Respiratory viruses and SARS-CoV-2 virus circulation in the provinces of Lucca and Massa Carrara during the 2022/2023 and 2023/2024 flu seasons

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Summary

Background and Aims: the study aimed to investigate the circulation of influenza viruses and SARS-CoV-2 in the 2022-23 and 2023-24 influenza seasons in the provinces of Lucca and Massa Carrara in the northwest of the Tuscany region.

Materials and Methods: a retrospective observational study was conducted on adult and pediatric patients who accessed hospital facilities in the considered area during the influenza periods (from September to April) of 2022-23 and 2023-24.

Results: the 2022-23 influenza season was characterized by the circulation of the A/H3N2 influenza virus (84% of the total), with a peak of positive samples from weeks 42 to 52 in 2022. Instead, the influenza A/(H1N1)pdm09 virus was responsible for 94% of influenza infections in the 2023-24 season, with a peak of positive samples between weeks 52 of 2023 and 3 of 2024.

Conclusions: effective virological diagnostics and constant monitoring of circulating viruses are mandatory to correctly address therapy and contain the viral circulation in the different influenza seasons.

Introduction

Influenza epidemics remain a serious public health problem and a significant source of direct and indirect costs both for the implementation of control measures and for the management of cases and complications of the disease. Worldwide, annual epidemics are estimated to result in approximately one billion cases of influenza, three to five million forms of severe illness, and 290,000 to 650,000 deaths [7,11,14,16,35]. Older people, young children, pregnant women, and people with chronic diseases are the groups at greatest risk for developing severe forms, but the entire population can be subject to significant complications, including pneumonia, myocarditis, and encephalitis, which can result in unfavorable outcomes [12,13,17,29,34]. There are two main types of influenza viruses A and B. Influenza A viruses are classified into subtypes based on two surface proteins, Hemagglutinin (HA) and Neuraminidase (NA). A combination of two subtypes of HA (H1 and H3) and two subtypes of NA (N1 and N2) are recognized among influenza A viruses as causing widespread human disease over the past decades [2,6]. In the 2022/2023 influenza season, the majority of infections were caused by the A/H3N2 influenza virus, also known as the «Australian» influenza virus, which is particularly aggressive in the elderly, pregnant women, and patients with chronic diseases. The first known case of the epidemic due to the H3N2 strain dates back to the Hong Kong flu in 1968, which arrived in Italy between 1969 and 1970 [37]. The H1N1 virus was identified as the causative agent of the 1918 Spanish flu, an influenza pandemic that killed millions of people worldwide between 1918 and 1920. It was the first of the 20th-century pandemics involving the H1N1 influenza virus. Studies have shown that the 1918 H1N1 pandemic virus may have originated before 1918, when a human H1 virus acquired avian neuraminidase and internal proteins genes. The genetic segments

