Original article

Influenza vaccine effectiveness to prevent medically attended laboratory confirmed influenza during season 2010-2011 in Romania: a case control study

Eficacitatea vaccinului gripal față de cazurile de gripă care s-au prezentat la medic în sezonul 2010-2011 în România: studiu caz-martor

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Abstract

We aimed to measure the seasonal trivalent influenza vaccine effectiveness against medically attended, laboratory confirmed influenza in Romania through a test-negative case-control study, part of ECDC-funded I-MOVE project. We included in the study 154 cases and 101 test-negative controls. We identified seven (4.5%) vaccine failures and 13 (12.9%) vaccinated controls. The overall adjusted vaccine effectiveness was 83% (95% confidence interval (CI): 23, 96), and 78.0% (95% CI: -119, 98) in the target group for vaccination. The results suggest a good protection of the 2010-2011 seasonal influenza vaccine against medically-attended laboratory confirmed influenza in Romania, taking into account the several limitations of the study. The participation in the I-MOVE multicentre case-control study allowed conducting the vaccine effectiveness study in Romania.

Keywords: *Influenza, vaccine effectiveness, case-control*

Rezumat

Scopul prezentului studiu caz-martor, care face parte din proiectul I-MOVE finanțat de ECDC, a fost de a măsura eficacitatea vaccinului gripal trivalent sezonier 2010-2011 față de cazurile de gripă care s-au prezentat la medic şi au fost confirmate prin examen de laborator. În studiu au fost incluse 154 de cazuri, dintre care şapte (4.5%) vaccinate antigripal si 101 martori, dintre care 13 (12.9%) vaccinați. Eficacitatea vaccinală ajustată a fost de 83% (IC 95%: 23, 96) în populația generală şi de 78.0% (IC 95%: -119, 98) în populația eligibilă la vaccinare. Cu toate limitele studiului, rezultatele sugerează o bună protecție a vaccinului gripal sezonier 2010-2011 față de cazurile de gripă care s-au prezentat la medic şi au fost confirmate cu laboratorul in România. Participarea la studiul multicentric caz-martor IMOVE ne-a ajutat să dezvoltăm studii de eficacitate vaccinală în România.

Cuvinte cheie: *gripa, eficacitate vaccinală, caz-martor*

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Introduction

In Romania, annual influenza vaccination has been recommended since 1977 for high risk groups for influenza complications and provided free of charge. For the season 2010-2011, influenza vaccination was recommended to people with chronic diseases (i.e. respiratory, cardiovascular, renal, hepatic diseases, diabetes and metabolic disorders), HIV infected persons, pregnant women, the elderly over 65 years old, institutionalized persons for social care and health care workers. The influenza vaccination campaign started at the end of October 2010 and continued throughout the season with a non-adjuvanted split virion vaccine, comprising the WHO recommended strains (1) for the season in the northern hemisphere. The vaccine was produced in Romania and authorized each year (if a new vaccine strain was introduced) by the National Medicines Agency (2) taking into account safety and immunogenicity studies. Other vaccine brands were available in pharmacies as well.

The influenza sentinel surveillance system has been set up in Romania since 1995 and joined the European influenza surveillance network (former EISS) since 2002 (3). During the season 2010-2011, the surveillance system comprised 21 sentinel surveillance units (in Bucharest and 20 districts), including 12 sentinel physicians (GP) per district and 20 GPs in Bucharest, 21 ambulance stations and 65 schools, colleges and universities (4). The network covered around 2% of the general population, from both urban and rural areas. The system was developed under the coordination of National Centre for Surveillance and Control of Communicable diseases and National Reference Laboratory within National Institute of Research and Development (NIRDMI) Cantacuzino that performed the laboratory confirmation for sentinel and non-sentinel specimens. The laboratory was accredited as National Influenza Centre (NIC) in 1969 and as WHO Influenza Regional Laboratory for the Balkan region in January 2009.

