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Analysis of Infections Caused by Influenza Viruses and Influenza-Like Viruses in the Population of People over 65 Years of Age in the 2019-2020 Epidemic Season in Poland

Authors' Contribution:
Study Design A
Data Collection B
Statistical Analysis C
Data Interpretation D
Manuscript Preparation E
Literature Search F
Funds Collection G

BCDEF Katarzyna Kondratiuk
BCDEF Ewelina Hallmann
BCDEF Katarzyna Łuniewska
BCDEF Karol Szymański
ABCDEFG Lidia B. Brydak

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

Corresponding Author: Ewelina Hallmann, e-mail: ehallmann@pzh.gov.pl
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Background: Influenza can be the most dangerous for people in risk groups, for example for seniors, in whom it can lead to serious and life-threatening complications. The aim of this research was to analyze the activity of influenza viruses and influenza-like viruses in patients over 65 years of age in the 2019-2020 epidemic season in Poland.

Material/Methods: A total of 1269 samples collected from patients over 65 years of age with suspected influenza or other respiratory viruses in the 2019-2020 epidemic season (from October 1, 2019, to September 30, 2020) were analyzed. The test material was nose and throat swabs collected during the 2019-2020 epidemic season. Quantitative polymerase chain reaction was used to determine the influenza virus type and subtype for positive samples.

Results: mong the confirmed infections with influenza viruses, cases due to influenza A were dominant, and the dominant subtype was influenza A subtype A/H1N1/pdm09. Infections with influenza-like viruses were also confirmed in the patients participating in the study, with the presence of genetic material of respiratory syncytial viruses confirmed most often.

Conclusions: In the 2019-2020 influenza season, cases of influenza A were dominant, and the dominant subtype was influenza A subtype A/H1N1/pdm09. Patients over 65 years of age constitute the majority of all patients treated for flu in Europe and worldwide. Seasonal vaccinations is the most effective method to reduce the number of cases, and thus the risk of post-influenza complications and deaths among seniors

Keywords: Influenza A virus • Influenza B virus • Respiratory Syncytial Viruses • Geriatrics • Epidemiology

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Background

Influenza is an acute illness caused by infection of the respiratory system with the influenza virus. The disease is caused by viruses from the *Orthomyxoviridae* family. Influenza viruses are classified into 3 types: A, B, and C. Type A is divided into subtypes based on the properties of their surface antigens, hemagglutinin and neuraminidase. Influenza A viruses exist in 18 hemagglutinin antigenic subtypes (H1-H18) and 11 neuraminidase subtypes (N1-N11) [1,2]. The most characteristic feature of the influenza virus is its variability, which distinguishes it from all known viruses of this type [3].

Influenza starts rapidly and from the start of illness is associated with the most frequent acute accompanying symptoms, such as a sudden high fever (over 37.8°C), malaise, chills, weakness, headache, joint and muscle pain, dry cough, throat inflammation, and sometimes bronchitis. In general, full recovery can take up to several weeks and patients can experience dry cough, weakness, and increased fatigue. Influenza can be most dangerous for people from the identified risk groups, regardless of their age, in whom the disease is a serious threat to health and even life. These include seniors because, in people over 65 years of age, all symptoms of the disease are usually more intense and can last longer than in young people. Seniors, owing to lowered immunity and frequently accompanying chronic diseases, among other reasons, are much more likely to have influenza complications.

The most effective method of preventing influenza is immunization, which is especially recommended for elderly people. The World Health Organization's (WHO) Advisory Committee on Immunization Practices, many international and national scientific societies, and the Ministry of Health emphasize that to effectively prevent influenza and its complications, care must be taken to prevent and build immunity in seniors through seasonal influenza vaccinations [4,5]. It is worth remembering that even if vaccinations do not prevent the disease, they can contribute to a much milder course of the disease and help prevent health-threatening complications. The most common complications after influenza in the age group of over 65 years includes pneumonia and bronchitis, secondary bacterial pneumonia caused mainly by *Staphylococcus aureus*, *Streptococcus pneumoniae*, and *Haemophilus influenzae*, exacerbation of chronic diseases (asthma, cystic fibrosis, diabetes, chronic renal failure), myalgia, myocarditis, pericarditis, meningitis or brain inflammation, heart failure, inflammation of the middle ear and paranasal sinuses, auditory receptor dysfunction (partial hearing loss or even deafness), neurological complications including Guillain-Barré syndrome, transverse myelitis, increased frequency of epileptic seizures, and transplant rejection [4,5]. Pharmacoeconomic studies indicate that influenza vaccination is a procedure with a high effectiveness rate, especially in the elderly population. The use of preventive vaccinations reduces the number of cases, hospitalizations, and mortality rate due to influenza and its complications [6]. In addition, preventive vaccinations reduce the use of antibiotics, which is also a favorable phenomenon given the increasing resistance of bacteria.