would have come from an avian virus that, by making a leap between species, would have adapted to humans, also acquiring an exceptional capacity for transmission from person to person. The 1918 H1N1 virus was the protagonist of another anomalous phenomenon at the same time as the human pandemic; when it began to circulate, it also spread to pigs, a species that was previously free from the flu. The swine H1N1 lineage derived from the human Spanish flu virus would later re-emerge in humans after 1922, initiating a new human H1N1 lineage [9,19,44]. The swine-derived influenza A/H1N1 virus has caused influenza infections since the 1950s following exposure and close contact with pigs. The A/H1N1 influenza virus includes multiple strains, among which pdm09 (Pandemic Disease Mexico 2009) is the main responsible for influenza infections in the 2023/2024 season in the southern and northern hemispheres [10]. Specifically, it is a quadruple reabsorbing virus, made up of two viruses of swine origin, a virus of avian origin, and a virus of human origin, which, since it emerged, has spread rapidly throughout the world, mainly infecting children, young people, adults and individuals with lung and heart disease [31,38]. Like other human influenza viruses, the circulation of the A/(H1N1)pdm09 influenza virus causes an increase in hospitalizations and mortality during the period of diffusion [25]. The symptoms of influenza virus A/(H1N1)pdm09 are similar to those of the classic seasonal flu; they mainly affect the respiratory tract and, especially in the early stages of the disease, are associated with fever, cough, cold, and generalized feelings of malaise [21,22,42]. In some cases, serious complications, such as pneumonia, which may require hospitalization and sometimes the use of intensive care, even in young subjects not affected by other pathologies, may occur. In seasons dominated by type A influenza viruses, higher hospitalization and mortality rates were recorded, with a higher average than in other years (excluding the 2009 H1N1 influenza epidemic) [40]. Influenza epidemics have always had an east-west transmissibility trend. As the seasons are opposite in the southern and northern hemispheres [8,20,45,46], the trend of flu epidemics, the number of cases, and the typology of the main viruses that will circulate in the autumn/winter season in our country, is based on the flu epidemics in the southern hemisphere and in particular in Australia [18,30]. The aim of the study was to analyze the main viruses circulating in the 2023/2024 influenza season compared to those circulated in the 2022/2023 one in the provinces of Lucca and Massa Carrara, also evaluating the trend of the virus SARS-CoV-2 in the post-Covid-19 pandemic period, based on the results of the processing of respiratory samples (nasopharyngeal swabs and respiratory secretions) of hospitalized patients. The data obtained were compared with the epidemiological data of the main viruses that circulated in the southern hemisphere in the same flu seasons to verify if the peaks recorded in the province of Lucca were caused by the same type of viruses.

Materials and Methods

Subjects

An observational retrospective study was conducted on patients hospitalized in the North West of Tuscany (San Luca Hospital in Lucca, Nuovo Ospedale Apuane, NOA, in Massa, and Versilia Hospital in Viareggio, with about 400 beds each, and 2 lower hospitals Lunigiana Fivizzano, Lunigiana Pontremoli). In the study were investigated all patients with microbiological diagnosis of respiratory virus infection for the 2022/23 (September 2022 to April 2023), and 2023/24 (September 2023 to April 2024) influenza seasons in Italy, for an exhaustive evaluation of the impact of respiratory viruses. Every year, data collection on flu-like syndromes and respiratory viruses by RespiVirNet, the Integrated Surveillance System

(epidemiological and virological), goes from the 42nd week to the 17th week. The period analyzed in the study, however, goes from the 39th week to the 17th week. Data collection for the study began in September and, therefore, does not coincide with the national surveillance period that began in October. All microbiological *in vitro* diagnostics were performed in the same laboratory (Clinical Chemistry Laboratory of Lucca). The report included both pediatric and adult subjects (age range from one month to 65 years). The subjects of the study were selected taking into account the provisions issued by the General Directorate of Health Prevention of the Ministry of Health on monitoring the trend of severe and complicated forms of influenza viruses for the 2023/2024 season, introduced with Circular of 19 November 2009 and integrated annually [33]. According to the provisions of the Circular of the Ministry of Health, the regions and autonomous provinces are required to report to the Ministry and the Istituto Superiore di Sanità (ISS) serious and complicated cases of laboratory-confirmed influenza, the conditions of which require hospitalization in the unit of intensive care and/or, the use of extracorporeal membrane oxygenation therapy [23]. The study included subjects with both severe and milder respiratory symptoms, who requested a test for the identification of influenza viruses, including subtyping for A(H1N1)pdm09 and A(H3N2). The patient cohort studied consisted of subjects with pneumonia who presented with symptoms such as chest pain, chills, fever, and shortness of breath. Patients with complicated pneumonia, on the other hand, showed more or less severe clinical pictures. The characteristic symptoms of progressively worsening forms of pneumonia may present a series of dysfunctions such as the presence of low oxygen levels in the bloodstream, which causes shortness of breath; detection of hypotension; the presence of a lung abscess, which forms when a small area of the lung dies and a collection of pus forms in its place or the identification of an empyema which is a collection of pus in the space between the lung and the chest wall; identification of severe lung injury, caused by severe infection or excessive inflammation in response to infection, may manifest as Acute Respiratory Distress Syndrome (ARDS). ARDS causes shortness of breath, usually with rapid, shallow breathing. Patients with ARDS usually require breathing assistance with a mechanical ventilator for a long period of time.

