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MAJOR ARTICLE

Multiple Influenza Virus Infections in 4 Consecutive Epidemiological Seasons: A Retrospective Study in Children and Adolescents

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Background. Recent observations provide evidence for group-specific immunity toward influenza A infections and raise the question of how often we can get the flu.

Methods. We retrospectively analyzed 2308 cases of children and adolescents with clinically manifested influenza and a positive PCR-test during the last 4 epidemiological seasons (2014–15 through 2017–18).

Results. In the 2015–16 epidemiological season, almost 12% of patients had experienced an influenza infection during the previous season; in the 2016–17 season, more than 14% had at least 1 infection during the previous 2 seasons, and in 2017–18 season, over 18% had 1 or more infections during the previous 3 seasons. The majority of these repetitive infections occurred in children between 3–8 years of age. 29 patients experienced 3 or 4 infections during these seasons, whereas 38 children had 2 influenza episodes within the same season. Epidemiological pattern of circulating viral strains changed yearly; however, we identified 5 patients with confirmed influenza B infections during the 2014–15 and 2017–18 seasons, when only subtype Yamagata was circulating in Austria.

Conclusions. Repetitive influenza infections in consecutive epidemiological seasons occurred quite frequently in children and adolescents. Observations like ours contribute to a better understanding of the immunity against influenza virus infections and could have implications for future vaccination strategies.

Key words: children; epidemiology; influenza; multiple infections.

We and others have previously reported on consecutive influenza infections in children during the same epidemiological season [1, 2]. This work raised the principal question on how often people can get influenza during their lifetimes and whether protection may exist in subsequent years after an infection with a similar influenza virus type or subtype.

Recent evidence suggests that protection against influenza A subtypes, A(H1N1)pdm09 or A(H3N2), depends on the first exposure in childhood. Infections in the first decade of life result in a long-lasting immune memory, and such individuals have the highest antibody titers directed against viruses encountered earlier in life [3, 4]. Of note, the 2 major phylogenetic groups of influenza A subtypes, with group 1 including A(H1N1)pdm09 and group 2 including A(H3N2), appear to be of specific importance in this setting [5]. It was proposed that the first infection with 1 of these subtypes results in protection from subsequent infection with viruses of the same phylogenetic group even when their hemagglutinins are modified [5]. This goes along with the observation made during the A(H1N1) pdm09 pandemic in 2009 where elderly subjects who were previously exposed to influenza viruses during previous epidemics in childhood presented with reduced susceptibility and lower morbidity as compared to subjects without such exposure [6].

The epidemiology and specifically the type and relative frequency of circulating virus strains has been contrastingly different between epidemiological seasons of recent years. Details can be found at the website of the European Center of Disease Control (https://www.ecdc.europa.eu/en/publications-data).

We thus questioned whether changes in the epidemiology of influenza infections over years may be partly explained by clinical manifest influenza in previous years and immunological protection against such influenza strains in subsequent epidemiological seasons. Therefore, we retrospectively analyzed the data from children with influenza in 4 consecutive epidemiological seasons.

METHODS

Our facility in Innsbruck is a diagnostic microbiological laboratory serving general practitioners and specialists (including several pediatricians) in Western Austria. The diagnosis of

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influenza virus was performed in respiratory specimens by means of a certified real-time polymerase chain reaction (PCR) test (Altona Diagnostics, Hamburg, Germany) [1]. This PCR test is able to specifically and separately detect influenza A and B viruses in respiratory specimens, but it does not differentiate between A(H1N1)pdm09 and A(H3N2) or other influenza viruses. According to the manufacturer's instruction, the analytical sensitivity for influenza A viruses amounts to 0.45 copies/µl (95% confidence interval: 0.28–1.22 copies/µl), and the analytical sensitivity is 2.42 copies/µl (95% confidence interval: 1.53–6.32 copies/µl) for the influenza B viruses specific system.

We performed a retrospective analysis of all positive cases (n = 2308) with influenza virus infection during 4 epidemiological seasons (2014–15, 2015–16, 2016–17, and 2017–18) which were sent in by primary care pediatricians and general practitioners from the county of Tyrol, Austria, and searched for subjects with multiple infections during these seasons. We also asked attending physicians of children with 3 or more PCR-confirmed influenza infections during these 4 years to answer several questions using a standardized questionnaire. We evaluated whether the affected subjects (1) had presented with typical clinical signs of influenza virus infection, (2) had received influenza-specific anti-viral therapy, (3) had been vaccinated against influenza prior to the respective seasons, and (4) suffered from any chronic underlying disease.