Many scientific studies have shown that influenza vaccination is a safe and effective method of preventing complications from influenza in high-risk groups. It is the main way to prevent influenza and therefore prevent an influenza epidemic. After vaccination, immunity develops after about 7 days, increases with time, and lasts from 6 to 12 months. Although vaccine efficacy can vary, studies show that influenza vaccination reduces the risk of influenza by 40% to 60% in the overall population during periods when most circulating influenza viruses are present [7]. The argument for seasonal influenza vaccinations among the population of people over 65 years of age is the possibility of improving the effectiveness of heart attack prevention in patients with chronic coronary syndromes, improving the prognosis for patients with heart failure, and reducing cardiovascular mortality [8,9]. Influenza vaccinations may prevent, for example, myocardial infarction [10] and stroke [11]. Influenza vaccination has also been shown to prevent recurrent heart ischemia [12] and primary cardiac arrest [10], showing important benefits in patients with cardiovascular disease. The results of Polish studies involving patients with acute cardiovascular events have been evaluated and included in the European cardiologic recommendations for influenza vaccinations [13]. The Department of Influenza Research, National Influenza Center, conducted over 207 scientific studies on the advisability of vaccinations, with particular emphasis on people of various age groups, including children and the elderly [14,15].

According to WHO recommendations, influenza vaccinations among seniors 65 years and older in the WHO European Region should be implemented in individual countries at the level of 75% of vaccination status in this age group [16]. According to the statistics kept by the WHO, the level of vaccination among Polish seniors is alarmingly low and places Poland at the bottom of the list of European countries in this respect. The vaccination rate in the group of people over 65 years of age was only 7.13% in 2020, despite there being free influenza vaccinations offered to seniors in recent years by local governments [17]. Therefore, the WHO urges all member countries to be especially vigilant against influenza, including seasonal influenza A subtypes or influenza viruses that may cause a pandemic, and to maintain routine influenza vaccination programs to protect vulnerable individuals. This is extremely important, especially now, in view of the current epidemiological situation related to the ongoing SARS-CoV-2 respiratory virus pandemic.

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The aim of the presented research was to analyze the activity of influenza viruses and influenza-like viruses in patients over 65 years of age in the 2019-2020 epidemic season in Poland.

Material and Methods

The study included patients over 65 years of age who had swabs from the nose and throat. A total of 1269 samples were obtained under the Sentinel and Non-Sentinel Influenza Surveillance System in the 2019-2020 epidemic season, between week 40 of 2019 and week 39 of 2020 (October 1, 2019, to September 30, 2020). The influenza A and B samples were tested at the Department of Influenza Research, National Influenza Center at National Institute of Public Health NIH – National Research Institute and Voivodship Sanitary Epidemiological Stations.

Isolation of RNA

Isolation of RNA was conducted using nasal and throat swabs suspended in 1 mL of saline. For this isolation, the Maxwell 16 Viral Total Nucleic Acid Purification kit (Promega Corporation, Madison, WI, USA) was used in accordance with the manufacturer instructions. From 200 µL of a clinical sample, 50 µL of RNA suspended in RNase-free water was obtained.