Molecular biology methods

The throat and/or nasal swabs of the patients considered were processed, and, for the most serious ones, with lower airway infections, the Bronchoalveolar Lavage (BAL) or Broncho-Aspiration (BAS) samples were also analyzed. Different analytical platforms were used for processing, depending on the timing required and the number. A diagnostic platform was represented by the BIOFIRE® FILMARRAY® Panels (bioMérieux, Craponne, France), which uses a Nested Multiplex PCR (nmPCR) protocol for the identification of 16 viral and 4 bacterial targets (at gender and/or species), in respiratory samples. The integrated FilmArray platform is a closed system, which is based on the use of a disposable and compact pouch inside which the reactions of extraction, purification, and amplification of the nucleic acid of the pathogen present in the samples take place biologically. The execution time of the analysis is approximately one hour. The second platform in use is the Standard M10 system (SD Biosensor, Gyeonggi-do, Korea) which automatically extracts and amplifies nucleic acids (DNA, RNA) from respiratory tract samples using the multiplex RT-PCR test, for the search of the SARS-CoV-2 genome or for the combined search of the Influenza A virus genome and B, Respiratory Syncytial Virus (RSV) and SARS-CoV-2. The analysis time is 60 minutes. The third analytical panel used is AllplexTM Respiratory Full Panel Assay

(Seegene Inc., Seoul, Korea), a multiplex One-step RT real-time PCR test, which simultaneously detects and identifies 16 viruses, 7 bacteria, and 3 subtypes of Flu A, processing up to 30 samples in one run. It allows us to examine the presence of a wider variety of viruses and bacteria from a single nasopharyngeal swab or from respiratory secretions. It has an analysis time of approximately four hours and was used, for tests that did not have urgency parameters.