The influenza surveillance in Austria is based on a sentinel physician network as described previously [7]. Briefly, between October (calendar week 40) and April (week 16 of the following year) sentinel physicians (general practitioners and pediatricians throughout Austria) collect nasopharyngeal swabs from patients presenting with influenza-like illness as defined by the ECDC [8].

The samples were submitted to and analyzed by the National Influenza Center Austria, Center of Virology, Medical University Vienna. Influenza detection, typing, and subtyping were performed using real-time reverse transcription-PCR [7]. Based on the data of the Austrian influenza sentinel network, we were able to evaluate the influenza virus type or subtype and lineage distribution of influenza A and B viruses in patients under the age of 18 years in the western part of Austria.

As this was a retrospective analysis of laboratory tests without any interventions on patients, ethical approval was not mandatory.

RESULTS

The epidemics of influenza virus infection in patients under the age of 18 years differed widely over the past epidemiological seasons. We observed the concomitant presence of influenza A (59% of cases) and B in the 2014–15 season, the dominance of infections with influenza B (64%) over influenza A in 2015–16, an almost exclusive appearance of influenza A in the 2016–17 season, while a dominance of influenza B infections (56%) was again apparent in 2017–18 season (Figure 1 and Table 1a). Thus, co-circulation of influenza A and B viruses was noted in 3 of the 4 seasons. Distributions were quite similar for the 2 populations tested in Innsbruck and in Vienna (Table 1a). When focusing on influenza A infections in children, most of the cases in the 2014–15 and 2015–16 seasons were primarily or almost exclusively due to A(H1N1)pdm09. In contrast, influenza A(H3N2) became the only subtype detected in children below the age of 18 during the 2016–17 season. In 2017–18, all cases again were caused by A(H1N1)pdm09 (Table 1b). Of note, only influenza B Yamagata viruses were found during the in 2014–15 season,

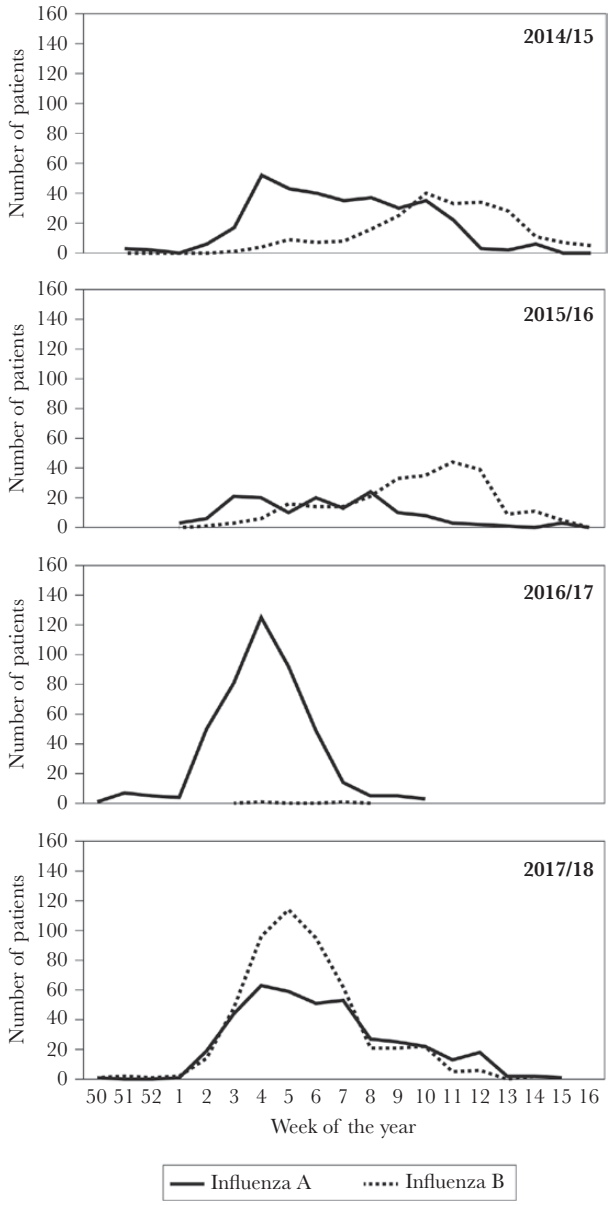

Figure 1. Number of positive real-time PCR results for influenza A and B virus infections in patients under the age of 18 years in our laboratory in Western Austria during 4 influenza seasons (2014–2018).