Real-Time Polymerase Chain Reaction

Real-time reverse transcription polymerase chain reaction was used to determine the influenza virus type and subtype. The reaction was performed with the SuperScript Platinum III kit (Invitrogen) using the Rotor-Gene Q thermal cycler (Qiagen). The probes and primers kits influenza A, influenza A/H3N2/, influenza A/H1N1/pdm09, and influenza B were provided by the International Reagent Resource of the Centers for Disease Control and Prevention. Briefly, 20 µL of reaction mixture comprised 5 µL of RNA, 0.4 µM of each primer, 0.4 µM of each probe, 10 µL of 2× reaction buffer mix, 0.4 µL of SuperScript III/Platinum Taq Mix, and 3.4 µL of water. To receive complementary DNA for isolated RNA, reverse transcription reaction was conducted in 50°C for 30 min. Next, the initial denaturation was performed at 95°C for 2 min. Amplification was performed under the following conditions: 45 cycles of denaturation (95°C for 15 s), annealing (55°C for 30 s), and elongation (72°C for 20 s). The positive controls for the reactions were isolated from viruses derived for the vaccine from the 2019-2020 epidemic season: A/Brisbane/02/2018 (H1N1) pdm09, A/Kansas/14/2017/ (H3N2), B/Colorado/06/2017 (B/Victoria/2/87 lineage), and B/Phuket/3073/2013 (B/Yamagata/16/88 lineage). The negative control was the RNase-free water from the SuperScript Platinum III kit (Invitrogen). The Voivodship Sanitary Epidemiological Stations carried out similar analyzes. The details of the methodology are shown in **Table 1**.

Results

A total of 1269 samples collected from patients over 65 years of age with suspected influenza or other respiratory viruses in the 2019-2020 epidemic season (from October 1, 2019, to September 30, 2020) were analyzed. As part of the sentinel program, 70 samples were tested (which constituted 5.52% of all the samples tested in this age group), while most samples (1199; 94.48% of all the samples taken for the study) came from the non-sentinel program. Positive samples, defined as samples in which the presence of the genetic material of influenza or influenza-like viruses was confirmed, were 28.68% (364) of all the tested samples. The percentage of positive samples in relation to all the tested samples in the age group over 65 years in the 2019-2020 epidemic season in Poland is shown in **Figure 1**.

After analyzing samples from patients over 65 years of age, 348 cases of influenza were confirmed, of which 345 cases were caused by the influenza A virus. The presence of the genetic material of the influenza B virus was confirmed in only 3 samples. Among cases caused by influenza A, the cases of influenza virus subtype A/H1N1/pdm09 dominated, with 45 confirmations, which constituted 13.04% of all confirmations of influenza infection type A. For influenza A virus of subtype A/H3N2/, 23 infections (6.67% cases due to influenza A) were confirmed. The remaining cases of influenza A virus were not subtyped, of which there were 277, which accounted for 80.29% of influenza A cases. The percentage share of influenza viruses and influenza A virus subtypes in the age group over 65 years in the 2019-2020 epidemic season in Poland is shown in **Figure 2**.

Among the tested people over the age of 65, infections caused by influenza-like viruses were also confirmed, although infections with influenza viruses constituted the vast majority. Among the analyzed trials, 16 cases caused by influenza-like viruses were confirmed. The greatest number of them were infections with the respiratory syncytial virus, which were found in 13 people, 81.25% of all the infections with influenza-like viruses. There were 2 cases of Coronavirus 229E/NL63 and 1 of adenovirus. The percentage of influenza-like viruses in the over 65 age group in the 2019-2020 epidemic season in Poland is shown in **Figure 3**. In the tested samples collected from people over 65 years of age in the 2019-2020 epidemic season, coinfections were also confirmed (a situation in which there is a simultaneous infection with 2 or more respiratory viruses, namely influenza and/or influenza-like viruses). The coinfections confirmed in this age group are presented in **Table 2**.

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Table 1. The diagnostic methods used in 16 Voivodship Sanitary Epidemiological Stations in the epidemic season 2019-2020 in Poland.