Results

From the analysis of the data obtained for the 2022/2023 and 2023/2024 influenza seasons, an increase in influenza cases is observed compared to the 2020/2021 pandemic season and post-2021/2022 COVID-19 pandemic [43]. During the 2022/2023 season, a total of 9648 samples were analyzed, and from the analysis of the trend of requests and the results obtained, an increase in some respiratory viruses was recorded compared to other viruses involved in respiratory infections. At the beginning of the 2022/2023 influenza season, in September 2022, 149 samples were analyzed, with 12 (8%) positive for viral strains involved in respiratory infections and only 1 positive, during the last week (39th), for type A influenza virus H1N1/pdm09 subtype (70-year-old intensive care patient). The samples analyzed for SARS-CoV-2 were 1271, and 167 (13%) tested positive. In October 2022 (weeks 40 to 44), 228 samples were analyzed, of which 67 (29%) were positive, 4 (6%) to influenza virus type A subtype H3N2, and 63 (94%) to other respiratory viruses. In the same period, 1771 samples were analyzed for SARS-CoV-2, with 523 (30%) positive. In November 2022 (weeks 45 to 48), 290 samples were analyzed, of which 114 (39%) were positive; 25 (12%) to influenza virus type A subtype H3N2, and 85 (88%) to other respiratory viruses. The samples analyzed for SARS-CoV-2 were 1541 with 388 (25%) positive. In December 2022 (weeks 49 to 52), 556 samples were analyzed, of which 233 (42%) tested positive; 83 (36%) for influenza virus type A subtype H3N2, and 150 (64%) for other respiratory viruses. The samples analyzed for SARS-CoV-2 were 988, with 276 (28%) positive. In January 2023 (weeks 1 to 5), 387 samples were analyzed, of which 182 (47%) were positive; 19 (10%) to influenza A virus subtype H3N2 and 163 (90%) to other respiratory viruses. There were 766 samples analyzed for SARS-CoV-2, and 154 (20%) tested positive. In February 2023 (from the 6th to the 9th week), 244 samples were analyzed, of which 104 (43%) were positive; 13 samples (13%) tested positive for influenza virus type A subtype (H1N1)/pdm09 and 2 (2%) for H3N2 subtype, while 89 (85%) tested positive for other respiratory viruses. The samples analyzed for SARS-CoV-2 were 559, with 99 (18%) positive. In March 2023 (from the 10th to the 13th week), 287 samples were analyzed, of which 59 (21%) were positive; 6 samples (10%) tested positive for influenza type A virus of subtype (H1N1)/pdm09 and 9 (15%) for influenza type B virus, while 44 (75%) tested positive for other respiratory viruses. The samples analyzed for SARS-CoV-2 were 187 with 50 (27%) positive. In April 2023 (from the 14th to the 17th week), 286 samples were analyzed, of which 65 (23%) were positive, 2 (3%) to influenza type B virus, and 63 (97%) to other respiratory viruses. The samples analyzed for SARS-CoV-2 were 138 with 50 (36%) positive. Starting from the end of February 2023, the circulation of influenza viruses recorded a new increase, although more limited, associated with an increased proportion of the identification of A/(H1N1)pdm09 strains with a peak in the 8th week, followed by a gradual decrease in the months of March 2023. From the end of February and during the first two weeks of March 2023, an increase in respiratory infections caused by the type B influenza virus was also recorded. The trend of positive cases for COVID-19 showed, however, an increase in the month of October, followed by a reduction in November 2022, then remaining constant until the month of March, then gradually increasing in the month of April 2023 (Figure 1). The 2023/2024 flu season started later than the previous year, and by analyzing in detail the progress of the requested investigations, variations in the viruses involved in respiratory infections were recorded during the months of the flu season considered. During the 2023/2024 season, a total of 3540 samples were analyzed. In September 2023, 167 samples were analyzed, and only 10 (6%) tested positive for viral strains involved in respiratory infections. In the same period, 105 samples were analyzed for SARS-CoV-2, and 100 (95%) tested positive. In October 2023 (from the 40th to the 44th week), 320 samples were analyzed, of which 179 (56%) tested positive for viruses involved in respiratory infections. There were 170 samples analyzed for SARS-CoV-2, and 168 (99%) tested positive. In November 2023 (weeks 45 to 48), 292 samples were analyzed, of which 89 (30%) were positive, 11 (12%) to influenza virus type A subtype H1N1/pdm09, and 78 (88%) to other respiratory viruses. There were 179 samples analyzed for SARS-CoV-2, and 168 (95%) tested positive. In December 2023 (weeks 49 to 52), 469 samples were analyzed, of which 152 (32%) were positive; 57 (38%) tested positive for influenza A virus subtype H1N1/pdm09, 2 (1%) positive for subtype H3N2 and 93 (61%) for other respiratory viruses. There were 250 samples analyzed for SARS-CoV-2 and 218 (87%) tested positive. In January 2024 (weeks 1 to 5), 659 samples were

Influenza season 2022/2023	39	40	41	42	43	44	45	46	47	48	49	50	51	52	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	TOTAL
Influenza A virus					1									1		1																3
Influenza B virus													1									2	3	3	1		2			2		14
Respiratory syncytial virus A							1	1	1	3	4	8	6	6	6	4	5	5	3	1	3	4	1		5	1	1					69
Respiratory syncytial virus B				1	1		1	1		1	2	4	11	11	12	13	9	11	3	8	9	7	7	2	2	2	3		1			122
Flu A-H1					1																											1
Flu A-H1pdm09	1												2	1						2	2	8	1	1	2	3						23
Flu A-H3				1	3	1	3	6	6	10	13	25	20	25	8	7	4					1	1									134
Adenovirus				1		1		2					1	2				1	1			1		1			1	1		1	1	15
Parainfluenza virus 1												4											1									5
Parainfluenza virus 2			1						1																							2
Parainfluenza virus 3							2	1	1	1		2	2	2				2		1		2	3	1		2	5	1	3	2	6	39
Parainfluenza virus 4						1			2																							3
Metapneumovirus						1		4	2	5		5	1	2	6	2	6	6	4	3		8	7	8	5	3	4	1	1	2	2	88
Bocavirus			1	2				1		1		1	3	1											1	1	1		1			14
Rhinovirus		7	7	6	7	5	6	8	10	10	13	11	10	11	11	12	9	10	9	2	9	9	7	5	7	5	4	2	5	5	3	225
Coronavirus NL63								1															1		3		1	1			2	9
Coronavirus 229E								1															4	1	1	1						8
Coronavirus OC43			1		1			1	1				3	3						1	1				1	1	1	1		1	1	21
SARS-CoV-2	40	50	50	100	223	100	100	100	88	100	100	76	50	50	30	35	40	29	20	35	30	14	15	10	12	13	15	15	15	10	10	1575
TOTAL POSITIVE	41	58	60	115	234	107	123	121	108	132	132	139	108	115	67	78	70	62	46	59	54	57	50	29	39	35	36	21	28	21	25	2370

