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While influenza vaccines aim to decrease the incidence of severe influenza among high-risk groups, evidence of influenza vaccine effectiveness (IVE) among the influenza vaccine target population is sparse. We conducted a multicentre test-negative case–control study to estimate IVE against hospitalised laboratory-confirmed influenza in the target population in 18 hospitals in France, Italy, Lithuania and the Navarre and Valencia regions in Spain. All hospitalised patients aged ≥18 years, belonging to the target population presenting with influenza-like illness symptom onset within seven days were swabbed. Patients positive by reverse transcription polymerase chain reaction for influenza virus were cases and those negative were controls. Using logistic regression, we calculated IVE for each influenza virus subtype and adjusted it for month of symptom onset, study site, age and chronic conditions. Of the 1,972 patients included, 116 were positive for influenza A(H1N1)pdm09, 58 for A(H3N2) and 232 for influenza B. Adjusted IVE was 21.3% (95% confidence interval (CI): -25.2 to 50.6; n=1,628), 61.8% (95% CI: 26.8 to 80.0; n=557) and 43.1% (95% CI: 21.2 to 58.9; n=1,526) against influenza A(H1N1) pdm09, A(H3N2) and B respectively. Our results suggest that the 2012/13 IVE was moderate against influenza A(H3N2) and B and low against influenza A(H1N1) pdm09.

Background

Antigenic drifts of influenza viruses expose the population to new but related influenza variants on a regular basis [1]. On the basis of a yearly revised composition of seasonal influenza vaccines, the World Health Organization (WHO) considers annual Influenza vaccination as the most efficient measure against influenza [2]. Every year, the seasonal influenza vaccine licensure is obtained based on immunogenicity data [3]. While these immunogenicity data are thought to be valid for healthy adults [4], the development of correlates of protection suited to vulnerable populations is still to be achieved [5].

The population targeted for influenza vaccination in Europe includes those at increased risk of exposure to influenza virus as well as of developing severe disease, especially disease resulting in hospitalisation or death [6]. Target groups for vaccination usually include adults over 59 or 64 years of age and people of any age with certain underlying medical conditions [7,8]. Measuring influenza vaccine effectiveness (IVE) in each influenza season is important for the following reasons: to identify vaccines types and brands with low IVE; to decide on alternative preventive strategies if early estimates of IVE are low (e.g. preventive use of antivirals among vulnerable individuals); and to help decide on the next season's vaccine content. Repeated evidence of suboptimal IVE among the population targeted for annual

Table 1

Generic protocol adaptations in each study site, hospital-based influenza vaccine effectiveness study, four European countries, 2012/13

Protocol adaptation	France	Italy	Lithuania	Spain	
				Navarre	Valencia
Additonal staff for the study	Yes	Yes	No	No	Yes
Services	Emergency ward	Emergency ward internal medicine unit	Emergency ward Infectious disease hospital	All	Emergency ward
Vaccine status ascertainment	Patient	Patient or GP	Patient or GP	Register	Register and oral
Ascertainment of type of vaccine used	Ecological data	Individual data	Ecological data	Individual data	Individual data
Exclusion based on place of residence	No	No	No	Yes	Yes
Inclusion of patients unable to sign the consent form	Yes	Yes	No	Yes	Yes
Type of respiratory specimen	Nasal	Nasal and pharyngeal	One pharyngeal and two nasal	Nasal and pharyngeal	Nasal and pharyngeal
Data entry validation	Coordination team	Coordination team	Coordination team	Coordination team	Double entry for laboratory results Weekly quality checks
Study periods[a]					
Influenza A(H1N1)pdm09	Week 1, 2013	Week 2, 2013	Week 52, 2012	Week 7, 2013	Week 47, 2012
	Week 10, 2013	Week 8, 2013	Week 9, 2013	Week 11, 2013	Week 15, 2013
Influenza A(H3N2)	Week 52, 2012	Week 3, 2013	Week 3, 2013	Week 4, 2013	Week 9, 2013
	Week 14, 2013	Week 6, 2013	Week 13, 2013	Week 13, 2013	Week 12, 2013
Influenza B	Week 50, 2012	Week 5, 2013	Week 4, 2013	Week 50, 2012	Week 51, 2012
	Week 13, 2013	Week 9, 2013	Week 15, 2013	Week 11, 2013	Week 15, 2013

GP: general practitioner.

[a] The International Organization for Standardization's week numbers were used, to ensure consistency across study sites.

influenza vaccination would also further advocate the need for vaccines that are more effective in this population. Moreover, there are ongoing scientific debates about the effect of repeated vaccination on the immunological response induced by the seasonal influenza vaccine [9-11] and further evidence is needed.

In 2011, we launched a pilot study to estimate the IVE against laboratory-confirmed influenza hospitalisation using a network of hospitals in the European Union (EU) [12]. During the 2012/13 influenza season, co-circulation of influenza A(H1N1)pdm09, A(H3N2) and B/Victoria- and B/Yamagata-lineage viruses was reported in Europe [13]. The objective of the study presented here was to measure the 2012/13 seasonal IVE against hospitalisation with subtype-specific laboratory-confirmed influenza in a hospital network in four EU countries: France, Italy, Lithuania and Spain.

Methods

We conducted a case–control study using the test-negative design [14] in 18 hospitals located in five study sites: France (five hospitals), Italy (two), Lithuania (two), and the Navarre (four) and Valencia (five) regions in Spain. Each study site adapted a generic protocol [15] to the local context (Table 1).

Study population

The study population was all community-dwelling adults (18 years of age or older), belonging to the target groups for vaccination as defined locally [16-20], admitted to one of the participating hospitals with no contraindication for influenza vaccination. Patients were excluded if they had previously tested positive for influenza virus in the 2012/13 season or resided outside the hospital catchment area (for the 11 hospitals with known catchment area).

