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Regular paper

The determination of haemagglutinin influenza antibodies in the Polish population in the epidemic season 2020/2021 during the SARS-CoV-2 pandemic

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The aim of the study was to prove the level of antibodies against haemagglutinin in the sera of people from seven age groups in the epidemic season 2020/2021 in Poland to determine the differentiation of the antibody level and the protection rate depending on age. The level of anti-haemagglutinin antibodies was established by haemagglutinin inhibition test (HAI). A total of 700 randomly selected sera from people belonging to 7 different age groups were tested. The results confirmed the presence of antibodies against the following influenza antigens: A/Guangdong-Maonan/SWL1536/2019 (H1N1)pdm09-like virus, A/Hong Kong/2671/2019 (H3N2)-like virus, B/Washington/02/2019 (B/Victoria lineage)-like virus and B/Phuket/3073/2013 (B/Yamagata lineage)-like virus. The level of haemagglutinin antibodies varied between the studied age groups, with the highest values in the 5–9 age group and the lowest in the 0–4 age group. It was also proven that the protection rate was the highest for the A/Hong Kong/2671/2019(H3N2)-like virus antigen, which exceeded the protection level in the 5 age groups. Considering the very low percentage of people vaccinated in the epidemic season 2020/2021 in Poland, which amounted to only 6.1%, the results should be interpreted as the immune system's response to an infection with influenza virus.

Keywords: hemagglutinin antibodies, influenza, protection rate, GMT

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Abbreviations: HAI, haemagglutinin inhibition test

INTRODUCTION

The flu virus belongs to the Orthomyxoviridae family. It infects the epithelial cells of the nose, larynx, trachea and bronchi damaging the epithelium of the respiratory system. There are 3 types of influenza virus: A, B and C, with types A and B causing seasonal flu infections in humans (Brydak, ch. II, 2008; Kowalczyk *et al.*, 2019). Influenza A is classified into different HxNx subtypes according to surface antigens: haemagglutinin (H), which has 18 subtypes, and neuraminidase (N), which has 11 subtypes (Tong *et al.*, 2013; Wu *et al.*, 2014). Influenza B virus also has these glycoproteins on its surface. There are two antigenically and phylogenetically different lines: Victoria and Yamagata (Ghebrehewet *et al.*, 2016). Influenza B does not cause a pandemic unlike influenza A (Caini *et al.*, 2015). The most common subtypes among humans are A/H1N1/, A/H1N1/pdm09, A/H2N2/, A/H3N2/ and B. However, from time to time, infections with the following subtypes can be found: A/H5N1/, A/H9N2/, A/H7N2/, A/H7N7/, which are more common in animals. Influenza B virus infects only humans (Brydak, ch. IV, 2008). During an infection, the human immune system produces antibodies against the virus surface glycoproteins (Johansson *et al.*, 1989). Haemagglutinin is responsible for the adsorption of a virus particle to a cell receptor in the host organism. This glycoprotein can bind to cell membranes and facilitate the integration of the viral envelope into the membranes of the infected cell. This process allows the virion to enter the cytoplasm of the cell and release its internal structures (Brydak, ch. II, 2008). Neuraminidase allows for the later split and release of the virus from the host cells (Paules & Subbarao, 2017). It should be noted that in respiratory infections, different clinical symptoms could be caused by the same virus, and on the other hand, the same set of symptoms could be caused by more than 200 different respiratory viruses (e.g., RSV, parainfluenza viruses, metapneumoviruses, adenoviruses, rhinoviruses, coronaviruses, enteroviruses, etc.) (Brydak, ch. VII, 2008). For this reason, the laboratory confirmation of an influenza virus infection is fundamental to influenza surveillance, and it is essential to assess the effectiveness of vaccines and antiviral drugs. Laboratory diagnosis of influenza involves confirming the presence of the influenza virus antigen in the material collected from the patient or detecting elevated levels of specific antibodies in the patient's serum (Brydak, ch. VII, 2008; Kowalczyk *et al.*, 2017). The constant evolution and variability of the virus are the causes of seasonal epidemics and occasional pandemics in the human population. Especially the A type viruses are constantly changing due to mutations, which has made it necessary to update the vaccine composition in every season (Carrat & Flahault, 2007; Tanner *et al.*, 2021). *Antigenic drift* is a point mutation in genes that changes the sequence of amino acids that modify antigenic sites; because of this process a seasonal epidemic takes place. *Antigenic shift*, on the other hand, occurs when, in the same cell infected with more than one strain of virus, one or more of their RNA fragments are replaced, creating a separate virus

Table 1. Strains used in vaccines in subsequent epidemic seasons.

