Hemagglutination inhibition antibody titer as a correlate of protection against influenza disease in the 2018/2019 epidemic season in Poland

Ewelina Hallmann-Szelińska, Karol Szymański, Katarzyna Łuniewska, Katarzyna Kondratiu and Lidia Bernadeta Brydak

Department of Influenza Research – National Influenza Center, National Institute of Public Health – National Institute of Hygiene, Warsaw, Poland

The aim of this study was to determine the level of antibodies against hemagglutinin of influenza viruses in the sera of people in the seven age groups in the epidemic season 2018/2019 in Poland. The level of anti-hemagglutinin antibodies was determined by hemagglutination inhibition assay (HAI). 1050 clinical samples from all over the country were tested. The level of antibodies against influenza viruses was highest in the 10–14 age group for A/Singapore/INFIMH-16-0019/2016 (H3N2) and B/Phuket/3073/2013 Yamagata lineage antigens. These results confirm the circulation of four antigenically different influenza virus strains, two subtypes of influenza A virus – A/Michigan/45/2015 (H1N1)pdm09 and A/Singapore/INFIMH-16-0019/2016 (H3N2) and two lineages of influenza B virus – B/Colorado/06/2017 – Victoria lineage and B/Phuket/3073/2013 Yamagata lineage.

Key words: hemagglutinin antibodies, protection rate, influenza, GMT, vaccination

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INTRODUCTION

The influenza virus belongs to the Orthomyxoviridae family. In humans, infections are caused by types A, B, and C of the influenza virus, of which the first two are epidemiologically more important (Paules & Subbarao, 2017).

Influenza accounts for three to five million severe cases and up to 650,000 deaths every year worldwide (WHO, 2019). Influenza viruses pose a threat to humans, especially those at high risk: people over 65 years of age; children under 2 years; pregnant women; people suffering from asthma, diabetes, cancer, or heart disease and people who stay permanently at home care or other long-term care facilities (CDC, 2019).

When localized on the surface of the viral membrane, HA remains inactive (referred to as HA0). The protein becomes activated inside the endosome upon the exposure to reduced pH which changes its structure. X-ray studies showed that inside the endosome each of the three HA0 polypeptide chains is cleaved into two fragments by the host proteases. After cleavage, the two pro-
tein fragments (HA1 and HA2) formed are connected by a disulfide bridge. The HA2 fragment is referred to as a fusion peptide because it has the ability to bind to the endosomal membrane. This fusion fragment is exposed under favorable conditions and travels approximately 10 nm toward the endosomal membrane. Studies have also shown that acidification of the endosome and the rate of structural change of HA and exposure of the fusion peptide are temperature-dependent and proceed faster with the increase in temperature. This suggests that the typical response shown by an organism to fight the virus actually favors its faster infection (Webster, 2013; Sriwilaijaroen et al., 2012; Skehel et al., 2000).

Administration of the influenza vaccine leads to the production of specific antibodies. These immunoglobulins block the ability of the virus to adsorb to the host cells by attaching to the globular portion of its HA glycoprotein (Fig. 2). The level of these antibodies is measured using the HA inhibition assay (HAI). Administration of the influenza vaccine leads to the production of specific antibodies. These immunoglobulins block the ability of the virus to adsorb to the host cells by attaching to the globular portion of its HA glycoprotein (Fig. 2). The level of these antibodies is measured using the HA inhibition assay (HAI). This test is carried out using chicken or turkey blood (Vemula et al., 2016; Krammer et al., 2014). The antibodies present in the blood bind to the shaft portion of the HA glycoprotein; however, these antibodies are rare in humans, and it is not possible to detect them and determine their titers using the HAI. In a person who received the vaccination, the produced immunoglobulins prevent the change in the conformation of the HA structure caused by the reduced pH in the endosome, thus disabling the virus from attaching to the cell (Krammer et al., 2014; Ekiert et al., 2010).

Antigenic shift, which is induced by collective immunity in the population as well as a decrease in the titer of the vaccine-specific antibody over time, necessitates the need for all-season vaccination. It is important to administer the vaccine before the virus increases in the population. However, a person can get vaccinated at any time during the season (ACIP, 2019; Houser et al., 2015).

The effectiveness of the vaccine may vary between the seasons and also depends on the health and condition of the person receiving the vaccine. Nonetheless, regular vaccination reduces the negative effects of influenza in-
Influenza virus strains were used in HAI in A/H3N2/Phuket/3073/2013-like virus Yamagata lineage in the 10 to 14 years age group (92.7%), followed by the age groups ≥65 (74.0%), 45–64 (71.3%), and 15–25 years (68.0%). In the age groups 0–4 and 5–9 years, a similar level of protection was achieved at 54.7% and ≥65 years, respectively. The lowest value of the protection coefficient was obtained for the age group 26–44 years at 47.3%.