Study teams actively screened all patients admitted for potentially influenza-related conditions. These conditions included the following: acute myocardial infarction or acute coronary syndrome; heart failure; pneumonia and influenza; chronic pulmonary obstructive disease; myalgia; altered consciousness, convulsions, febrile-convulsions; respiratory abnormality; shortness of breath; respiratory or chest symptoms; acute cerebrovascular disease; sepsis; and systemic inflammatory response syndrome. Among them, study teams invited patients with an onset of influenza-like

Table 2

Definition of the categories of chronic conditions according to the variables collected, hospital-based influenza vaccine effectiveness study, four European countries, 2012/13

Categories of chronic conditions	Chronic conditions	Study sites that collected the information
Cardiovascular disease	Cardiovascular disease[a]	FR, IT, LT, VA
	Heart disease	FR, IT, LT, NV, VA
	Stroke	FR, IT, LT, NV
	Transient ischemic attack	IT
	Peripheral arterial disease	IT, VA
Respiratory disease	Lung diseases[a]	FR, IT, LT, NV
	Asthma	IT, VA, LT
	Chronic obstructive pulmonary disease	IT, LT
	Emphysema	IT, LT
	Mucoviscidosis	FR, IT, LT
	Bronchitis	VA, LT
Metabolic and endocrine disorders	Diabetes	FR, IT, NV, VA
	Nutritional deficiency	FR, IT, LT
	Endocrine disease	FR, IT, LT, VA
Haematological disease or cancer	Haematological cancer	FR, IT, LT, NV
	Anaemia/spleen condition	FR, IT, LT, VA
	Drepanocytosis	FR, IT
	Cancer	FR, IT, LT, NV, VA
Immunodeficiency	Immunodeficiency	FR, IT, LT, NV, VA
	Rheumatological disease	FR, IT, LT, NV
Hepatic disease		FR, IT, LT, NV, VA
Renal disease		FR, IT, LT, NV, VA
Obesity[b]		FR, IT, LT, NV, VA
Neuromuscular disorder		FR, IT
Dementia		FR, IT, LT, NV, VA

FR: France; IT: Italy; LT: Lithuania; NV: Navarre, Spain; VA: Valencia, Spain.

[a] May include the conditions from the same category listed below.

[b] Defined as body mass index ≥30 kg/m².

illness (ILI) symptoms (one systemic and one respiratory symptom) within the past seven days to participate. Those accepting to participate were swabbed and tested for influenza. Reverse transcription polymerase chain reaction (RT-PCR) was used to detect influenza viruses and to classify them as influenza A(H3N2), influenza A(H1N1)pdm2009 or influenza B. Patients positive for influenza were classified as cases of a given influenza type/subtype and those testing negative were controls.

We defined the study period as at least 15 days after the beginning of each site-specific seasonal influenza vaccination campaign until the end of the influenza season as declared by local influenza surveillance systems. For each of the influenza type/subtype analyses, we excluded the controls with onset of symptoms before the week of the first laboratory-confirmed case or after the week of the last laboratory-confirmed case. We used the International Organization for Standardization's week numbers [21] to ensure consistency across study sites.

We considered patients as vaccinated against seasonal influenza if they had received at least one dose of the 2012/13 influenza vaccine more than 14 days before onset of ILI symptoms. Patients not vaccinated or vaccinated less than 15 days before ILI onset were considered as unvaccinated.

Data collection

We collected data on the ILI episode, demographics, chronic diseases (Table 2), number of hospitalisations in the previous 12 months, number of consultations at the general practitioner (GP) in the previous three months, smoking status, vaccination against influenza in 2012/13 and 2011/12 and, for those aged 65 years and over, functional status before ILI onset using the Barthel score [22]. The data were gathered from hospital medical records, face-to-face interviews with the patient and/or patient's family and laboratory databases. The vaccination status was obtained from vaccination registers in two study sites, interview with the patients and/or patient's family in two sites and contact with the patient's physician in one site.

Table 3

Number of records received by the pooled analysis coordinator and included in the pooled analysis by study site, hospital-based influenza vaccine effectiveness study, four European countries, 2012/13

Type of record	Number of records per study site					
	France[a]	Italy	Lithuania[b]	Navarre, Spain	Valencia, Spain	Total
Eligible records	433	84	184	93	1,535	**2,329**
Non-target groups for vaccination	78	14	96	18	102	**308**
Missing laboratory results	2	0	0	0	43	**45**
Unknown vaccination status	3	0	1	0	0	**4**
Total records used for the analyses	350	70	87	75	1,390	**1,972**
Influenza A(H1N1)pdm09						
Cases	20	10	20	9	57	**116**
Controls	213	39	24	24	1,213	**1,513**
Influenza A(H3N2)						
Cases	38	4	9	2	5	**58**
Controls	229	24	29	33	204	**519**
Influenza B						
Cases	62	13	25	17	115	**232**
Controls	219	31	28	45	971	**1,294**

[a] In France, one specimen of influenza A virus could not be subtyped.

[b] In Lithuania, one patient was coinfected with A(H3N2) and A(H1N1)pdm09 viruses.

Data analysis

Study sites transmitted anonymised datasets to the pooled analysis coordinator, through a password-secured web-based platform. We ran a complete case analysis, excluding records for which laboratory results, vaccination status or potential confounding variables were missing.

To test for heterogeneity between study sites, we used Cochran's Q-test and the I^2 index [23]. The Q-test provides a p value that indicates the presence or not of heterogeneity. The I^2 index quantifies the proportion of the variance attributable to differences between study sites. It is common to consider that I^2 around 25%, 50% and 75% indicate low, medium and high heterogeneity, respectively.

We conducted separate analyses for each type/subtype of influenza. We estimated the pooled IVE as 1 minus the odds ratio (OR) (expressed as a percentage) of being vaccinated in cases versus controls, using a one-stage method with study site as fixed effect in the model [24].

We assessed the presence of effect modification by comparing the time- and study site-adjusted OR (assuming that the test-negative design case–control study is a density case–control study implying adjustment for the time of symptom onset) across strata of characteristics using the homogeneity test. We considered a variable as a confounder when the percentage change between the unadjusted and adjusted OR was greater than 15%.

We conducted a multivariable logistic regression analysis. In addition to study site and month of symptom onset, we adjusted the models for the covariates identified as potential confounders in the stratified analysis as well as the presence of at least one underlying condition and the age that we modelled as a restricted cubic spline with four knots [25]. The likelihood ratio test was used to decide on the final models. We conducted stratified analyses by age group (less than 65 years, 65–79 years and 80 years and above).

To study the effect of previous influenza vaccination on laboratory-confirmed influenza, we conducted a stratified analysis using four vaccination status categories: vaccination in none of the seasons (2011/12 and 2012/13), 2012/13 vaccination only, 2011/12 vaccination only and vaccination in both seasons and computed and compared IVE for each of these categories using vaccination in none of the seasons as a reference.

We carried out sensitivity analyses excluding the weeks when less than 10% of the patients included were positive for influenza, excluding patients who received antivirals between the onset of symptoms and swabbing and by restricting the analysis to patients swabbed within four days of symptoms onset. To avoid the inclusion of patients with acute manifestation of chronic respiratory illnesses rather than respiratory infection, we restricted our analysis to patients with no underlying respiratory conditions.