Epidemic season	A/H1N1/pdm09	A/H3N2/	B/Victoria lineage	B/Yamagata lineage
2017/2018 (WHO, 2017)	A/Michigan/45/2015 (H1N1) pdm09-like virus	A/Hong Kong/4801/2014 (H3N2) – like virus	B/Brisbane/60/2008	B/Phuket/3073/2013
2018/2019 (WHO, 2018)	A/Michigan/45/2015 (H1N1) pdm09-like virus	A/Singapure/INFIMH-16-0019/2016 (H3N2) – like virus	B/Colorado/06/2017--like virus	B/Phuket/3073/2013--like virus
2019/2020 (WHO, 2019)	A/Brisbane/02/2018 (H1N1) pdm09-like virus	A/Kansas/14/2017/ (H3N2)-like virus	B/Colorado/06/2017--like virus	B/Phuket/3073/2013--like virus
2020/2021 (WHO, 2020)	A/Guangdong-Maonan/ SWL1536/2019 (H1N1) pdm09-like virus	A/Hong Kong/2671/2019 (H3N2)--like virus	B/Washington/02/2019--like virus	B/Phuket/3073/2013--like virus

and thus causing a pandemic. Therefore, it is necessary to vaccinate against the currently circulating strains of the influenza virus every season (Table 1) (Brydak, ch. IV, 2008). Haemagglutinin binds to sialic acid receptors on host cells and it is the main target of neutralizing antibodies (Chen & Subbarao, 2009). The serum anti-HA antibody levels are measured by the haemagglutination inhibition assay (HAI) (Gilbert *et al*., 2019). The method is based on the ability of anti-HA antibodies to inhibit virus-induced erythrocyte agglutination (WHO, 2011). HAI measures the highest serum dilution that prevents haemagglutination of erythrocytes induced by exposure to influenza virus (Gilbert *et al*., 2019). The haemagglutination inhibition assay (HAI) has a wide range of uses and it has long been used for assessing resistance to influenza.

Anti-HA antibody titers of 1:40 have been found to correspond to a 50% reduction of risk of influenza infections in the population (Hobson *et al*., 1972).

The presence of anti-haemagglutinin (anti-HA) antibodies not only protects against an infection with specific strains of influenza virus, but can also reduce the disease symptoms in case of an infection with another variant of the virus. This is attributed to the occurrence of cross-reactive antibodies. It has been shown that subtype-specific anti-HA antibodies can reduce the infectivity of other virus subtype by disrupting the proliferation and release of viral particles during an infection (Bednarska *et al*., 2015). The goal of vaccinating against influenza every season is to generate the protective levels of antibodies. This is currently the most effective method of reducing the disease-related morbidity and mortality. Antibodies detected in the sera of unvaccinated people arise from past infections (Brydak, ch. X, 2008; Hallmann-Szelińska *et al*., 2018); therefore, each vaccination against seasonal influenza increases immunity to infections and in consequence reduces the risk of complications caused by influenza. During the 2020/2021 epidemic season, according to WHO recommendations (WHO, 2020) for the northern hemisphere, influenza vaccines contained the following virus strains:

- A/Guangdong-Maonan/SWL1536/2019 (H1N1) pdm09-like virus;
- A/Hong Kong/2671/2019 (H3N2)-like virus;
- B/Washington/02/2019 (B/Victoria lineage)-like virus;
- B/Phuket/3073/2013 (B/Yamagata lineage)-like virus.