The protection rate is defined as the percentage of people with anti-hemagglutinin antibodies at a protection level of at least 1:40 after vaccination or infection (Brydak et al., 2003). The values of the protection coefficients for individual HAs of influenza virus strains in different age groups are presented in Fig. 4. It should be noted that for people over 60 years of age, the protection rate had a value of ≥60%, while for people aged 18–60 years old it had a value of ≥70% (Brydak, 2008).

In the analyzed epidemic season, the highest protection levels were recorded for HAB strain B/Phuket/3073/2013-like virus Yamagata lineage in the 10 to 14 years age group (92.7%), followed by the age groups ≥65 (74.0%), 45–64 (71.3%), and 15–25 years (68.0%). In the age groups 0–4 and 5–9 years, a similar level of protection was achieved at 54.7% and 60.0%, respectively. The lowest value of the protection coefficient was obtained for the age group 26–44 years at 47.3%.

In the case of HAB strain B/Colorado/06/2017-like virus Victoria lineage the highest protection rate was recorded in the age group 10–14 years (47.3%) and a lower value in the age group 26–44 years (36.6%). On the other hand, the lowest level of the protection rate

Table 1. Influenza virus strains used for the hemagglutination inhibition test (HAI) in the 2018/2019 epidemic season.

| Influenza virus strains          | Epidemi season 2018–2019 |
|---------------------------------|---------------------------|
| A/H1N1/pdm09                    |                           |
| A/H3N2/                          |                           |
| A/Michigan/45/2015 (H1N1) pdm09-like virus |                   |
| A/Singapore/INFIMH-16-0019/2016 (H3N2)-like virus |                   |
was recorded in the age groups 15–25 and 45–64 years at 10.0%. In the groups of younger children, the protection coefficient ranged from 22.67% (0–4 years old) to 11.33% (5–9 years old) (Fig. 4). Among older people ≥65 years old the protection rate was 12.67%.

For H1 strain A/Michigan/45/2015(H1N1)pdm09-like virus the highest protection level was observed in the age group 10–14 years (51.3%) and a lower level in the age group 5–9 years (48.0%). In the remaining four age groups, the protection rate was at a similar level as follows: 0–4 years: 20.0%; 15–25 years: 23.3%; 26–44 years: 24.0%; and ≥65 years: 14.7%. The lowest protection rate was recorded in the age group 45–64 years at 8.7%.

For H3 strain A/Singapore/INFIMH-16-0019/2016 the protection rate was high among children: 10–14 years old: 91.3%; 5–9 years old: 69.3% and 0–4 years old: 51.3%. In the age group 15–25 years, the value of the protection rate was 68.7%. In the remaining age groups 26–44, 45–64, and ≥65 years, the protection rate was between 21.0% and 35.0%.

Figure 3. Geometric mean titers (GMT) of anti-hemagglutinin antibodies within the age groups in the 2018/2019 influenza epidemic season in Poland.

Figure 4. The proportion of individuals with a protective anti-hemagglutinin antibody titer level within the age groups in the 2018/2019 influenza epidemic season in Poland.
DISCUSSION

In the 2017/2018 epidemic season, the influenza A/H1N1/pdm09 vaccine strain was changed from A/California/7/2009 to A/Michigan/45/2015 for the first time since the 2010/2011 epidemic season. The epidemic season 2018/2019 is, therefore, the second season in which this strain is included in the vaccine. The GMT values of strain A/Michigan/24/2015 in the analyzed season were higher among children in the age groups 0–4 years (72.0) and 5–9 years (91.3) compared to the previous season (0–4 years: 47.6; 5–9 years: 51.1). By analyzing the GMT values of the influenza A/H1N1/pdm09 strain from the 2013/2014 epidemic season to the currently discussed 2018/2019 season it was found that the GMT values of antibody titers were higher among the children of 0–14 years than in adults irrespectively of the type of strain contained in the vaccine (Hallmann-Szelitiska et al., 2019; Kowalczyk et al., 2019; Hallmann-Szelitiska et al., 2018; Kowalczyk et al., 2017; Bednarska et al., 2015). This may be due to the fact that children experience flu infections more frequently than adults (Glezen et al., 1980; Izurieta et al., 2000). According to the NIPH-NIH data, the incidence of infections by influenza and influenza-like viruses in the epidemic season 2018/2019 in children of 0–4 years of age was 59,417.5 and in the age group 5–14 years was 24,828.7, while the incidence among adults of 15–64 years of age was 8166.4 and in the age group ≥65 was 6650.9. In addition, it should be emphasized that the rate of vaccination against flu is very low in the Polish population. In 2018, only 0.63% were vaccinated in the age group 0–4 years, 0.96% in the 5–14 years, 1.58% in the 15–64 years, and 8.31% in the ≥65 years. In the analyzed epidemic season, the protection rate was lowest for H1. Analysing all the age groups, the lowest protection rate was observed in the 45–64 years group at 8.7%, but in the previous season, it was 4.7% in the same group. However, the highest protection rate was found in the age group 10–14 years for all the HAs tested. In the 2017/2018 epidemic season, the highest values of the protection coefficient were recorded in the same age group at 38.0% for H1 and 74.7% for H3 (Hallmann-Szelitiska et al., 2019), while the lowest values were recorded in the age group 45–64 years for four HAs and oscillated from 4% for H3 to 24.7% for B Yamagata lineage. By contrast, in the analyzed season, the lowest values of the protection rate were recorded in the age group 45–64 years at 8.7% for H1 and 21.3% for H3. For HAB Victoria, the protection rate was 10% in the age groups 15–25 and 45–64 years and was the lowest value recorded for this protein. The highest value of the protection rate was recorded for H3 at 91.3% and B Yamagata line at 92.7% in the age group 10–14 years.