Table 1. Relative Distribution of Influenza A and B in the 2 Cohorts (a) and of Subtypes or Lineages in Patients Covered by the Sentinel Network (b)

a

	% A		% B	
Laboratory	Innsbruck	Vienna	Innsbruck	Vienna
2014/15	59	55	41	45
2015/163	36	44	64	56
2016/17	100	100	0	0
2017/18	44	32	56	68

b

	% A(H1N1)pdm09	% A(H3N2)	% B Victoria	% B Yamagata
2014/15	41	14	0	45
2015/16	44	0	56	0
2016/17	0	100	0	0
2017/18	32	0	0	68

whereas influenza B Victoria viruses accounted for all influenza B infections in the subsequent year. Influenza B was almost completely absent in the 2016–17 season, but again only B Yamagata viruses were detected and accounted for almost 70% of all influenza infections in 2017–18.

We also analyzed the 2308 cases of children and young adults under the age of 18 with confirmed influenza virus infections in respect to their risk to acquire an influenza infection in subsequent years or dual infections in the same epidemiological season. In Table 2, the numbers for multiple infections in all 4 seasons and the number of patients with repeated infections are shown.

Among the 395 children who tested positive for influenza in 2015–16, 47 children (11.9%) had a confirmed influenza infection in the previous season. In 2016–17, as many as 63 of 443 (14.2%) children diagnosed positive for influenza suffered from influenza in 1 or both of the previous epidemic seasons. Finally, 165 out of 911 (18.1%) PCR-positive influenza patients in the 2017–18 season had at least 1 confirmed influenza infection in the 3 previous seasons (Table 2).

Among those subjects with repetitive infections in subsequent seasons, we found patients with influenza A infection followed either by influenza A or B infection in subsequent years as well as patients with influenza B infections followed by either influenza A or B infection in next seasons (Table 2).

Noteworthy is that we also identified 5 cases of children who had infections with influenza B in the 2014–15 and 2017–18 seasons, when most likely only viruses of the Yamagata line circulated according to epidemiological data (2 patients with only these 2 infections and cases 2, 21, and 22 in Figure 2).

We also identified subjects with repeated influenza A infections in subsequent years; however, we cannot provide subtype distribution, which would have been of great interest, due to our test system.

We can assume, however, with a high probability that the subtype distributions in our patient groups are quite similar to those found in the Austrian sentinel data for the respective epidemiological season (Table 1b).

We also identified a total of 29 patients with more than two, that is, 3 or 4 influenza infections during those 4 epidemiological seasons (Figure 2). One patient (no. 12) had 4 infections—2 influenza B infections in the first 2 seasons followed by 2 influenza A infections in the next 2 seasons. Eight of these patients had 2 infections in the same season, 2 patients in 2014–15, 1 patient in 2015–16, and 5 patients in 2017–18. We contacted the treating physicians to receive clinical data from those 29 patients with repeated influenza virus infections. We received data on temperature scores from 19 patients. Only 1 of these patients had lower temperature at 2 presentations (37.4 and 37.8°C) whereas all others presented with fever above 38°C during each episode of influenza. Among those 29 patients who suffered in total an amount of 85 influenza virus infections during the 4 seasons,

Table 2. Number of Multiple Infections During 4 Influenza Seasons[a]

		2014/15		2015/16		2016/17		2017/18	
		A	B	A	B	A	B	A	B
2014/15	A	0	13	7	23	11	0	17	32
	B	0	0	7	8	26	0	8	5
2015/16	A			0	2	4	0	1	14
	B			1	0	28	0	17	8
2016/17	A					0	0	34	36
	B					0	0	0	0
2017/18	A							0	10
	B							12	0
Number of infections		13		48		69		194	
Number of patients		13		47		63		165	

[a]Example of data interpretation. First row (season 2014–15): 13 patients with influenza A in the season 2014–15 also suffered from subsequent influenza B virus infection in the same season. Second column (season 2015–16): 7 and 23 patients with influenza A virus infection in the season 2014–15 had an infection with influenza A (7) or influenza B virus (23), respectively, in the following season, 2015–16. 7 and 8 patients with influenza B virus infection in the season 2014–15 suffered from influenza A (7) or influenza B (8) virus infection, respectively, in the following season 2015–16.

Number of patients and number of infections differ; some patients had more than 1 infection in previous years.