During the 2020/2021 epidemic season, 6.1% of the Polish population were vaccinated (Brydak, 2021). Seasonal vaccination protects against an infection with currently circulating viruses and provides cross-protection that can reduce viral replication and, in this way, alleviate the course of the disease. Influenza viruses pose a threat to people of all ages and latitudes, especially those at high risk: people over 65; children up to 2 years old; pregnant women; people with asthma, diabetes, cancer, heart disease and people living in nursing homes or other long-term care facilities. These people particularly should be vaccinated against influenza every season (Brydak, ch. X, 2008). The epidemic season in the northern hemisphere lasts from the beginning of October of a given year to the end of September of the following year (52 weeks). The peak of influenza incidence usually occurs in Europe between January and March (Brydak, ch. VIII, 2008). In week 28/2021 (05–11.07.2021), 943 flu cases were reported to the European Surveillance System (TESSy) across the entire European region of the World Health Organization (WHO); 51% were influenza A viruses, with A/H3N2/ and A/H1N1/pdm09 being approximately equally represented, and 49% were B viruses with only 16 assigned to a lineage – 13 to the B/Victoria lineage and 3 to the B/Yamagata lineage. Compared to the same period in 2020, there are 99.4% fewer infections detected, possibly due to the COVID-19 pandemic and measures applied to prevent it (ECDC, 2021).

MATERIALS AND METHODS

Sera were obtained from patients from 7 age groups (0–4, 5–9, 10–14, 15–25, 26–44, 45–46 and 65+) at 7 sanitary and epidemiological stations in Poland. The samples were stored at –30°C until the test. A total of 700 samples (100 per age group) were tested. The antibody level was determined using the haemagglutination inhibition assay (HAI), as the antigen of the influenza viruses recommended by WHO for the season 2020/2021 (Table 2) (WHO, 2020) were used, which were propagated in chicken embryos, prepared and diluted according to the WHO protocol (WHO, 2011).

The study was performed in accordance with the WHO Laboratory Flu Surveillance Manual (2011).

Table 2. Influenza viruses used in the haemagglutinin inhibition assay (HAI) (WHO, 2020)

Epidemic season	A/H1N1/pdm09	A/H3N2/	B/Victoria lineage	B/Yamagata lineage
2020/2021	A/Guangdong-Maonan/ SWL1536/2019 (H1N1) pdm09-like virus	A/Hong Kong/2671/2019 (H3N2)-like virus	B/Washington/02/2019--like virus	B/Phuket/3073/2013-like virus

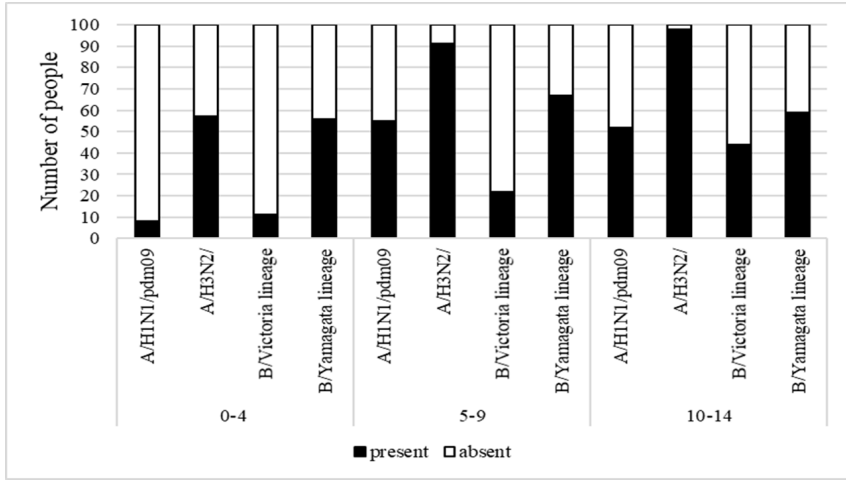

Figure 1. The presence of antibodies in the serum from the 0–4, 5–9 and 10–14 age groups in the epidemic season 2020/2021

The following parameters were used to analyze the results:

Geometric mean titers of anti-haemagglutinin antibodies in the tested sera of patients with positive antibodies (GMT).

Protection rate, i.e. the minimum percentage of individuals with anti-haemagglutinin antibody titers ≥40.

The SPSS12.0 PL suite was used for statistical analysis.

The chi-square test was used for intergroup comparisons for qualitative variables (having antibodies by virus type, achieving protective levels of antibodies in age groups). The significance of differences in the level of antibodies in the analyzed age categories was assessed using the Kruskal-Wallis test. A significance level of 0.05 was adopted in all statistical analyses.