Voivodship Sanitary Epidemiological Station	Diagnostic equipment	Kits
Białystok	LightCycler 96 (Roche)	Real Time Ready Influenza A/H1N1/pdm09 Detection Set, RealTime Ready RNA Virus Master, LightCycler Multiplex RNA Virus Master, Light Mix Modular EAV RNA Extraction Control (Roche)
Bydgoszcz	LightCycler 480 II (Roche)	Multiplex RNA Virus Master (Roche); sondy i startery Modular Dx Kit Inf M2, Modular Dx Kit InfA H3, InfB, Light Mix Kit CC_ Hexaplex 480 II; kontrola wewn IC – Roche RNA Process Control Kit Trial Pack
Gdańsk	–	FTD Flu (Fast Track Diagnostics)
Gorzów Wlkp.	LightCycler 480 II (Roche)	FTD Flu (Fast Track Diagnostics)
Katowice	LightCycler 480 II (Roche)	PowerChek Pandemic A/H1N1/pdm09, A/H3N2 Real Time RT-PCR Kit (Kogene Biotech); FTD Flu (Fast Track Diagnostics)
Kielce	–	Allplex Respiratory Panel 1 (Seegene)
Kraków	Rotor-Gene Q MDx 5 plex Real-time PCR firmy Bioer	One tube multiplex PCR for influenza A/H1N1/pdm09, B, H3, H5 and H7 (Fast Track Diagnostics) Vitassay qPCR Flu+RSV+SARS-CoV-2
Lublin	CFX96 Bio-Rad	FTD Flu (Fast Track Diagnostics)
Łódź	–	Bosphore H1N1Detection Kitv3 (Anatolia Geneworks)
Olsztyn	CFX96 Bio-Rad	cDNA Synthesis Premix (Seegene) RV16 Detection (Seegene) Respiratory Panel 1 (Seegene) SARS-CoV-2/Flu A/ Flu B/RSV Assay (Seegene)
Opole	Real Time PCR MIC-4 (bio molekular systems - Syngen) termocykler Real Time PCR System SLAN 96S (Argenta)	FLU A+B (VIASURE, Syngen)
Poznań	GeneXpert (Cepheid) 7500 Real-Time PCR (Applied Biosystems)	Xpert Flu A,B, A/H1N1/pdm09
Rzeszów	Applied Biosystems 7500 Real-Time PCR System Roche Light Cycler 480 II – RBC Bioscience – MagCore HF 16 Plus	Ribo-prep nucleic adic extraction kit (AmpliSens), MagCore Super/HF 16 Plus nucleic Acid Extraction Kit (RBC Bioscience), FTD Flu (Fast-Track Diagnostics), FTD Flu differentiation (Fast-Track Diagnostics)
Szczecin	Rotor-Gene (Qiagen)	PowerChek Pandemic H1N1/H3N2 Real Time RT-PCR Kit (Kogene Biotech)
Warszawa	GeneXpert (Cepheid)	Xpert Flu A,B, A/H1N1/pdm09, Xpert Xpress Flu-RSV
Wrocław	Rotor-Gene (Qiagen) LightCycler 96 (Roche)	Viasure FLU A+B Real Time PCR Detection Kit (CerTest Biotec) Viasure FLU Typing I (H1H3) Real Time PCR Detection Kit (CerTest Biotec) RealAccurate Quadruplex Influenza PCR Kit (PathoFinder)

Prepared by Brydak L.B. based on National Institute of Public Health NIH – National Research Institute, 2022.

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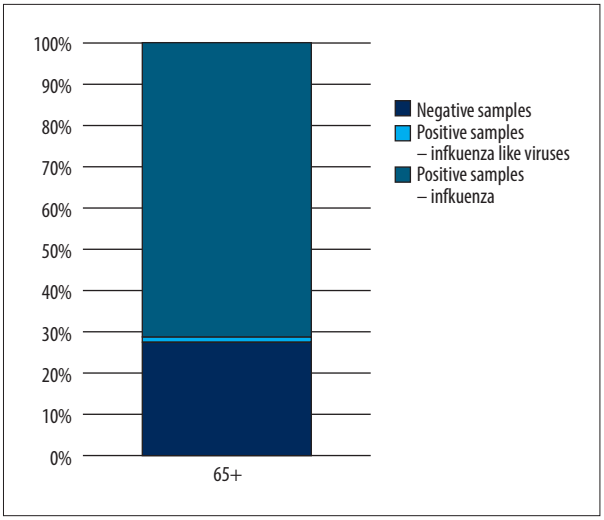

Figure 1. The percentage of positive samples in relation to all tested samples in the 65 year and older age group in the 2019-2020 epidemic season in Poland. Prepared by L.B. Brydak, based on National Institute of Public Health NIH – National Research Institute, 2022.

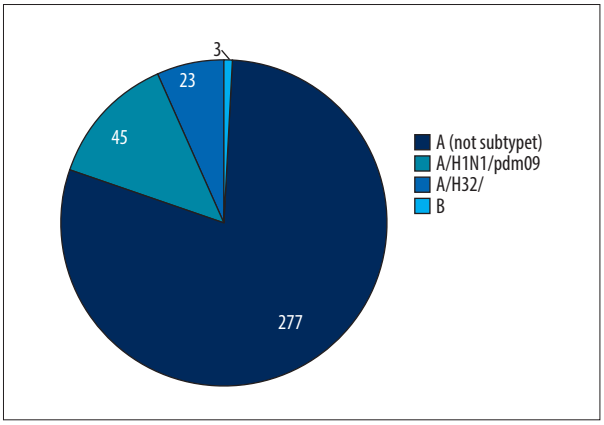

Figure 2. The percentage share of influenza viruses and their subtypes in children and in the 65 year and older age group in the 2019-2020 epidemic season in Poland. Prepared by L.B. Brydak, based on National Institute of Public Health NIH – National Research Institute, 2022.