Patient no. (yrs)	2014/15		2015/16		2016/17		2017/18	
	A	B	A	B	A	B	A	B
1 (5)	xxxxx	ooooo	xxxxx					
2 (3)	xxxxx	ooooo						ooooo
3 (1)	xxxxx		xxxxx					ooooo
4 (2)	xxxxx			ooooo	xxxxx			
5 (4)	xxxxx			ooooo	xxxxx			
6 (1)	xxxxx			ooooo	xxxxx			
7 (2)	xxxxx			ooooo				ooooo
8 (11)	xxxxx			ooooo				ooooo
9 (5)		ooooo	xxxxx		xxxxx			
10 (2)		ooooo		ooooo	xxxxx			
11 (7)		ooooo		ooooo	xxxxx			
12 (1)		ooooo		ooooo	xxxxx		xxxxx	
13 (5)	xxxxx				xxxxx			ooooo
14 (2)	xxxxx				xxxxx			ooooo
15 (5)	xxxxx				xxxxx			ooooo
16 (3)	xxxxx				xxxxx			ooooo
17 (2)		ooooo			xxxxx		xxxxx	
18 (5)		ooooo			xxxxx		xxxxx	
19 (2)		ooooo			xxxxx		xxxxx	
20 (5)		ooooo			xxxxx		xxxxx	
21 (3)		ooooo			xxxxx			ooooo
22 (2)		ooooo			xxxxx			ooooo
23 (3)	xxxxx						xxxxx	ooooo
24 (1)	xxxxx						xxxxx	ooooo
25 (3)			xxxxx	ooooo				ooooo
26 (3)				ooooo	xxxxx		xxxxx	
27 (7)					xxxxx		xxxxx	ooooo
28 (3)					xxxxx		xxxxx	ooooo
29 (3)					xxxxx		xxxxx	ooooo

Figure 2. Distribution of influenza A (xxxxx) and influenza B (ooooo) virus infections in patients with 3 or 4 (patient no. 12) infections during 4 consecutive influenza seasons. Age of patients in years at first infection is given in brackets.

only 11 of those influenza virus infections were treated with neuraminidase inhibitors. Among the 29 children, 27 were never vaccinated, 1 child received 2 vaccinations at the age of 1 year in 2010 (trivalent inactivated vaccine), and 1 child received 2 vaccinations at the age of 4 years in 2009 (trivalent inactivated vaccine). 27 children had no chronic disease or clinical evidence of immune deficiency, whereas 2 children had chronic remittent diseases: 1 child suffered from familial Mediterranean fever and 1 child from PFAPA syndrome (periodic fever, aphthous stomatitis, pharyngitis, and cervical adenitis).

We also analyzed the age distribution of children with subsequent infections and found that these occurred most frequently between the age of 3 and 8 years (Figure 3).

In contrast to our previous observations [1], we found consecutive infections with influenza A and B only rarely in 2015–16 (2 influenza B infections following an influenza A infection and 1 A following B). In 2016–17, this of course was not possible as only influenza A infections occurred in this epidemic season. However, in 2017–18 again, 22 consecutive infections during the same season could be detected. An influenza A virus infection was subsequently followed by an influenza B virus infection in 10 subjects, and the reverse order of influenza virus infection was observed in 12 cases, confirming that 2 subsequent infections in 1 season are not unusual if both influenza A and B occur in the same season in relevant numbers.

DISCUSSION

A changing epidemiology of influenza A and B infections in Europe has been observed over the past years. This is in agreement with our data demonstrating that subtle alterations of circulating influenza strains occur in infected children between

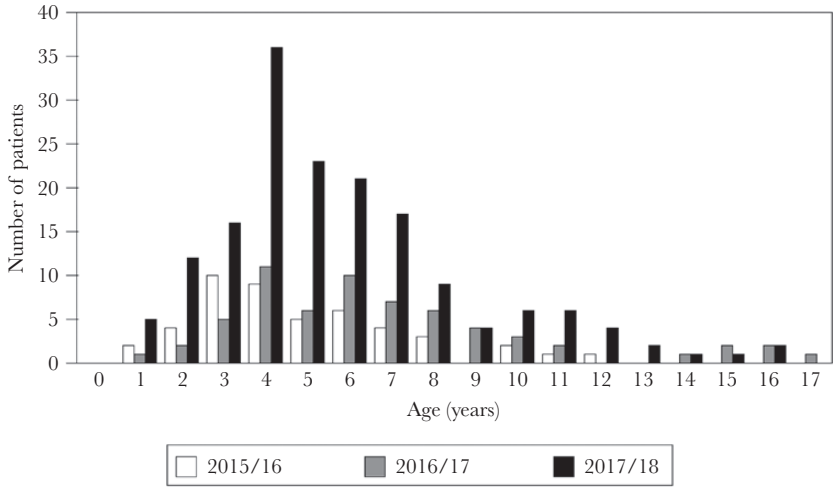

Figure 3. Age distribution of children and adolescents who had repeated infections in 2 or more consecutive influenza seasons.

different epidemiological seasons. We also found that repetitive infections occurred in a relatively high percentage of children in subsequent years.