We ran all analyses with Stata v12 (Stata Corp LP, College Station, TX, United States).

Table 4

Characteristics of influenza A(H1N1)pdm09 (n=116), influenza A(H3N2) (n=58) and influenza B (n=232) cases and corresponding test-negative controls included in the study, hospital-based influenza vaccine effectiveness study, four European countries[a], 2012/13 (n=1,972)

Charactertistic	A(H1N1)pdm09		A(H3N2)		B	
	Controls[b] (n=1,513)	Cases (n=116)	Controls[c] (n=519)	Cases (n=58)	Controls[d] (n=1,294)	Cases (n=232)
	Number (%)[e]	Number (%)[e]	Number (%)[e]	Number (%)[e]	Number (%)[e]	Number (%)[e]
Median age in years	77.0	63.0*	75.0	73.0	77.0	75.2
Age group in years						
18–64	339 (22.4)	60 (51.7)*	146 (28.1)	14 (24.1)	301 (23.3)	60 (25.9)
65–79	563 (37.2)	42 (36.2)*	175 (33.7)	22 (37.9)	473 (36.6)	92 (39.7)
80–103	611 (40.4)	14 (12.1)*	198 (38.2)	22 (37.9)	520 (40.2)	80 (34.5)
Sex						
Male	851 (56.2)	67 (57.8)	294 (56.6)	24 (41.4)*	718 (55.5)	108 (46.6)*
Vaccine status						
2012/13 seasonal influenza vaccination	866 (57.2)	39 (33.6)*	296 (57.0)	20 (34.5)*	734 (56.7)	88 (37.9)*
2011/12 seasonal influenza vaccination	835 (55.3)	37 (31.9)*	296 (57.5)	25 (44.6)	702 (54.5)	102 (44.5)*
Presence of comorbidities						
Metabolic and endocrine disorders	546 (36.1)	41 (35.3)	195 (37.6)	24 (41.4)	462 (35.7)	72 (31.0)
Cardiovascular disease	768 (50.8)	49 (42.2)	247 (47.6)	26 (44.8)	636 (49.1)	103 (44.6)
Renal disease	198 (13.1)	9 (7.8)	84 (16.2)	8 (13.8)	165 (12.8)	27 (11.7)
Respiratory disease	750 (49.6)	50 (43.5)	243 (46.8)	25 (43.1)	634 (49.0)	80 (34.6)*
Neuromuscular disorder	82 (5.6)	7 (8.0)	27 (5.9)	3 (6.4)	70 (5.7)	7 (3.7)
Hepatic disease	65 (4.3)	2 (1.7)	14 (2.7)	0 (0.0)	57 (4.4)	8 (3.5)
Immunodeficiency	102 (6.7)	8 (6.9)	40 (7.7)	5 (8.6)	87 (6.7)	16 (6.9)
Haematological disease or cancer	321 (21.7)	16 (14.5)	96 (19.2)	12 (21.8)	279 (21.6)	30 (13.0)*
Any chronic condition (of all chronic conditions collected in the study site)	1,404 (92.8)	106 (91.4)	473 (91.1)	52 (89.7)	1,195 (92.3)	192 (82.8)*
More than one chronic condition	1,013 (67.0)	62 (53.4)*	340 (65.5)	37 (63.8)	853 (65.9)	113 (48.7)*
Obesity[f]	423 (28.1)	26 (22.6)	127 (24.7)	10 (17.9)	359 (27.9)	54 (23.5)
Pregnancy	10 (0.7)	1 (1.3)	7 (2.0)	0 (0.0)	11 (1.0)	8 (4.7)*
Low functional status[g] (among patients ≥65 years)	232 (19.8)	9 (16.1)	5 (14.8)	4 (9.1)	187 (18.9)	34 (19.8)
Other potential confounders						
More than one GP visit in previous 3 months	738 (49.1)	46 (39.7)	261 (51.3)	26 (46.4)	649 (50.7)	109 (48.0)
Hospitalisations in previous 12 months	582 (38.5)	32 (27.6)*	205 (39.6)	22 (37.9)	502 (38.8)	70 (30.2)*
Smoker status						
Current	277 (18.3)	39 (33.6)*	108 (20.8)	13 (22.4)	243 (18.8)	32 (13.9)*
Former	580 (38.3)	35 (30.2)*	173 (33.4)	16 (27.6)	485 (37.5)	58 (25.1)*
Never	656 (43.4)	42 (36.2)*	237 (45.8)	29 (50.0)	565 (43.7)	141 (61.0)*
Potential for misclassification						
Swabbing delay<4 days	745 (49.2)	69 (59.5)*	233 (44.9)	24 (41.4)	621 (48.0)	90 (38.8)*
Antiviral treatment before swabbing	18 (1.2)	12 (10.4)*	17 (3.3)	5 (8.6)	18 (1.4)	17 (7.3)*

GP: general practitioner.

* p value for difference between cases and controls<0.05.

[a] France, Italy, Lithuania and Spain (Navarre and Valencia regions).

[b] Comparisons were made with controls recruited between the week of the first case of influenza A(H1N1)pdm09 and the week of the last case of influenza A(H1N1)pdm09 (1,513 controls).

[c] Comparisons were made with controls recruited between the week of the first case of influenza A(H3N2) and the week of the last case of influenza A(H3N2) (519 controls).

[d] Comparisons were made with controls recruited between the week of the first case of influenza B and the week of the last case of influenza B (1,294 controls).

[e] Unless otherwise indicated.

[f] Defined as body mass index ≥30 kg/m².

[g] Determined using the Barthel score [22].