Discussion

Monitoring the viruses circulating in the population is of great importance in the virological aspect because it allows for the correct selection of viruses included in the vaccine for the coming epidemic season and thus contributes to the effectiveness of influenza vaccination in a given season. Updating seasonal influenza vaccines is essential every season, as influenza viruses have exceptionally high mutation rates. In the 2019-2020 epidemic season, the influenza vaccine included the following strains: A/Brisbane/02/2018 (H1N1) pdm09, A/Kansas/14/2017 (H3N2), B/Colorado/06/2017, and B/Phuket/3073/2013. The

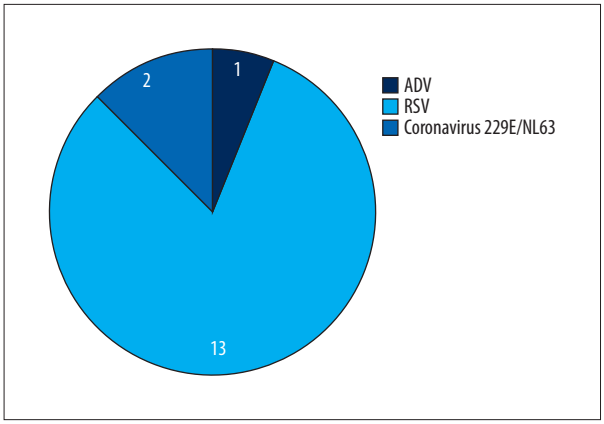

Figure 3. The percentage share of influenza-like viruses in the 65 year and older age group in the 2019-2020 epidemic season in Poland. Prepared by L.B. Brydak, based on National Institute of Public Health NIH – National Research Institute, 2022.

Table 2. Coinfections of respiratory viruses in the 2019-2020 epidemic season among the population of people over 65 years of age in Poland.

No.	Patient's age	Influenza viruses
1	86-year-old	A+RSV
2	70-year-old	CorOC43+RSV A
3	72-year-old	A+B
4	77-year-old	A+RSV A+RSV B

Prepared by Brydak L.B. based on National Institute of Public Health NIH – National Research Institute, 2022.

monitoring of circulating viruses is also of great clinical importance. Distinguishing influenza, which includes many health consequences, from harmless respiratory infections is extremely important. Therefore, it is important to quickly confirm the presence of the influenza virus using molecular biology methods [18], which allows the use of new generation anti-influenza drugs, including neuraminidase inhibitors such as zanamivir, oseltamivir, and generic versions of oseltamivir, shortly after the onset of symptoms; it is also advisable to confirm an influenza infection to limit the formation of mutants resistant to anti-influenza drugs [5,19]. Infections with influenza-like viruses have similar symptoms to influenza infections but cause less serious complications than infections with the influenza virus. It has been shown that patients over 65 years of age comprise 63% of all patients treated for influenza, and that as much as 85% of deaths in this age group are caused by a severe course of influenza or its complications. Studies from the United States indicate that the mortality rate for influenza and pneumonia makes them the sixth most common cause of death and fifth in the group of elderly people [20].

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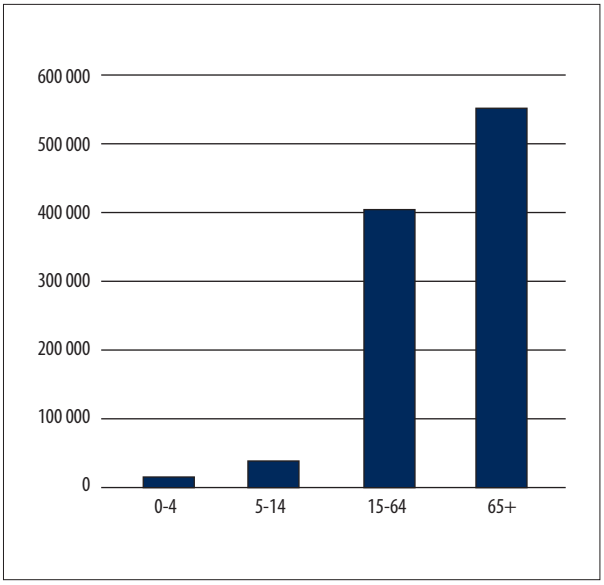

Figure 4. Number of influenza-vaccinated persons by age groups in 2019 in Poland. Prepared by L.B. Brydak, based on National Institute of Public Health NIH – National Research Institute, 2022.