RESULTS

The presence of antibodies against all the 4 analyzed antigens was found in the sera of all the age groups studied (Fig. 1 and Fig. 2). The percentages of people with antibodies varied in a statistically significant way for all the 4 viruses, $p<0.001$. Antibodies against B/Washington/02/2019 (B/Victoria lineage)-like virus were found in the serum of the smallest number of patients (37.1%). In contrast, antibodies against A/Hong Kong/2671/2019 (H3N2)-like virus were detected in the largest number of samples. The geometric mean titers of anti-HA antibodies in the sera of people with antibodies from different age groups in the 2020/2021 epidemic season are presented in Fig. 3. The highest level of antibodies against haemagglutinin of A/Guangdong-Maonan/SWL1536/2019 (H1N1) pdm09-like virus was found in the 5–9 age group (GMT-50). The GMT of anti-H1 antibodies was slightly lower in the 15–25 (GMT-41) and 26–44 (GMT-36) age groups. The lowest level of anti-H1 antibodies was observed in the 0–4 (GMT-11) and 10-14 (GMT-11) age groups. In the oldest age groups (45–64 and 65+), the results were average and similar (21 and 20, respectively). In the case of the A/Hong Kong/2671/2019 (H3N2)-like virus antigen, the largest differences between the age groups were observed. The highest average titers were recorded in the 5–9 (GMT-186) and 10–14 (GMT-172) age groups; the lowest in the 45–64 (GMT-30) and 0–4 (GMT-31) age groups. The highest level of antibodies against B/Washington/02/2019 (B/Victoria lineage)-like virus was found in the 5–9 age group (GMT-55), while the lowest was in the 0–4 age group (GMT-12). The results for the remaining categories ranged from GMT-15 (for the 10-14 age group) to GMT-27 (for the 26–44 age group). On the other hand, for the B/Phuket/3073/2013 (B/Yamagata lineage)-like virus line, the difference in the antibody level among the examined age groups was the lowest. The high-

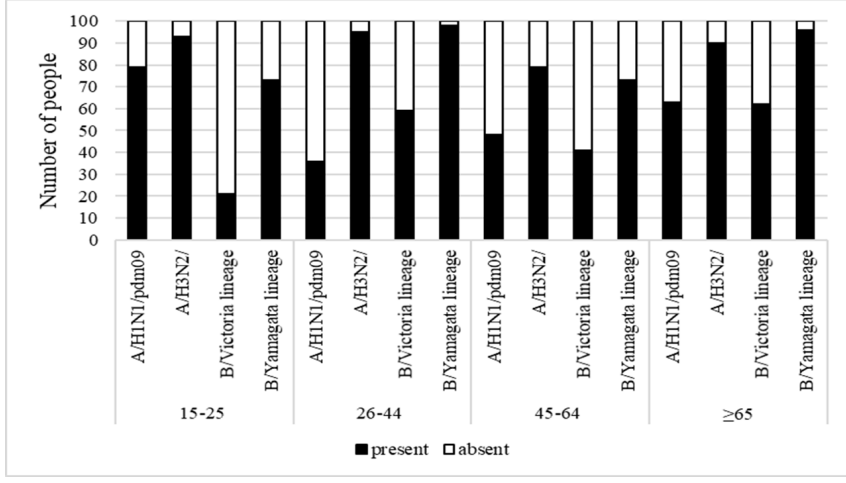

Figure 2. The presence of antibodies in the serum of the 15–25, 26–44, 45–64 and 65+ age group in the epidemic season 2020/2021