Table 5

Influenza vaccine effectiveness against influenza A(H1N1)pdm09, A(H3N2) and B, adjusted for various covariables by age group, hospital-based influenza vaccine effectiveness study, four European countries[a], 2012/13

Groups assessed	A(H1N1)pdm09	A(H3N2)	B
All target groups			
Number of cases and controls	1,628	577	1,526
Number of cases; number of vaccinated cases	116; 39	58; 20	232; 88
Number of controls; number of vaccinated controls	1,512; 865	519; 296	1,294; 734
Variables used for adjustment of vaccine effectiveness	Percentage influenza vaccine effectiveness (95% CI)		
Study site	47.0 (18.8 to 65.4)	54.4 (16.1 to 75.2)	46.5 (27.7 to 60.4)
Study site and month of symptom onset	45.7 (16.4 to 64.8)	53.0 (13.2 to 74.5)	44.3 (24.3 to 59.0)
Study site, month of symptom onset and age	20.9 (–25.3 to 50.1)	61.9 (27.2 to 80.1)	46.9 (26.8 to 61.5)
Study site, month of symptom onset, age and presence of chronic conditions	21.3 (–25.2 to 50.6)	61.8 (26.8 to 80.0)	43.1 (21.2 to 58.9)
Patients aged 18–64 years belonging to target groups			
Number of cases and controls	372[b]	143[c]	346[d]
Number of cases; number of vaccinated cases	60; 9	14; 3	60; 7
Number of controls; number of vaccinated controls	312; 105	129; 39	286; 91
Variables used for adjustment of vaccine effectiveness	Percentage influenza vaccine effectiveness (95% CI)		
Study site and month of onset	42.5 (–28.3 to 74.3)	26.1 (–215.9 to 82.7)	68.4 (25.7 to 86.6)
Study site, month of onset and presence of chronic conditions	41.8 (–30.7 to 74.1)	NA[c]	66.0 (19.3 to 85.7)
Patients aged 65–79 years			
Number of cases and controls	504[e]	181[f]	565
Number of cases; number of vaccinated cases	42; 18	22; 7	92; 40
Number of controls; number of vaccinated controls	462; 276	159; 91	473; 287
Variables used for adjustment of vaccine effectiveness	Percentage influenza vaccine effectiveness (95% CI)		
Study site and month of onset	44.2 (–9.0 to 71.4)	55.7 (–22.8 to 84.0)	37.3 (–2.1 to 61.5)
Study site, month of onset and presence of chronic conditions	43.8 (–10.7 to 71.5)	52.4 (–33.9 to 83.1)	28.2 (–18.9 to 56.6)
Patients aged 80–103 years			
Number of cases and controls	623[g]	216[h]	600
Number of cases; number of vaccinated cases	14; 12	22; 10	80; 41
Number of controls; number of vaccinated controls	609; 412	194; 147	520; 348
Variables used for adjustment of vaccine effectiveness	Percentage influenza vaccine effectiveness (95% CI)		
Study site and month of symptom onset	–171.7 (–1,170.7 to 41.9)	73.8 (30.0 to 90.2)	46.4 (9.6 to 68.2)
Study site, month of symptom onset and presence of chronic conditions	NA[g]	73.8 (29.9 to 90.2)	44.8 (6.7 to 67.4)

CI: confidence interval; NA: not applicable.

[a] France, Italy, Lithuania and Spain (Navarre and Valencia regions).

[b] A total of 27 controls dropped because no cases in November among patients less than 65 years.

[c] A total of 17 controls dropped because no cases in December and April and in Italy among patients less than 65 years. No adjustment for chronic disease because all A(H3N2) cases aged less than 65 years had chronic conditions.

[d] A total of 15 controls dropped because no cases in April among patients less than 65 years.

[e] A total of 101 controls dropped because no cases in December among patients aged 65–79 years.

[f] A total of 16 controls dropped because no cases in December and in Navarre, Spain, among patients aged 65–79 years.

[g] Two controls dropped because no cases in Lithuania among patients aged 80 years and over. No adjustment for chronic disease because all A(H1N1)pdm09 cases aged 80 years and over had chronic conditions.

[h] Four controls dropped because no cases in April among patients aged 80 years and over.

Results

Overall, 2,329 eligible patients, of whom 2,021 belonged to the target groups for influenza vaccination, were recruited in the 18 study hospitals (Table 3). A total of 45 (2.2%) and four (0.2%) patients were excluded due to missing laboratory results and missing vaccination status, respectively. We included a total of 1,972 patients in the analysis: 1,390 from Valencia (177 cases), 350 from France (121 cases), 87 from Lithuania (53 cases), 75 from Navarre (28 cases) and 70 from Italy (27 cases).

Influenza A(H3N2), A(H1N1)pdm09 and B co-circulated in all study sites (Table 1). The study site having included patients for the longest period of time was Valencia (week 47, 2012 to 15, 2013) and for the shortest period was in Italy (week 2–8, 2013). The period of

Table 6

Crude and adjusted vaccine effectiveness against influenza A(H1N1)pdm09 (n=1,625), A(H3N2) (n=571) and B (n=1,518) by vaccination status, hospital-based influenza vaccine effectiveness study, four European countries[a], 2012/13

Influenza type	Number of cases	Number of controls	Crude VE[b] (95% CI)	Adjusted VE[c] (95% CI)
A(H1N1)pdm09 (n=1,625)				
No vaccination in 2012/13 and 2011/12	71	539	–	–
2012/13 vaccination only	8	135	26.2 (–62.9 to 66.6)	6.2 (–110.4 to 58.2)
2011/12 vaccination only	6	108	39.8 (–47.1 to 75.4)	26.6 (–81.6 to 70.3)
2011/12 and 2012/13 vaccinations	31	727	52.8 (24.3 to 70.6)	27.9 (–20.5 to 56.9)
A(H3N2) (n=571)				
No vaccination in 2012/13 and 2011/12	30	183	–	–
2012/13 vaccination only	1	36	65.3 (–176.6 to 95.7)	68.3 (–157.2 to 96.1)
2011/12 vaccination only	6	40	5.1 (–156.4 to 64.9)	12.3 (–140.7 to 68.1)
2011/12 and 2012/13 vaccinations	19	256	49.2 (1.7 to 73.8)	59.6 (18.5 to 80.0)
B (n=1,518)				
No vaccination in 2012/13 and 2011/12	121	478	–	–
2012/13 vaccination only	6	109	69.5 (27.6 to 87.2)	68.3 (24.5 to 86.7)
2011/12 vaccination only	21	82	0.4 (–73.1 to 42.7)	–5.6 (–84.5 to 39.6)
2011/12 and 2012/13 vaccinations	81	620	39.3 (15.5 to 56.3)	37.3 (10.7 to 56.0)

CI: confidence interval; VE: vaccine effectiveness.

[a] France, Italy, Lithuania and Spain (Navarre and Valencia regions).
[b] Adjustment for study site and month of symptom onset.
[c] Adjustment for study site, month of symptom onset, age and comorbidities.

recruitment was the longest for A(H1N1)pdm2009 (21 weeks) and the shortest for A(H3N2) (15 weeks).