Figure 1. Weekly trend of samples testing positive for influenza viruses divided by type/subtype and other respiratory viruses circulating in the provinces of Lucca and Massa Carrara in the 2022/2023 influenza season.

analyzed, of which 255 (39%) were positive; 98 (38%) to influenza A virus subtype H1N1/pdm09, 2 (1%) to subtype H3N2 and 155 (61%) to other respiratory viruses. There were 84 samples analyzed for SARS-CoV-2, and 59 (66%) tested positive. In February 2024 (from the 6th to the 9th week), 334 samples were analyzed of which 163 (49%) were positive; 28 (17%) for influenza virus type A, subtype (H1N1)/pdm09 and 3 (2%) for influenza virus type H3N2, while 132 (81%) tested positive for other respiratory viruses. There were 50 samples analyzed for SARS-CoV-2 and 27 (54%) tested positive. In March 2024 (from the 10th to the 13th week), 299 samples were analyzed, of which 107 (36%) were positive; 3 samples (3%) tested positive for influenza A virus subtype (H1N1)/pdm09; 12 samples (11%) tested positive for influenza B while 92 (86%) tested positive for other respiratory viruses. There were 24 samples analyzed for SARS-CoV-2, and 5 (21%) tested positive. In April 2024 (from the 14th to the 17th week), 220 samples were analyzed, of which 51 (23%) were positive; 15 samples (29%) tested positive for influenza B, while 36 (71%) tested positive for other respiratory viruses. There were 10 samples analyzed for SARS-CoV-2 and 6 (60%) tested positive (Figure 2). Overall, in the 2022/2023 influenza season, of all the samples sent to the laboratory with requests for further investigation for respiratory tract infection of suspected viral origin, 794 (35%) tested positive: 160 (17%) for influenza virus type A and 14 (1.5%) for type B. Among the samples positive for influenza A virus, 23 (14%) belong to the H1N1/pdm09 subtype and 134 (84%) to the H3N2 subtype. Furthermore, 225 samples (28%) positive for rhinovirus were found; 15 (2%) positive for adenovirus; 49 (5%) positive for parainfluenza viruses; 88 (12%) positive for metapneumovirus; 38 (4%) samples positive for human coronaviruses other than SARS-CoV-2; 69 (7%) samples positive for respiratory syncytial virus A 122 (13%) for respiratory syncytial virus B; 14 (1.5%) samples positive for Bocavirus. Overall, in the 2023/2024 influenza season, of all the samples sent to the laboratory with requests for further investigation for respiratory tract infection of suspected viral origin, 795 (38%) tested positive: 213 (27%) for influenza virus type A and 22 (3%) for type B. Among the samples positive for influenza A virus, 200 (94%) belong to the H1N1/pdm09 subtype and 7 (6%) to the H3N2 subtype. Furthermore, 236 samples (30%) positive for rhinovirus were found; 34 (4%) were positive for adenovirus; 54 (7%) were positive for parainfluenza viruses; 54 (7%) positive for metapneumovirus; 46 (6%) samples positive for human coronaviruses other than SARS-CoV-2; 94 (12%) samples positive for respiratory syncytial virus A 21 (3%) for respiratory syncytial virus B; 21 (3%) samples positive for bocavirus (Figure 3).