To summarize, the circulation of four strains of influenza virus contained in the vaccine for a given epidemic season was confirmed in Poland during the 2018/2019 epidemic season. For strain A/Michigan/45/2015 (H1N1) pdm09-like virus the highest levels of anti-hemagglutinin antibodies were detected in the age groups 0–4 years old and 5–9 years old. For strain A/Singapore/INFIMH-16-0019/2016 (H3N2)-like virus the highest level of antibodies was recorded in the age group 10–14 years. Whereas, for influenza B type, for strain B/Colorado/06/2017-like virus Victoria lineage the level of anti-hemagglutinin antibodies was at a similar level in the age groups: 0–4 years, 10–14 years, 26–44 years and not gaining a protection value (value under 40). For strain B/Phuket/3073/2013-like virus Yamagata lineage a protection value of antibody titers was obtained in the 10–14 years age group.

A study by Argentinean scientists showed that vaccination with proteins leads to CD4 T-cells and infection recalls their memory. In addition, the hemagglutinin released by infected or dying cells, rather than infectious virions, is the main source of antigen for antigen presentation after the infection (Knowlden, et al. 2019).

Reflecting the prevailing virological and epidemiological situation is possible thanks to the supervision of influenza, in which clinical samples are obtained from all over the country. Surveillance provides the information on the evolution of viruses and the correlation between antigenic differences and changes in sialic acid receptor binding properties of the HA glycoprotein. As a result of the mutation preventing the antibody from binding, may also be observed changes in sialic acid receptor binding (Lin, et al. 2012). In addition allows the selection of vaccine strains for the next epidemic season and monitoring the appearance of new variants, as well as checking the drug resistance of influenza viruses.

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Conflicts of Interest

The authors declare no conflicts of interest in relation to this article.

REFERENCES

Advisory Committee on Immunization Practices (2019) https://www.cdc.gov/mmwr/volumes/68/rr/rr6803a1.htm (Accessed 31.10.2019)

Bednarska K, Nowak MA, Kondratuk K, Hallmann-Szelitiska F, Brydak LB (2015) Incidence of circulating antibodies against hemagglutinin of influenza viruses in the epidemic season 2013/2014 in Poland. Adv Exp Med Biol 857: 45–50. https://doi.org/10.1007/5884_2015_118

Brydak LB (2008) Influenza, pandemic flu, myth or real threat? Rytm Warsz 1:492

Brydak LB (2012) Influenza – an age old problem. Hygia Public Health 47: 1–5

European Centre for Disease Prevention and Control (ECDC) (2019) https://www.cdc.gov/ihr/higstrk/index.htm (Accessed, 28.10.2019)

European Centre for Disease Prevention and Control (ECDC) (2019) https://www.ecdc.europa.eu/en/seasonal-influenza/facts/factsheet (Accessed 28.10.2019)

Ekert DC, Bhubha G, Ellsiger MA, Friesen RH, Jongeneelen M, Throsby M, Wilson IA (2009) Antibody recognition of a highly conserved influenza virus epitope. Science 324: 246–251. https://doi.org/10.1126/science.1171491

FluSurfer (2019) https://flusurver.bii.a-star.edu.sg/

Glezen WP, Paredes A, Taber LH (1980) Influenza in children: relationship to other respiratory agents. JAMA 243: 1345–1349. https://doi.org/10.1001/jama.243.13.1345

Hallmann-Szelitiska F, Ciesiak K, Kowalczyk D, Szmysarka K, Brydak LB (2018) Antibodies to influenza virus hemagglutinin in the 2016/2017 epidemic season in Poland. Adv Exp Med Biol 1108: 69–74. https://doi.org/10.1007/5884_2018_232