Of the 1,972 patients included in the pooled analysis, 116 patients tested positive for influenza A(H1N1) pdm09, 58 for A(H3N2) and 232 for influenza B. Two patients were coinfected with types A and B and one patient was coinfected with A(H3N2) and A(H1N1) pdm09. One specimen of influenza A could not be subtyped.

Influenza A(H1N1)pdm09 cases were younger (63 vs 77 years, p<0.05) than controls. A lower proportion of A(H1N1)pdm09 cases had more than one underlying condition (53.4% vs 67.0%, p<0.05), had been hospitalised in the previous year (27.6% vs 38.5%, p<0.05) and a higher proportion were current smokers (33.6% vs 18.3%, p<0.05) compared with controls (Table 4).

Influenza A(H3N2) cases and controls were similar for all characteristics except for the proportion of male patients (41.4% vs 56.6%, p<0.05).

Compared with controls, a lower proportion of influenza B cases had underlying conditions (82.8% vs 92.3%, p<0.05), had been hospitalised in the previous year (30.2% vs 38.8%, p<0.05) and were smokers (13.9% vs 18.8% of current smokers, p<0.05).

The 2012/13 vaccine coverage was 57.2% among all controls (all influenza-negative patients included in the study), 33.6% among A(H1N1)pdm09, 34.5% among A(H3N2) and 37.9% among influenza B cases (Table 4).

The p values associated with the Q-test and the I^2 index using models adjusted for age, month of symptom onset and chronic condition, testing for heterogeneity between study sites, were respectively 0.19 and 40.0% for A(H3N2), 0.10 and 48.3% for A(H1N1)pdm09 and 0.08 and 56.2% for influenza B.

The overall adjusted A(H1N1)pdm09 IVE was 21.3% (95% confidence interval (CI): –25.2 to 50.6; n=1,628); 41.8% (95% CI: –30.7 to 74.1; n=372) among the 18–64 year-old patients and 43.8% (95% CI: –10.7 to 71.5; n=504) among those aged 65–79 years. Among patients aged 80 years and older, there were 14 A(H1N1)pdm09 cases, including 12 vaccine failures (Table 5). Restricted to those aged less than 80 years-old, the adjusted IVE was 35.2% (95% CI: –9.1 to 61.5; n=1,004). Adjusted IVE against A(H1N1)pdm09 was 6.2% (95% CI: –110.4 to 58.2; n=753) among patients vaccinated in the 2012/13 season only, 26.6% (95% CI: –81.6 to 70.3; n=724) for those vaccinated in 2011/12 and 27.9% (95% CI: –20.5 to 56.9; n=1,368) for those vaccinated in both seasons (Table 6).

Table 7

Adjusted[a] vaccine effectiveness against influenza A(H3N2), influenza A(H1N1)pdm09 and B viruses according to various restrictions, hospital-based influenza vaccine effectiveness study, four European countries[b], 2012/13

Restriction	A(H1N1)pdm09		A(H3N2)		B	
	Total number/ number of cases	Adjusted VE (95% CI)	Total number/ number of cases	Adjusted VE (95% CI)	Total number/ number of cases	Adjusted VE (95% CI)
No restriction	1,628/116	21.3 (–25.2 to 50.6)	577/58	61.8 (26.8 to 80.0)	1,526/232	43.1 (21.2 to 58.9)
No antiviral treatment started between symptom onset and swabbing	1,598/104	18.6 (–30.7 to 49.3)	555/53	59.4 (21.7 to 79.0)	1,491/215	40.5 (17.3 to 57.2)
Swabbing delay≤4 days	1,147/88	14.9 (–47.1 to 50.8)	359/36	60.4 (10.0 to 82.5)	1,037/151	45.3 (18.8 to 63.2)
Weeks when ratio controls to cases was<9:1	1,019/109	29.8 (–15.1 to 57.2)	542/56	62.7 (27.5 to 80.8)	1,142/221	44.3 (21.6 to 60.4)
Patients with no chronic respiratory conditions	829/66	38.9 (–20.3 to 69.0)	304/33	57.8 (–4.3 to 82.9)	812/152	50.7 (24.1 to 68.0)

CI: confidence interval: VE: vaccine effectiveness.

[a] Adjustment for study site, month of symptom onset, presence of any chronic condition and age.

[b] France, Italy, Lithuania and Spain (Navarre and Valencia regions).

The overall adjusted IVE against A(H3N2) was 61.8% (95% CI: 26.8 to 80.0; n=577) (Table 5). The adjusted IVE was 52.4% (95% CI: –33.9 to 83.1; n=181) among 65–79 years patients and 73.8% (95% CI: 29.9 to 90.2; n=216) among those 80 years and older. Among patients aged less than 65 years, all cases had chronic conditions. In this age group, the IVE adjusted for month of symptom onset and study site was 26.1% (95% CI: –215.9 to 82.7; n=143). Adjusted IVE was 68.3% (95% CI: –157.2 to 96.1; n=250) among patients vaccinated in 2012/13 only and 59.6% (95% CI: 18.5 to 80.0; n=488) among patients vaccinated in 2011/12 and 2012/13 (Table 6).

The overall adjusted IVE against influenza B was 43.1% (95% CI: 21.2 to 58.9; n=1,526), 28.2% (95% CI: –18.9 to 56.6; n=565) among patients aged 65–79 years and 66.0% (95% CI: 19.3 to 85.7; n=346) among those younger than 65 years (Table 5). Adjusted IVE against influenza B was 68.3% (95% CI: 24.5 to 86.7; n=714) among patients vaccinated in 2012/13 only and 37.3% (95% CI: 10.7 to 56.0; n=1,300) in those vaccinated in both seasons (Table 6).

There were few changes in the IVE when conducting the sensitivity analyses (Table 7). The IVE against A(H1N1) pdm09 was higher when restricted to patients with no chronic respiratory conditions (38.9% (95% CI: –20.3 to 69.0) vs 21.3% (95% CI: –25.2 to 50.6)).

Discussion

Our results suggest that in the population targeted for the influenza vaccination, the 2012/13 IVE for laboratory-confirmed hospitalised influenza was 21.3% against A(H1N1)pdm09, 61.8% against A(H3N2) and 43.1% against B.

The adaptation of a generic protocol by 18 European hospitals enabled us to pool data and obtain a sample of 1,972 hospitalised ILI patients targeted for influenza vaccination. In a season with co-circulation of the three viruses, this large sample size allowed us to compute type-/subtype-specific estimates of IVE against hospitalised influenza and to further attempt to stratify by age group. However, stratified analyses led to estimates with broad confidence intervals. Consequently, some results of the stratified analyses can only be used to generate hypotheses.