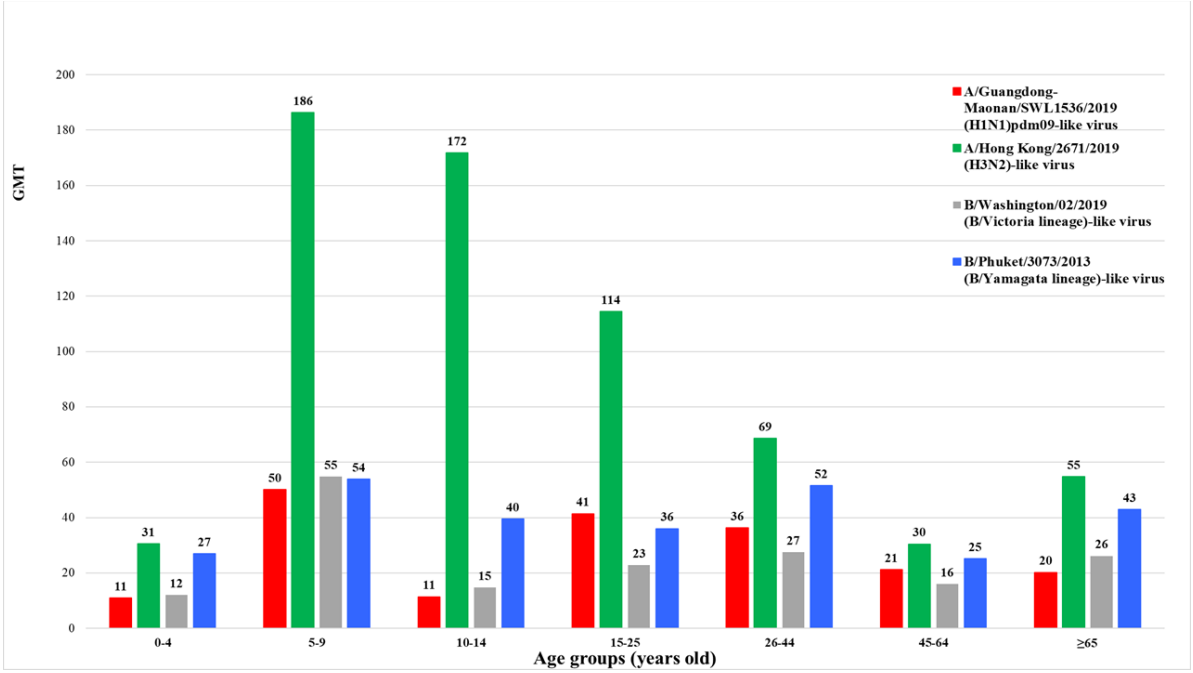

Figure 3. The geometric mean titers of anti-haemagglutinin antibodies (GMT) in the epidemic season 2020/2021 according to age groups in Poland

est level of antibodies was found in the 5–9 age group and amounted to GMT-54, while the lowest for the 45-64 age group and amounted to GMT-25. In summary, the highest geometric mean antibody titres were observed in the 5–9 age group, while the lowest in the 0–4 age group. In all the age groups, the highest level of immune response was demonstrated for the A/Hong Kong/2671/2019 (H3N2)-like virus antigen. The differences in the antibody level among the analyzed age groups are statistically significant for all the 4 antigens tested (p<0.001). The protective factor is the percentage of people with a protective anti-HA antibody titre of at least 40. This level may be the result of either prior vaccination or a history of the disease. Depending on age, it should reach different values: ≥70% in the population of people aged 18–60 years and ≥60% for people over 60 years of age (Brydak, ch. XII, 2008). The values of protective coefficients for individual HA strains of influenza virus in different age categories are presented in Fig. 4. In none of the analyzed groups, the protection rate for the A/Guangdong-Maonan/SWL1536/2019 (H1N1)pdm09-like virus and B/Washington/02/2019 (B/Victoria lineage)-like virus antigens reached the protection