Discussion

In Italy, an average of 8,000 deaths from influenza and its complications are recorded every year (https://www.epicentro.iss.it/influenza/sorveglianza-mortalita-influenza), and with the increase in the average lifespan of individuals, it will determine a significant increase of direct and indirect costs both for the implementation of control measures and for the management of cases and complications of the disease. In Italy, for an exhaustive evaluation of the impact of respiratory viruses on the population, it was established 2023-2024 season system RespiVirNet, the Integrated Surveillance System (epidemiological and virological) of cases of flu-like syndromes and respiratory viruses (formerly InfluNet). RespiVirNet is based on general practitioners, paediatricians, and regional reference laboratories for respiratory viruses. Surveillance is coordinated by the Istituto Superiore di Sanità (ISS) with the support of the Ministry of Health. The data collected highlights an increase in cases of influenza from type A influenza viruses, both in the 2022/2023 and 2023/2024 seasons, compared to those recorded in previous seasons characterized by the COVID-19 pandemic [36,39]. In the 2022/2023 influenza season, an increase in the circulation of the A/H3N2 influenza virus was observed in the provinces of Lucca and Massa Carrara, while in the 2023/2024 winter season there was an increase of influenza A/(H1N1)pdm09 (Figure 4), confirming the association with the circulation of influenza viruses in the southern hemisphere, and in particular in Australia, which for the 2022 and 2023 influenza season had already indicated as predominant strains respectively influenza viruses the A/H3N2 and A/(H1N1)pdm09. Also, in this period, serious complications (pneumonia and respiratory failure) and deaths associated with cases of influenza virus type A are recorded [26]. Since 2022, the population has been more receptive to respiratory infections from influenza A viruses, and this may be associated with immunity debt, deriving from the lower viral circulation linked to the measures adopted to combat SARS-CoV-2 infection [1,4,5,28]. The easing of anti-COVID-19 measures starting in the autumn of 2021 has gradually brought the circulation of influenza viruses back to previous levels. Although there was an easing of anti-COVID-19 measures starting from the autumn of 2021, in the 2021/2022 season, the influenza epidemic remained at low intensity, presumably thanks to the virtuous behaviors learned during the pandemic, while its intensity significantly increased in season 2022/2023 and 2023/2024. From the analysis of the results obtained from the tests carried out on hospitalized patients in our area of expertise, it is highlighted that

| Influenza season 2023/2024	39	40	41	42	43	44	45	46	47	48	49	50	51	52	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	TOTAL
Influenza A virus													1	1	1		2	1														6
Influenza B virus			1																							2	4	6	4	4	1	22
Respiratory syncytial virus A		1	1	2	3							2	4	9	12	8	3	9	6	3	7	12	2	1	3	3	1	1			1	94
Respiratory syncytial virus B								1	1	1							1	2	1	2	3	3		2	2	2						21
Flu A-H1																																0
Flu A-H1pdm09							1		2	8	7	5	15	30	25	34	14	13	12	17	7	3	1	2			1		2	1		200
Flu A-H3													2		1	1				2			1									7
Adenovirus							1	3	1	1			3	5	2				2	1		2		2	3	5	1		1	1	1	34
Parainfluenza virus 1								1															1									2
Parainfluenza virus 2					1																1	1		2								5
Parainfluenza virus 3			1		3	1	2			1			3			1	1	2		1	1			2	1	3		2	3	1	2	31
Parainfluenza virus 4	1		1	1		1		2	1	3			2				2				1		1							1		16
Metapneumovirus		1				1		1	1		1	1	1	2	2	1	2		1	2	4	5		7	5	8	6	1	1			54
Bocavirus								1		1			2	1				5	4	1	2	1	1		2							21
Rhinovirus	1	8	10	8	9	4	6	8	6	6	8	7	11	12	8	7	5	14	8	9	9	13	12	9	9	8	7	3	5	2	4	236
Coronavirus NL63					1																		2		1							4
Coronavirus 229E														1	3							1		1		2				2	1	11
Coronavirus OC43					1								3				3	7	1	1	4	2	3		2	1	1		1	1		31
SARS-CoV-2	10	14	27	36	45	46	42	39	40	40	40	52	65	61	20	10	10	9	10	11	10	4	2	1	1	2	1	1	2	1	2	654
TOTAL POSITIVE	12	24	41	47	63	54	53	54	52	61	56	68	103	123	81	74	38	58	44	53	46	49	22	29	30	35	22	14	22	11	10	1449