The test-negative design has been mainly discussed and validated for GP-based studies [26,27]. It is assumed that by restricting the study population to patients consulting for ILI, the health-seeking behaviour confounding effect (associated with propensity to get vaccinated and to go to the GP in case of influenza) is controlled for. Since in our study sites all people needing hospitalisation are likely to be hospitalised, we believe that confounding due to health-seeking behaviour is minimised.

In hospital-based studies, several outcomes could be used. If we were to measure IVE against influenza confirmed-severe acute respiratory infection (SARI), we would need to make sure that for both cases and controls a respiratory infection was the cause of admission. We have chosen a broader case definition and a more sensitive inclusion criteria to cover a larger part of the influenza disease burden. As a consequence, some of the ILI in the seven days before admission may correspond to an exacerbation of underlying respiratory conditions. This could lead to an overestimation of the

IVE. Restricting our analysis to patients with no underlying respiratory conditions provides similar results and does not support this hypothesis. Furthermore, we adjusted for the presence and number of previous hospitalisations for underlying conditions.

The inclusion of patients swabbed more than four days after symptoms onset or after antiviral treatment had started could have led to misclassification biases if viral clearance occurred before swabbing. However, analyses confined to patients swabbed within four days of symptom onset and to patients who did not receive antiviral treatment did not change the results.

Studies using the test-negative design may underestimate the IVE when the ratio of controls to cases is high, especially if the laboratory tests have low specificity [28]. In our study, all cases were confirmed by RT-PCR, which has high specificity [29]. In the analyses restricted to weeks when the control to case ratio was lower than 9:1 resulted in very similar IVE estimates.

The data quality was high with only 49/2,021 records with missing outcomes or exposures in the database. We believe that ascertainment of vaccination status through patient interviews in two of the five study sites has not introduced differential information bias as data were collected before laboratory testing.

Due to the small sample size in some study sites, the test of heterogeneity may have had no power to detect heterogeneity even if differences exist between study sites. Different IVE across study sites could be due to variations in circulating strains, different vaccines by study site or different measured and unmeasured confounding factors. Further typing of circulating strains would be valuable to discuss site-specific IVE with regard to the level of matching between vaccine and locally circulating strains. Different access to vaccination according to age and underlying condition and to hospitalisation [30] could partly explain variations in IVE across study sites. Finally, the presence of random errors cannot be ruled out due to low sample size by study site. A larger sample size would be needed to carry out a two-stage pooled analysis [24].

Our results suggest that, in people belonging to target groups for vaccination, the 2012/13 IVE varied by subtype and age group. However, we cannot exclude the possibility that the variability of IVE results by age group mainly reflects sample size limitations. Small stratum-specific sample sizes (and very small number of cases) lead to unstable results and do not allow for biological interpretation of age-specific results. Our results would suggest that IVE against A(H3N2) was higher among older age groups. This observation would be in contradiction to the principles of immune senescence. In addition to the sample-size limitations, and as discussed above, we cannot exclude a selection bias for our controls, which we adjusted for. However we used the same control group for the three subtypes and age-specific results vary by subtype. We consider that it is unlikely that confounding factors would differ by subtype.

When looking at the effect of repeated vaccination (over two consecutive seasons), similar patterns were observed for influenza A(H3N2) and B. The highest point estimate IVE was in patients vaccinated in 2012/13 only, the lowest in those vaccinated in 2011/12 only and intermediate among those vaccinated both seasons. Such findings are consistent with recent reports from the Unites States and Australia [9,10,31]. The 2011/12 vaccine included an A/Perth/16/2009(H3N2)-like virus and a B/Brisbane/60/2008-like virus, while the 2012/13 vaccine included an A/Victoria/361/2011(H3N2)-like virus and a B/Wisconsin/1/2010-like (Yamagata lineage). On the basis of European virological surveillance data [13], the main circulating strains during the 2012/13 season were an A/Victoria/361/2011(H3N2) (with some A/Texas/50/2012 circulation reported) and B/Wisconsin/1/2010-like (with some B/Estonia and B/Massachusets/2/2012 circulation reported). These data support the absence of protection by the 2011/12 seasonal vaccine on the 2012/13 circulating strains as they were not matched.

Some authors have discussed the hypothesis of attenuated immunological responses as a result of repeated vaccination. From a school-based study, Davies et al. [32] suggested that a natural infection in season 1 produces antibodies that have a larger potential to form high post-vaccination titres in season 2 than vaccine-induced antibodies. Smith et al. [33] hypothesised that large antigenic distances between vaccines in seasons 1 and 2, and between vaccine in season 1 and epidemic strain in season 2, significantly increase the risk of infection among repeated vaccinees compared with those receiving the vaccine in season 2 only. Considering the antigenic differences between the 2011/12 vaccine and the 2012/13 circulating strains, this hypothesis could explain our results, suggesting a higher IVE against influenza A(H3N2) and B among patients vaccinated in 2012/13 only compared with those vaccinated in 2011/12 and 2012/13. Further studies, including a longer history of vaccine uptake and natural infections would be of great value to better understand the effect of repeated vaccination on the immunological response to a new influenza seasonal vaccine and the level of clinical protection conferred to individuals.

Our results suggest a low IVE against A(H1N1)pdm09, especially among the elderly [34]. A total of 14 cases of influenza A(H1N1)pdm09 occurred among patients older than 80 years. While the majority of these cases (n=12) were vaccinated patients, small numbers make the IVE estimates hard to interpret in that age group. The IVE was similar for those vaccinated in 2011/12 only or in both seasons. There was no effect for those vaccinated in 2012/13 only. The recommended A/California/7/2009(H1N1)pdm09-like virus strain

was the same for the 2011/12 and 2012/13 vaccines and matched the 2012/13 circulating strains (some A/California/06/2009 also reported). Long-lasting immune response induced by trivalent inactivated vaccines was previously described [35] and some recent results suggest that frequent previous vaccinations may be effective for the current influenza season [11]. The absence of protection among patients vaccinated in 2012/13 only is difficult to understand and interpret; it may reflect the presence of associated (and unmeasured) negative confounders for which repeated vaccination may be a surrogate. In addition, other studies [36-38] suggest a decreasing effect in the season difficult to reconcile with a long-term effect between seasons. Considering the small sample size in some of the vaccination groups in our study, we cannot exclude the possibility that this observation is due to chance.