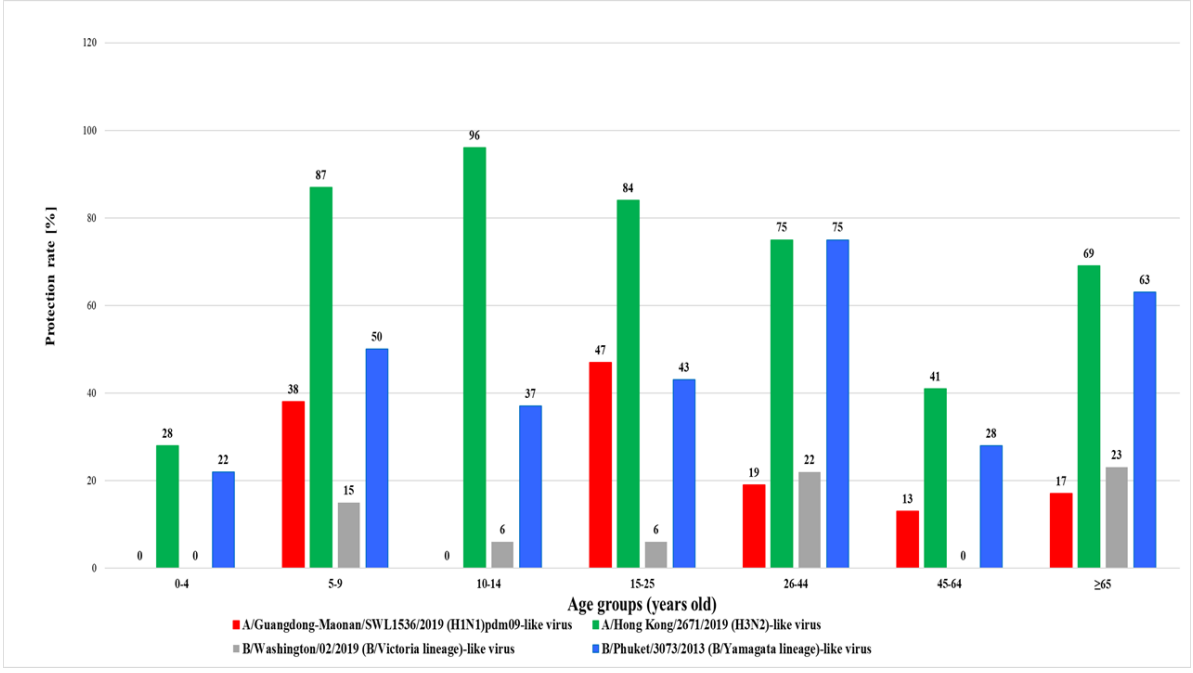

Figure 4. The percentage of cases with protective anti-haemagglutinin antibody titers (%) in the 2020/2021 epidemic season according to age groups

level. The protection rate for A/Hong Kong/2671/2019 (H3N2)-like virus exceeded 70% for the age groups 5–9, 10–14, 15–25, 26–44 and 60% for the 65+ age group. On the other hand, for B/Phuket/3073/2013 (B/Yamagata lineage)-like virus, the appropriate level of protection occurred only in the 26–44 and 65+ age groups. In other cases, the protection rate did not reach the recommended protection level. For all the virus types, the differences between the age groups are statistically significant ($p<0.001$).