Hallmann-Szelitiska F, Szmysarka K, Luniewska K, Masny A, Kowalczyk D, Salamatin R, Brydak LB (2019) Occurrence of influenza hemagglutinin antibodies in the Polish population during the epidemic season 2017/18. Adv Exp Med Biol Oct 22. https://doi.org/10.1007/5884_2019_443

Houser K, Subbarao K (2015) Influenza vaccines: challenges and solutions. Cell Host & Microbe 17: 295–300. https://doi.org/10.1016/j.chom.2015.02.012

Izurieta HS, Thompson WW, Kramarz P, Shay DK, Davis RL, DeStefano F, Black S, Shinfeld H, Fukushima K (2000) Influenza and the rates of hospitalization for respiratory disease among-
fants and young children. *N Engl J Med* **342**: 232–239. https://doi.org/10.1056/NEJM200001273420402

Jang YH, Seong BL (2019) The quest for a truly universal influenza vaccine. *Front Cell Infect Microbiol* **9**: 344. https://doi.org/10.3389/fcimb.2019.00344

Knowlden ZAG, Richards KA, Moritzky SA, Sant AJ (2019) Peptide epitope hot spots of CD4 T cell recognition within influenza hemagglutinin during the primary response to infection. *Pathogens* **8**: 220. https://doi.org/10.3390/pathogens8040220

Kondrich J, Rosenthal M (2017) Influenza in children. *Curr Opinion Pediatrics* **29**: 297–302. doi:10.1097/mop.0000000000000495

Kowalczyk D, Szmyński K, Cieślak K, Brydak LB (2017) Circulation of antibodies against influenza virus hemagglutinins in the 2014/2015 epidemic season in Poland. *Adv Exp Med Biol* **968**: 35–40. https://doi.org/10.1007/5584_2016_191

Kowalczyk D, Szmyński K, Cieślak K, Hallmann-Szelińska E, Brydak LB (2019) Circulation of influenza virus in the 2015/2016 epidemic season in Poland: serological evaluation of anti-hemagglutinin antibodies. *Adv Exp Med Biol* **1150**: 77–82. https://doi.org/10.1007/5584_2018_271

Kramer F, Palese P (2013) Influenza virus hemagglutinin stalk-based antibodies and vaccines. *Curr Opinion Virol* **3**: 521–530. https://doi.org/10.1016/j.coviro.2013.07.007

Lin YP, Xiong X, Wharton SA, Martin SR, Coombs PJ, Vachieri SG, Christodoulou E, Walker PA, Liu J, Skehel JJ, Gamblin SJ, Hay AJ, Daniels RS, McCauley JW (2012) Evolution of the receptor binding properties of the influenza A(H3N2) hemagglutinin. *Proc Natl Acad Sci USA* **109**: 21474–21479. https://doi.org/10.1073/pnas.1218441110

Neumann G, Noda T, Kawaoka Y (2009) Emergence and pandemic potential of swine-origin H1N1 influenza virus. *Nature* **459**: 931–939. https://doi.org/10.1038/nature08157

Paules C, Subbarao K (2017) Influenza. *Lancet* **390**: 697–708. https://doi.org/10.1016/S0140-6736(17)30129-0

Skehel JJ, Wiley DC (2000) Receptor binding and membrane fusion in virus entry: the influenza hemagglutinin. *Annu Rev Biochem* **69**: 531–569. https://doi.org/10.1146/annurev.biochem.69.1.531

Sriwijaiaroen N, Suzuki Y (2012) Molecular basis of the structure and function of H1 hemagglutinin of influenza virus. *Proc Jpn Acad Ser. B* **88**: https://doi.org/10.2183/pjab.88.226

Tyrell DAJ, Hornsfall FL (1952) A procedure which eliminates nonspecific inhibitor from human serum but does not affect specific antibodies against influenza viruses. *J Immunol* **69**: 563–574

Webster RG (2013) *Textbook of influenza*. Wiley Blackwell, ISBN 978-0-470-67048-4

WHO – Global Influenza Surveillance Network (2011) Manual for the laboratory diagnosis and virological surveillance of influenza. *WHO Proc* pp 1–153. Geneva

WHO (2018) Recommended composition of influenza virus vaccines for use in the 2018–2019 northern hemisphere influenza season. Available from http://www.who.int/en/WHO (2019). http://apps.who.int/flumart/Default?ReportNo=12 (Accessed 28.10.2019)

WHO (2019) http://www.euro.who.int/en/health-topics/communicable-diseases/influenza/vaccination/influenza-vaccination-coverage-and-effectiveness (Accessed 31.10.2019)

Vemula SV, Zhao J, Liu J, Wang X, Biwas S, Hewlett I (2016) Current approaches for diagnosis of influenza virus infections in humans. *Viruses* **8**: 96. https://doi.org/10.3390/v8040096