Since the season 2008-2009, Romania participated along with other countries in the European Center for Disease Prevention and Control (ECDC) funded I-MOVE project, aimed to monitor influenza vaccine effectiveness in the European Union (EU). Thus, 2008-2009 seasonal influenza vaccine effectiveness was evaluated for the first time in Romania in the elderly with an adjusted influenza vaccine effectiveness (VE) of 86.8% (95% confidence interval (CI): 38.0, 97.2) (5,6). During the pandemic season 2009-2010, the study intended to estimate the pandemic influenza vaccine effectiveness (PIVE) in all age groups, but the sample size recruited did not allow the calculation of country-specific PIVE (7). In these conditions, we used the laboratory database as an alternative for PIVE estimates, and estimated PIVE at 56% (95%CI: -54; 87) and when we excluded those vaccinated with the seasonal vaccine to 70% (95%CI:-2; 91), suggesting a misclassification of the vaccination status reported in the laboratory database (8).

The present study aimed to estimate the seasonal influenza VE in Romania during the season 2010-2011, using a case-control study design. As secondary objectives, we estimated influenza VE by influenza type/subtype and in the target group for vaccination.

Methods

We conducted a case-control study using the test-negative design, between mid-November 2010 (week 46/2010, two weeks after the start of the vaccination campaign) and April 2011 (week 15/2011, when the last positive case was recruited in the study). The study was embedded within the influenza surveillance system and it was carried out adapting the ECDC generic protocol (9).

All 285 influenza sentinel physicians from the 21 sentinel districts were invited to participate in the study. Each sentinel physician has assigned between 1500 and 2200 patients for primary care (catchment area).

Any patient that consulted the participating sentinel physician for influenza like illness (ILI) according to the EU case definition (10) and was resident within the GPs' catchment area was swabbed and included in the study. ILI patients laboratory positive for any type of influenza were considered study cases. ILI patients swabbed and tested negative for any type of influenza were included in the study as test-negative controls. Patients were excluded if they refused to participate in the study, were contra-indicated for influenza vaccination, were unable to give informed consent (as an adult) or were institutionalised in a residential home.

Participating physicians used a standardized and structured questionnaire for data collection. We collected demographic information (age, sex, residence), clinical signs and symptoms according to the case definition, date of symptom onset and swabbing, pregnancy, presence of chronic conditions (diabetes, obesity, endocrine diseases, heart disease, haematological cancer, immunodeficiency, chronic pulmonary diseases, cirrhosis, non-haematological neoplasia, renal diseases), smoking history (none, past, current smoker), 2010-2011 seasonal Influenza vaccination (data of vaccination and brand of vaccine), pandemic vaccination 2009-2010 (including brand of pandemic vaccine), seasonal influenza vaccination in the previous three seasons (2007-2010), number of hospitalisations for chronic conditions during the previous year, number of visits at family physician during previous year, antiviral treatment and brand of antiviral used.

An individual was considered vaccinated if there were more than 14 days delay between the vaccination date and the date of symptom onset. To ascertain vaccination, the patient had to be registered as vaccinated in the GP registry or to have a vaccination document signed by medical personnel showing the vaccination status.

Data was validated at district level by the epidemiologist from Public Health Department and checked for duplicates, errors or missing data. The investigator from the NIC entered data from questionnaires, after a second validation, in an EPIDATA database.

We calculated means for continuous variables and frequencies for categorical variables. We used Pearson chi2 or Fisher exact test when appropriate to test the statistical significance ($p<0.05$). Logistic regression was used to calculate the adjusted OR and its correspondent 95% confidence interval (CI). Variables that changed the crude estimate with more than 10% were included in the model. The vaccine effectiveness was computed as (1 – OR)*100. The statistical analysis was performed with Stata 11 (StataCorp. 2007. Stata Statistical Software: Release 11. College Station, TX: StataCorp LP).