Increasing the number of study sites in this network would allow a sufficient sample size to be reached early enough in the season to prompt the use of alternative prevention measures if a low IVE against hospitalised cases is observed among the target group. Early estimates of IVE against hospitalised influenza are also a useful complement to guide the decision-making of WHO experts regarding the composition of the next season's vaccines. A larger sample size and good documentation of vaccine brands used would allow the computing of brand-specific IVE. To further study the effect of previous seasonal vaccination will require documenting past vaccination over several seasons. In addition, ways to measure past natural immunity may also be needed to better understand the complex immunity of influenza natural infection and vaccination.

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Conflict of interest

No conflict of interest. Sanofi Pasteur, GlaxoSmithKline, Sanofi Pasteur MSD supported the study. They had no role in study design, data collection, pooled analysis and publication.

Authors' contributions

Marc Rondy was involved in the original methodological design of the study (generic protocol). He coordinated the European hospital IVE network, undertook the statistical analysis on which the research article is based and led the writing of the research article. Alain Moren initiated the original methodological design of the study. He coordinated the European hospital IVE network and contributed to the writing of the research article. Odile Launay, Joan Puig-Barberà, Giedre Gefenaite, Jesús Castilla, Katleen de Gaetano Donati, Florence Galtier, Eelko Hak, Marcela Guevara and Simona Costanzo were responsible for the coordination of the study at the local level. They were in charge of the data collection and management. They read, contributed and approved the manuscript final version. The European hospital IVE network contributors were in charge of supervising the study at the hospital level and collected the data published in this research article.

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* Authors' correction

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References

1. Carrat F, Flahault A. Influenza vaccine: the challenge of antigenic drift. Vaccine. 2007;25(39-40):6852-62. Available from: http://dx.doi.org/10.1016/j.vaccine.2007.07.027 PMID:17719149
2. Influenza vaccines. Wkly Epidemiol Rec. 2005;80(33):279-87. Available from: PMID: 16171031
3. Committee for Proprietary Medicinal Products (CPMP). Note for guidance on harmonization of requirements for influenza vaccines. London: European Medicines Agency; Mar 1997. CPMP/BWP/214/96. Available from: http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2009/09/WC500003945.pdf
4. Coudeville L, Bailleux F, Riche B, Megas F, Andre P, Ecochard R. Relationship between haemagglutination-inhibiting antibody titres and clinical protection against influenza: development and application of a bayesian random-effects model. BMC Med Res Methodol. 2010;10(1):18. Available from: http://dx.doi.org/10.1186/1471-2288-10-18 PMID:20210985
5. McCullers JA, Huber VC. Correlates of vaccine protection from influenza and its complications. Hum Vaccin Immunother. 2012;8(1):34-44. Available from: http://dx.doi.org/10.4161/hv.8.1.18214 PMID:22252001
6. World Health Organiation (WHO) Strategic Advisory Group of Experts on immunization (SAGE). Background paper on influenza vaccines and immunization. SAGE Working Group. Geneva: WHO; 2012. [Accessed 19 Apr 2013]. Available from: http://www.who.int/immunization/sage/meetings/2012/april/1_Background_Paper_Mar26_v13_cleaned.pdf
7. Mereckiene J, Cotter S, D'Ancona F, Giambi C, Nicoll A, Levy-Bruhl D, et al. VENICE project gatekeepers group. Differences in national influenza vaccination policies across the European Union, Norway and Iceland 2008-2009. Euro Surveill. 2010;15(44):19700. Available from: PMID:21087586
8. World Health Organization (WHO). Recommended composition of influenza virus vaccines for use in the 2012-2013 northern hemisphere influenza season. February 2012. Geneva: WHO; 2012. Available from: http://www.who.int/influenza/vaccines/virus/recommendations/201202_recommendation.pdf
9. Ohmit SE, Petrie JG, Malosh RE, Cowling BJ, Thompson MG, Shay DK, et al. Influenza vaccine effectiveness in the community and the household. Clin Infect Dis. 2013;56(10):1363-9. Available from: http://dx.doi.org/10.1093/cid/cit060 PMID:23413420
10. Ohmit SE, Thompson MG, Petrie JG, Thaker SN, Jackson ML, Belongia EA, et al. Influenza vaccine effectiveness in the 2011-2012 season: protection against each circulating virus and the effect of prior vaccination on estimates. Clin Infect Dis. 2014;58(3):319-27. doi: 10.1093/cid/cit736 PMID:24235265
11. McLean H, Sundaram M, Kieke B, Meece J, McClure D, Thompson M, et al. Current and previous season vaccination have similar effectiveness for preventing medically attended influenza A (H3N2) and B: results from a community cohort over 8 seasons. Poster presentation at Options for the control of Influenza VIII, Cape Town, South Africa, 5-10 Sep 2013.
12. Rondy M, Puig-Barbera J, Launay O, Duval X, Castilla J, Guevara M, et al. 2011-12 seasonal influenza vaccines effectiveness against confirmed A(H3N2) influenza hospitalisation: pooled analysis from a European network of hospitals. A pilot study. PLoS ONE. 2013;8(4):e59681. Available from: http://dx.doi.org/10.1371/journal.pone.0059681 PMID:23565159
13. European Centre for Disease Prevention and Control (ECDC). Influenza virus characterisation. Summary Europe, July 2012. Stockholm: ECDC; 2013. Available from: http://ecdc.europa.eu/en/publications/Publications/influenza-virus-characterisation-July-2013.pdf.
14. Valenciano M, Kissling E, Ciancio BC, Moren A. Study designs for timely estimation of influenza vaccine effectiveness using European sentinel practitioner networks. Vaccine. 2010;28(46):7381-8. Available from: http://dx.doi.org/10.1016/j.vaccine.2010.09.010 PMID:20851086
15. Seyler T, Rondy M, Valenciano M, Moren A. Protocol for hospital-based case control studies to measure seasonal influenza vaccine effectiveness against laboratory confirmed influenza hospitalisations across the European Union and European Economic Area Member States. May 2014. I-MOVE. Available from : https://drive.google.com/viewerng/viewer?a=v&pid=sites& srcid=ZGVmYXVsdGRvbWFpbnxlcGlmbHV8Z3g6NGY3ZGYyZDQ1OGQ3OGIxOA
16. Haut Conseil de la Sante´ Publique. Le Calendrier des vaccinations et les recommandations vaccinales 2012 selon l'avis du Haut Conseil de la santé publique. [2012 vaccination schedule and recommendations from the Haut conseil de la santé publique in France]. Bulletin épidémiologique hebdomadaire. 2012;14-15:163-87. French. Available from: http://www.sante.gouv.fr/IMG/pdf/beh_14_15_1_.pdf.
17. Ministero della Salute. Prevenzione e controllo dell'influenza: raccomandazioni per la stagione 2012-2013. [Prevention and control of influenza: recommendations for the season 2012-2013]. Roma: Ministero della Salute; 2012. Italian. Available from: http://www.salute.gov.it/portale/news/p3_2_1_1_1.jsp?lingua=italiano&menu=notizie&p=dalministero&id=463
18. Vaccine European New Integrated Collaboration Effort (VENICE) II Consortium. Seasonal influenza vaccination survey in EU/EEA, influenza season 2009-10. Final report. VENICE; 2011. Available from: http://venice.cineca.org/Final_Seasonal_Influenza_Vaccination_Survey_2010.pdf
19. Instituto de Salud Pública de Navarra. Protocolo de vacunacion antigripal 2012-2013, [2012-13 Influenza vaccination protocol]. Boletin informative. 2012;69:1-4. Spanish. Available from: http://www.navarra.es/NR/rdonlyres/AECCD760-AB2A-4841-818A-FA53478FD6DC/233865/BOL69INT2012_2013.pdf
20. Vacunacion antigripal estacional 2012. [Influenza vaccination, 2012 season]. Direccio General d'Investigacio i Salut Publica, Generalitat Vaclenciana. Spanish.
21. International Organization for Standardization (ISO). ISO 8601:2004: Data elements and interchange formats -- Information interchange -- Representation of dates and times. Geneva: ISO; 2004.
22. Mahoney FI, Barthel DW. Functional evaluation: the Barthel index. Md State Med J. 1965;14:61-5. Available from: PMID:14258950
23. Huedo-Medina TB, Sánchez-Meca J, Marín-Martínez F, Botella J. Assessing heterogeneity in meta-analysis: Q statistic or I2 index? Psychol Methods. 2006;11(2):193-206. Available from: http://dx.doi.org/10.1037/1082-989X.11.2.193 PMID:16784338
24. Stukel TA, Demidenko E, Dykes J, Karagas MR. Two-stage methods for the analysis of pooled data. Stat Med. 2001;20(14):2115-30.
25. Harrell FE. Regression modeling strategies: with applications to linear models, logistic regression, and survival analysis. New York, NY: Springer; 2001.
26. Foppa IM, Haber M, Ferdinands JM, Shay DK. The case test-negative design for studies of the effectiveness of influenza vaccine. Vaccine. 2013;31(30):3104-9. Available from: http://dx.doi.org/10.1016/j.vaccine.2013.04.026 PMID:23624093
27. Jackson ML, Nelson JC. The test-negative design for estimating influenza vaccine effectiveness. Vaccine. 2013;31(17):2165-8. Available from: http://dx.doi.org/10.1016/j.vaccine.2013.02.053 PMID:23499601
28. Orenstein EW, De Serres G, Haber MJ, Shay DK, Bridges CB, Gargiullo P, et al. Methodologic issues regarding the use of three observational study designs to assess influenza vaccine effectiveness. Int J Epidemiol. 2007;36(3):623-31. Available from: http://dx.doi.org/10.1093/ije/dym021 PMID:17403908
29. Petric M, Comanor L, Petti CA. Role of the laboratory in diagnosis of influenza during seasonal epidemics and potential pandemics. J Infect Dis. 2006;194(s2) Suppl 2;S98-110. Available from: http://dx.doi.org/10.1086/507554 PMID:17163396
30. World Health Organization (WHO) Regional Office for Europe. In-patient care discharges per 100. European Health for All database (HFA-DB). Copenhagen: WHO Regional Office for Europe. [Accessed 28 Dec 2014]. Available from: http://www.euro.who.int/en/data-and-evidence/databases/european-health-for-all-database-hfa-db

