. 3.50 million of citizens and numbers of confirmed SARS-CoV-2 infections presented by the Polish Ministry of Health, we calculated that in September 2020, just before the so-called second wave of pandemic, approximately 4-fold more infections occurred than were reported by the government, i.e. 32,550 SARS-CoV-2 infections based on the seroprevalence vs. 7,985 COVID-19 cases from official statistics. These discrepancies may result from the asymptomatic SARS-CoV-2 infection, as mentioned above, reluctance to coronavirus diagnostic tests, which are potentially linked with mandatory 10 days of quarantine. The hesitation in testing might result from the lack of trust for the healthcare workers in the Polish society. Based on the YouGov data, in case of the ongoing pandemic, the Poles trust the most family and friends, in contrary to other European nations who ranked medical experts as the most trusted (21).

In comparison to Poznan, the anti-SARS-CoV-2 seroprevalence at similar time points, in other metropolitan cities were higher, i.e., in Madrid, Spain, was equal to 13.6% (22); in Geneva, Switzerland, it was 10.8% (23), and in Tehran, Iran, it was 16.3% (20). This situation ensured that as of September 2020, the Polish population remained largely immunologically naïve to the virus. The low seroprevalence in Poland highlights also the importance of the vaccination against COVID-19. It is estimated, that the spread and transmission of SARS-CoV-2 will be stopped with 60-70% of the
population being vaccinated (24). It is impossible and extremely dangerous to reach
this level of herd immunity based on the SARS-CoV-2 infections.

In addition to the estimation which percentage of Poznan metropolitan area citizens
underwent novel coronavirus infection, we found the SARS-CoV infection risk
factors in Polish population. Namely, contact with SARS-CoV-2 infected individuals,
age over 65 y.o., non-compliance with epidemiological recommendations, travelling
abroad and occupation related with constant contact with people (i.e., physicians,
nurses) are linked with higher seroprevalence. Our data confirmed previously
published results (19,25).

Moreover, it should be noted that besides many strengths, such as relatively big
group of study participants and anti-SARS-CoV-2 antibodies detection with two
independent techniques, our study has some limitations. First of all, we observed
overrepresentation of woman in the study, i.e., 59.7% vs. 51.5% in the Polish
population, probably due to higher willingness to participate in online surveys (26).
However, since we did not detect significant differences in the seroprevalence among
gender, this fact should not bias the final results. In addition, the individuals
travelling abroad was also overrepresented in the study. According to the Polish
Tourism Organisation, 54% of the Polish population went for some vacations, and
18.3% of them travelled abroad in 2019 (27). This fact may lead to some bias and
overestimation of the seroprevalence. On the other hand, there is underrepresentation
of study participant over 65, i.e., 5.1% vs. 22% in Polish society. This fact can be
explained by the online survey and problems of older people with modern
technology (28). In addition, there were many volunteers, who wanted to participate,
but due to the COVID-19 pandemic, high risk of severe COVID-19 in this age group,
and recommendation to stay at home and isolate from others, they cancelled the
meetings and were replaced by next random person from the list. Finally, our results
can be bias due to the sensitivity and specificity of serological tests. Currently, it was
published that many serological tests, including EuroImmun ELISA used in our
study, cannot detect the anti-SARS-CoV-2 antibodies at low levels, characteristic for
so-called non-responders (29). This might explain why we did not detect the antibodies in 4 of 7 study participants with positive results of RT-PCR test. The problem with specificity of serological tests was observed also by us in case of anti-SARS-CoV-2 IgA antibodies. In any case of individuals with positive result of IgA antibodies, we did not observed seroconversion. The false positive results of the presence of IgA antibodies were mostly correlated with allergies, which was not described by the manufacturer. It should be also highlighted that ELISA is a very good screening method, but the results should be confirmed by immunoblot. As shown in our study for the first time, the ELISA false positive signals can be as high as almost 50% of all positive results.

5. Conclusions

To our knowledge, this is the first study which demonstrate the anti-SARS-CoV-2 antibodies seroprevalence in the general population in Poland after the first wave of COVID-19 pandemic. Our findings confirm the low SARS-CoV-2 incidence in Poland probably due to the effectiveness of early countermeasures. However, based on the seroprevalence of 0.93%, it should be noted that the official numbers of novel coronavirus infections were underestimated and the approximately 4-fold more infections occurred than were reported by the Polish Ministry of Health. The low anti-SARS-CoV-2 seroprevalence implies that the population had little herd immunity heading into the second and third wave of the pandemic, and therefore herd immunity contributed little to prevent the high numbers of SARS-CoV-2 infections and COVID-19 related deaths in Poland during these subsequent waves. Finally, taking into account all above mentioned limitations of our study, the obtained seroprevalence may be underestimated. Therefore, further studies on the SARS-CoV-2 burden in Poland are needed.
**Supplementary Materials**: Figure S1. SARS-CoV-2 infections and COVID-19 related deaths in Poland during the COVID-19 pandemic. Data collected and published online by Michal Rogalski. Blue – time of blood collection from study participants in Poznan metropolitan area.

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**Informed Consent Statement**: Informed consent was obtained from all subjects involved in the study.

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