Figure 2. Weekly trend of samples testing positive for influenza viruses divided by type/subtype and other respiratory viruses circulating in the provinces of Lucca and Massa Carrara in the 2023/2024 influenza season.

the trend of the influenza epidemic in the last two seasons has been 'atypical' compared to the influenza seasons before of pandemic caused by SARS-CoV-2 in which recorded a single epidemic peak between the end of January and the beginning of February [3,32]. In the 2022/2023 season, there was a maximum peak of respiratory infections caused by influenza A virus subtype H3N2, concentrated in weeks 48-52 of 2022, to which other viruses such as rhinovirus, respiratory syncytial virus A and B, SARS-CoV-2 and also parainfluenza viruses, adenoviruses and other human coronaviruses other than SARS-CoV-2. Furthermore, in 2022 and 2023, unlike normal seasonal trends, influenza viruses continued to circulate until April, albeit with a more limited spread. In the 2023/2024 influenza season, an increase in influenza type A virus was observed, the prevalence of the A/(H1N1)pdm09 subtype, with a maximum peak of infections between the 52nd week of 2023 and the 2nd week of 2024. Comparing the trend and number of cases of respiratory infections diagnosed in the influenza seasons considered, it is highlighted that there was an increase in positive cases compared to the total samples analyzed in the 2023/2024 season (Figure 5). Faced with an increase in requests and samples analyzed in the last season, it can be said that there has been greater appropriateness of the requests, which reflects a targeted clinical and diagnostic framework for the patient.

The same consideration can also be extended in the case of samples positive for SARS-CoV-2; in fact, during the 2022/2023 influenza season, the epidemiological trend of the SARS-CoV-2 virus reached contagion levels significantly higher than the previous two influenza seasons. The main cause is certainly the spread of the Omicron variant, which is much more contagious than the previous variants (alpha and delta) but, fortunately, less capable of producing severe clinical pictures. At the same time, the possibility of diagnosis via antigenic nasopharyngeal swab was introduced [27], while until December 2021, the diagnosis was possible only with a molecular swab. An increase in diagnosed cases, confirmed by molecular swabs, has been observed since September 2022. Analyzing the trend of infections caused by SARS-CoV-2 on a monthly basis, after the peak recorded in October 2022, a series of smaller waves with a constant decrease until April 2023. In the 2023/2024 influenza season, was recorded appearance of two Omicron variants, Eris, which is very widespread, and JN.1, which became the dominant variant in January 2024 [41]. It has been estimated, in fact, that cyclically, every 4-6 months, we witness the appearance of different variants. This entails the possibility of reinfection after 6 months, and for this reason, the SARS-CoV-2 infection rates remain stable, interspersed with waves with a greater number of infections. COVID presents a cyclical nature which, unlike what happens with the flu, is not connected only to the winter season, and this forces continuous surveillance of the resulting infections. In the 2023/2024 season, starting from September 2023, a constant increase in SARS-CoV-2 infections was recorded, with a peak between the end of December 2023 and the 1st week of January 2024 and then suddenly decreasing at the end of January 2024.

	Season 2022/2023	Season 2023/2024	P
Influenza A virus	3 (0,4%)	6 (1%)	<0,01
Influenza B virus	14 (2%)	22 (3%)	<0,05
Respiratory syncytial virus A	69 (9%)	94 (12%)	<0,01
Respiratory syncytial virus B	122 (15%)	21 (3%)	<0,01
Flu A-H1	1 (0,1%)	0	<0,01
Flu A-H1pdm09	23 (3%)	200 (25%)	<0,01
Flu A-H3	134 (17%)	7 (1%)	<0,01
Adenovirus	25 (3%)	34 (4%)	NOT SIGNIFICANT
Parainfluenza virus 1	5 (0,3%)	2 (0,2%)	<0,01
Parainfluenza virus 2	2 (0,3%)	5 (1%)	<0,01
Parainfluenza virus 3	39 (5%)	31 (4%)	NS
Parainfluenza virus 4	3 (0,4%)	16 (2%)	<0,01
Metapneumovirus	98 (12%)	54 (7%)	<0,01
Bocavirus	14 (2%)	21 (3%)	NOT SIGNIFICANT
Rhinovirus	245 (31%)	236 (30%)	NOT SIGNIFICANT
Coronavirus NL63	9 (1%)	4 (1%)	NOT SIGNIFICANT
Coronavirus 229E	8 (1%)	11 (1%)	//
Coronavirus OC43	21 (3%)	31 (4%)	<0,01
TOTAL POSITIVE	794 (100%)	795 (100%)	