31. Ohmit SE, Petrie JG, Malosh RE, Cowling BJ, Thompson MG, Shay DK, et al. Influenza vaccine effectiveness in the community and the household. Clin Infect Dis. 2013;56(10):1363-9. Available from: http://dx.doi.org/10.1093/cid/cit060 PMID:23413420
32. Davies JR, Grilli EA. Natural or vaccine-induced antibody as a predictor of immunity in the face of natural challenge with influenza viruses. Epidemiol Infect. 1989;102(2):325-33. Available from: http://dx.doi.org/10.1017/S0950268800030004 PMID:2703026
33. Smith DJ, Forrest S, Ackley DH, Perelson AS. Variable efficacy of repeated annual influenza vaccination. Proc Natl Acad Sci USA. 1999;96(24):14001-6. Available from: http://dx.doi.org/10.1073/pnas.96.24.14001 PMID:10570188
34. Karageorgopoulos DE, Vouloumanou EK, Korbila IP, Kapaskelis A, Falagas ME. Age distribution of cases of 2009 (H1N1) pandemic influenza in comparison with seasonal influenza. PLoS ONE. 2011;6(7):e21690. Available from: http://dx.doi.org/10.1371/journal.pone.0021690 PMID:21747947
35. Sasaki S, He X-S, Holmes TH, Dekker CL, Kemble GW, Arvin AM, et al. Influence of prior influenza vaccination on antibody and B-cell responses. PLoS ONE. 2008;3(8):e2975. Available from: http://dx.doi.org/10.1371/journal.pone.0002975 PMID:18714352
36. Kissling E, Valenciano M, Larrauri A, Oroszi B, Cohen JM, Nunes B, et al. Low and decreasing vaccine effectiveness against influenza A(H3) in 2011/12 among vaccination target groups in Europe: results from the I-MOVE multicentre case-control study. Euro Surveill. 2013;18(5):20390. Available from: PMID:23399425
37. Pebody R, Andrews N, McMenamin J, Durnall H, Ellis J, Thompson CI, et al. Vaccine effectiveness of 2011/12 trivalent seasonal influenza vaccine in preventing laboratory-confirmed influenza in primary care in the United Kingdom: evidence of waning intra-seasonal protection. Euro Surveill. 2013;18(5):20389. Available from: PMID:23399424
38. Castilla J, Martínez-Baz I, Martínez-Artola V, Reina G, Pozo F, García Cenoz M, et al. Decline in influenza vaccine effectiveness with time after vaccination, Navarre, Spain, season 2011/12. Euro Surveill. 2013;18(5):20388. Available from: PMID:23399423