Given the present epidemiologic situation with 2 influenza A subtypes and 2 influenza B lineages circulating during the last seasons, we propose that it is not unlikely to experience infections with all 4 influenza viruses within a few years.

Our study has some limitations. Due to the retrospective design, information on clinical data are limited. However, all patients presented with clinical symptoms compatible for influenza virus infections. We do not have information as to whether all children with influenza in a previous epidemiological season and influenza-like illness in subsequent seasons consulted their general practitioner or pediatrician and thus the numbers of consecutive or subsequent infections, or both, could be even higher than reported herein.

Based on evidence from the literature, it had been suggested that an infection of children with a specific influenza virus strain confers protection against infection with a related or identical virus in subsequent years [5, 9]. According to that data, prior infection in early childhood causes efficient protection by recalling adaptive immune memory upon viral challenge with a genetically similar virus [3, 5]. Thus, it is plausible that re-infection with influenza strains from closely related influenza viruses does not become clinically apparent, and subjects who do not suffer from influenza-like symptoms have an efficient boost of their anti-influenza immune response [9]. It is suggested that any type of influenza exposure may cause protective immunity lasting for several years. This goes also along with epidemiological and vaccination studies that indicate the importance of cross-reactivity and the order of infection for protective immune response against influenza A [3, 5] but also shows the persistence of antibodies against influenza proteins for many years [10].

According to the paradigm that the "first flu is forever" [11], it would be important to know if subsequent infections with other influenza A or B virus subtypes in childhood result in a similarly long-lasting immunological memory resulting in partial or complete protection from clinical infection. Our results from 4 influenza seasons are partly in accordance with this prediction but also challenge those results. During the observation period, we found a number of patients with 3 or even 4 infections. Those children had either 1 or 2 influenza A virus infections and 1 or 2 influenza B virus infections, but no child had more than 2 influenza A or 2 influenza B virus infections, respectively. We also observed consecutive infections within the same season. Of interest, none of these children had received a vaccination in the previous 3 seasons and the vast majority (27 of 29) never in lifetime. Further, most children (27 of 29) with repetitive influenza infections (3 or 4 in 4 seasons) were otherwise healthy and had no underlying chronic disease.

Due to our test system, we were not able to differentiate between influenza A subtypes, so it is not possible to draw conclusions whether re-infection with influenza A in subsequent years occurred with the same or another viral subtype. Apart from the 2016–17 season when A(H3N2) was dominant in all other seasons, both A(H1N1)pdm09 and A(H3N2) were circulating. However, the observation of repeated infections with influenza B provides important information. This is due to the fact that in the respective epidemiological seasons only 1 influenza B virus was exclusively circulating in Austria with the Yamagata strain found in 2014–15 and 2017–18 and Influenza B Victoria detected in 2015–16. We identified several children with influenza B infections in 2014–15 and 2015–16 indicating that there is no or only limited immunological cross protection between these different influenza B viruses [12–14]. Of even more import, we also identified 5 children who had confirmed clinically-evident influenza B infection in 2014–15 and 2017–18 when almost exclusively the Yamagata strain was found in Austria [15]. This could imply that there is no long-lasting protective immunity after an infection with a specific influenza B

virus strain. However, we cannot exclude that antigenic patterns of influenza B Yamagata have changed in Austria over the years, thereby undermining protective immunity. Nevertheless, phylogenetic analysis of these viruses did not provide evidence for subtle alterations of Influenza B Yamagata over time [16]. However, to definitely address the questions of duration of immune protection against specific viral strains, large prospective population-based trials will be necessary.

According to our data, it appears that consecutive and subsequent infections in children occur mainly in the first decade of life with a peak between 3 and 8 years at a time when long-lasting immune memory is most likely to be developed [9]. Therefore, the question of how often we can get influenza in our lifetimes arises specifically when we experienced infections during the first decade of life. Subsequent studies will have to analyze influenza A virus subtypes to get more information regarding the efficacy and duration of immune protection after previous infection with the same strain. Such data may help to verify the hypothesis whether the changing epidemiological pattern of circulating influenza strains in different seasons is determined partly by short or long-lasting protective immunity against the same virus subtype that emerge from clinical apparent infections in the previous season.

This raises the question of whether either early vaccination of children (with inactivated or live vaccines) may cause long-lasting protection, or its opposite: if children who experienced infection with a specific influenza subtype do not require vaccination against this virus family during subsequent years or even during their lifetime. The answer would have important implications for vaccination strategies and the selection of subjects benefiting from vaccination against seasonal influenza, that is, by providing vaccination only for specific strains against which no persistent immune protection has been developed during childhood [17].

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