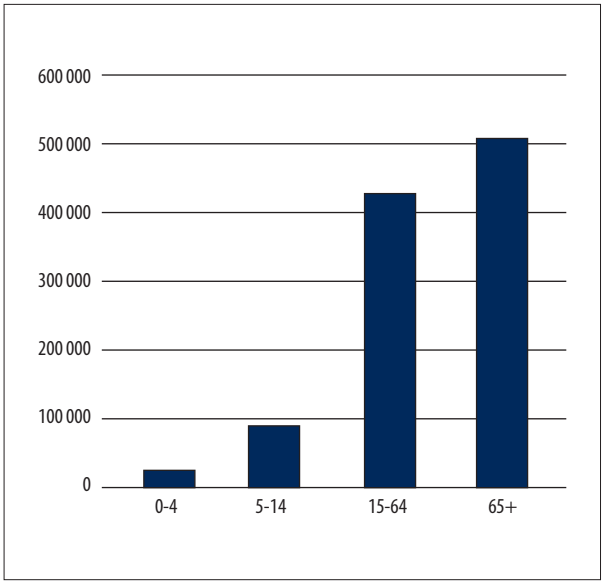

Figure 5. Number of influenza-vaccinated persons by age groups in 2020 in Poland. Prepared by L.B. Brydak, based on National Institute of Public Health NIH – National Research Institute, 2022.

In the 2019-2020 epidemic season, the circulation of influenza A, type B, and influenza-like viruses with varying severity was recorded. Among the tested people over 65 years of age, infections with influenza viruses and influenza-like viruses were confirmed. Influenza A was dominant. In the discussed 2019-2020 season, 2 subtypes of influenza A viruses, A/H1N1/pdm09 and A/H3N2/, were recorded as codominant, while in the previous 2018-2019 epidemic season, the subtype A/H1N1/pdm09 was most frequent in people over 65 years of age [21].

It is estimated that 291 243 645 843 people die worldwide every year due to respiratory complications after contracting influenza. Among them, people over the age of 75 years are most at risk. In this age group, there are 51.3 to 99.4 deaths per 100 000 people, compared with 13.3 to 27.8 per 100 000 people aged 65 to 74 years, and 1.0 to 5.1 per 100 000 people aged under 65 years [22,23]. In the 2019-2020 epidemic season, 43 deaths were recorded in Poland due to influenza complications in the population of people over 65 years of age. These deaths are recorded as deaths caused by influenza complications, especially pneumonia and concomitant chronic diseases, in particular cardiovascular diseases. Flu vaccination is the most effective and cost-effective way to prevent flu. Therefore, now, during the COVID-19 pandemic, resources should be invested in prevention and education related to vaccines. This has the potential to increase the percentage of the vaccinated population and hopefully achieve a population effect in the future. In 2019 and 2020 in Poland, the highest level of vaccination was recorded in the age group over 65 years [24,25] (**Figures 4, 5**). A meta-analysis on vaccination by Australian researchers Vu et al showed a 33% decrease in the number of hospitalizations due to pneumonia and influenza in this age group, and a reduction by as much as 47% [14,26] in mortality rate due to hospitalization in the course of pneumonia and influenza.

Limitations of the Study

This work is based solely on the analyses of samples that were reported to the Sentinel Influenza Surveillance System. Not all the tested samples are reported to the system; therefore, the number of patients studied in Poland in these seasons could be much higher.

Conclusions

Influenza poses a serious threat to people over the age of 65, because they often have chronic diseases. Patients over 65 years of age constitute the majority of all patients treated for flu, both in Europe and worldwide. This age group also has the highest number of deaths due to severe flu or its complications. Vaccination is the most effective method of preventing and controlling infectious diseases in the age group over 65, so resources should be invested in prevention and education related to vaccines. Today, in the era of the COVID-19 pandemic, the recommendation of influenza vaccination has particular importance.

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Declaration of Figures' Authenticity

All figures submitted have been created by the authors who confirm that the images are original with no duplication and have not been previously published in whole or in part.

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