Figure 3. Comparison of changes (number and percentage) in the main influenza viruses observed between the 2022/2023 and 2023/2024 seasons.
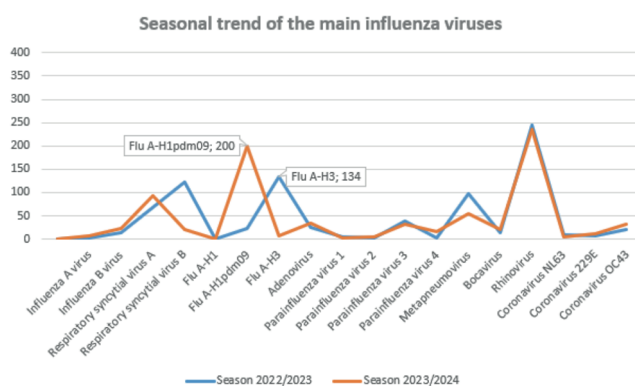
Figure 4. Trend of positive samples for influenza viruses and other respiratory viruses in the 2022/2023 influenza season and in the 2023/2024 season.
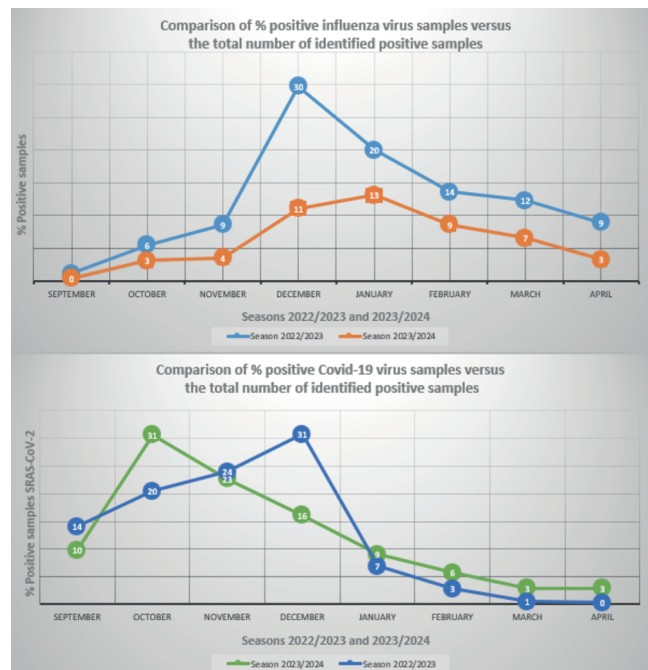
Figure 5. Comparison of the percentage trend of positive samples compared to the total of positive samples for the 2022/2023 and 2023/2024 seasons of both the viruses causing respiratory infections and SARS-CoV-2.

Conclusions

Epidemiological studies have highlighted a continuous variation in the antigenic structure of influenza viruses. The importance of maintaining global surveillance programs for the detection of virological changes, for clinical and epidemiological purposes, of circulating influenza viruses, which allows a timely assessment of the overall risk within the population, is therefore confirmed. Considering the extreme variability of influenza viruses, and the high contagiousness of the infection, it can be stated that the flu vaccination remains a decisive measure for controlling their circulation, especially after the resumption of normal relational life, at the end of the containment measures. As highlighted by the Ministry of Health with the issue on 21 April 2023 of the Circular «Prevention and control of influenza: recommendations for the 2023-2024 season» [15,24], vaccines are confirmed as a primary tool in influenza prophylaxis. An interesting development, on a territorial basis, will be a deep evaluation of the associations between the vaccination status of the population and viral circulation in the individual district, recoverable from a closer connection between vaccination registers by age groups and clinical and diagnostic findings of viral circulation.

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