DISCUSSION

In the epidemic season 2020/2021, 3 out of 4 influenza strains in the vaccine composition were changed. Only the B/Phuket/3073/2013 (B/Yamagata lineage)-like virus strain has been present in the vaccine for several seasons. Even though it is the second most common virus in the analyzed samples, it achieves the level of protection only in 2 age groups: 26–44 (75%) and 65+ (63%). Moreover, in the 2017/2018 and 2018/2019 seasons, the protection rate was higher than in the 2020/2021 season (Hallmann-Szelińska *et al*., 2020; Hallmann-Szelińska *et al*., 2019). The most frequently detected strain in the tested sera was A/Hong Kong /2671/2019 (H3N2)-like virus, for which both the geometric mean of anti-haemagglutinin antibody titers in all the age groups was the highest, and the protection rate reached the age-appropriate level of protection in 5 out of 7 analyzed age categories: 5–9 (87%), 10–14 (96%), 15–25 (84%), 26–44 (75%) and 65+ (69%). Considering the very small percentage of vaccinated people (6.1% of the Polish population), it can be assumed that this is the result of a past disease. In the 2017/2018 and 2018/2019 seasons in Poland, the most frequently detected type of influenza virus (Hallmann-Szelińska *et al*., 2020; Hallmann-Szelińska *et al*., 2019) among the viruses included in vaccines for a given epidemic season was subtype A/H3N2/. However, many A/H3N2/ virus strains agglutinate through their neuraminidase disrupting the interaction between anti-HA antibodies, the virus and RBC (Mögling *et al*., 2017). An alternative for these virus strains is testing HAI using guinea pig RBC and a neuraminidase inhibitor, such as oseltamivir, to prevent NA-dependent agglutination (Waldocka *et al*., 2021). Anti-NA antibodies, even at high titers, only support immunity because they do not prevent influenza on their own. However, anti-NA antibodies have been shown to block the replication process, moderate the severity of infection and reduce the incidence of the disease (Brydak, ch. II, 2008). In the 2020/2021 season, the percentage of vaccinated people in Poland amounted to 6.1% (Brydak, 2021). This means a significant increase compared to the previous seasons: 2019/2020 – 4.1%, 2018/2019 – 3.9%, 2017/2018 – 3.6%, 2016/2017 – 3.3% (NIPH-NIH RI (b), 2021). However, the protection rate in some age groups did not reach the level of protection, even despite the possibility of cross-protection. It should be remembered that one or two amino acid differences in HA may change the profile of newly induced antibodies or the ability of previously produced antibodies to neutralize (Skarlupka *et al*., 2020), therefore vaccines from previous seasons may be ineffective against viruses circulating in following seasons. It is important to get vaccinated each season. Based on serological screening of sera from people of different age groups in the 2020/2021 epidemic season, it can be concluded that the obtained results confirm the circulation of four antigenically different strains of influenza virus: A/Guangdong-Maonan/SWL1536/2019(H1N1)pdm09-like virus, A/Hong Kong/2671/2019 (H3N2)-like virus, B/Washington/02/2019(B/Victoria lineage)-like virus and B/Phuket/3073/2013(B/Yamagata lineage)-like virus. However, there has been only 1 confirmed case of type B influenza (NIPH-NIH RI (a) (2021). The emergence of severe acute respiratory syndrome caused by SARS-CoV-2 in late 2019 and the COVID-19 pandemic that has lasted since 2020 have had a serious impact on the activity of the influenza virus. Worldwide, significant reductions in the influenza virus activity have been reported, with only minor outbreaks recorded in some tropical regions. Possible causes include non-pharmaceutical interventions, decreased population movement and limited travel opportunities, but virus-virus interactions, sometimes referred to as "viral interference", are also possible (WHO, 2021). One potential explanation for this pattern is competition between SARS-CoV-2 and influenza virus. This competition can occur through multiple mechanisms such as immunological interactions, viral competition, and decreased susceptibility due to isolation (Zipfel *et al*., 2021). While these factors may have contributed to the reduction in the incidence of influenza viruses during the 2019/2020 and 2020/2021 epidemic seasons, it is likely that the changes and behavioral interventions that occurred in the COVID-19 pandemic were more significant. Non-pharmaceutical interventions, such as school and business lockdown, working from home, restricting collective events, and wearing face masks have been key tools in reducing the impact of the COVID-19 pandemic. Given the common route of transmission of SARS-CoV-2 and influenza virus, the same protective behavior can significantly reduce the transmission of both of viruses. As school-aged children play an important role in transmitting influenza virus, school lockdowns are considered to have a significant impact on the disease dynamics by reducing contact (Zipfel *et al*., 2021). However, the results obtained from the study of sera of school-aged patients in the 2020/2021 season, despite distance learning, did not show differences from the previous epidemic seasons (Hallmann-Szelińska *et al*., 2019, Hallmann-Szelińska *et al*., 2020). SARS-CoV-2 (the virus that causes COVID-19) and influenza virus have similar modes of transmission and clinical symptoms, so it is difficult to distinguish between the two. Epidemiological data suggest that SARS-CoV-2 is more contagious than influenza (Petersen *et al*., 2020), however, due to the similar clinical manifestations of both respiratory viruses, it is important that doctors test for both influenza infection and SARS-CoV-2 when assessing patients with a flu-like disease during the influenza season.

IN SUMMARY

- The results confirmed the presence of antibodies against the following influenza antigens: A/Guangdong-Maonan/SWL1536/2019 (H1N1)pdm09-like virus, A/Hong Kong/2671/2019 (H3N2)-like virus, B/Washington/02/2019 (B/Victoria lineage)-like virus and B/Phuket/3073/2013 (B/Yamagata lineage)-like virus;
- The most frequently detected strain in the tested sera was A/Hong Kong/2671/2019 (H3N2)-like virus;
- The haemagglutinin antibody level differed between the age groups studied, with the highest values in children 5–9 years old, intermediate in children aged 9–14 and adults, and the lowest in the 0–4 age group;
- The appearance of SARS-CoV-2 and all the methods of preventing the spread of this virus have had a major impact on reducing the activity of influenza virus.

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Data interpretation	D	B D F	Karol Szymański
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Funds Collection	G	A D E F G	Lidia B. Brydak

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