For the primary analysis, we restricted the data to patients meeting the EU case definition, with symptom onset starting the week 52 when the first influenza case was confirmed in the study, and with a delay between symptom onset and swabbing of less than eight days, reporting the overall influenza VE. For the secondary analyses, we further restricted data to estimate VE by circulating strains and in target groups for influenza vaccination.

The study was approved by the Cantacuzino Institute ethical committee. No personal data were transmitted with questionnaires at the national level and patients (parents or legal tutor in case of children) gave the verbal informed consent to be swabbed.

Laboratory testing

Specimens were collected at the GPs offices by the district epidemiologist and sent by courier to the National Influenza Reference Laboratory where all specimens were tested. Real-time PCR was used for type A and B matrix gene and then for subtype H1 and H3 gene. After confirmation by PCR, a percentage of positive samples were submitted for isolation on certified MDCK cells or embryonated eggs. Antigenic characterization of isolated strains was performed by haemagglutinin inhibition assay. The neuraminidase inhibitor sensibility testing (NAI) was performed with chemiluminescence kit,

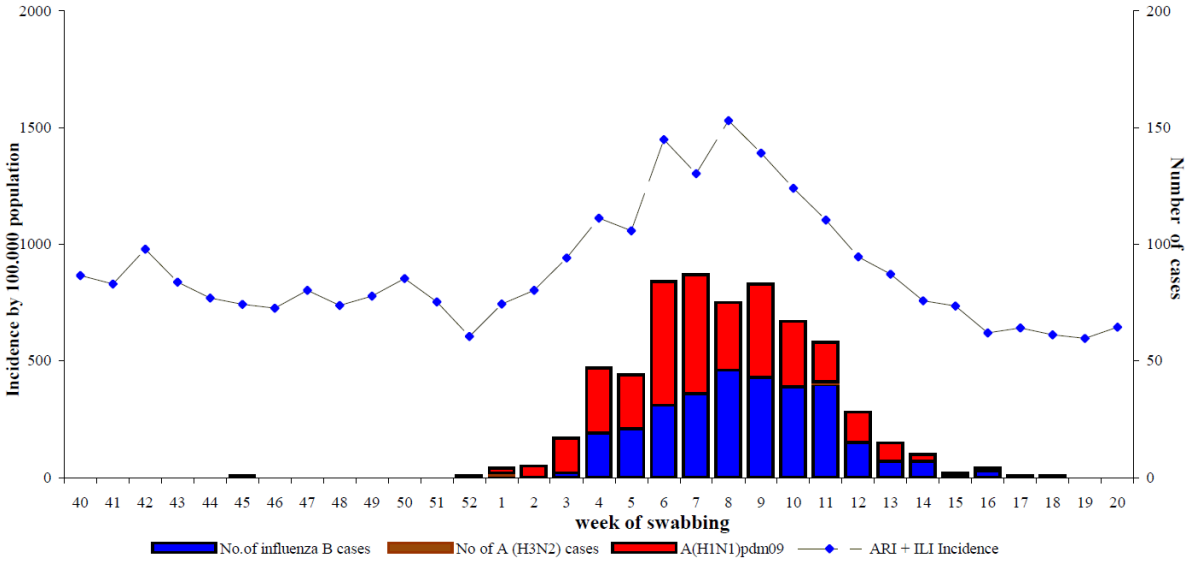

Figure1. Number of influenza cases and ARI/ILI incidence/100,000 population as reported in the surveillance system, Romania, season 2010-2011

used to determine the IC50 values to oseltamivir carboxilate. Sequencing of NA and/or HA genes were done at the beginning and the end of the season, and during the influenza peak.

Results

The influenza epidemic started in Romania in the first day of January 2011 (epidemiologic week 52/2011) and reached the peak in the week 9/2011. The respiratory tract infections' incidence decreased after week 10/2011, but sporadic influenza activity was registered until week 18/2011. A co-circulation of influenza virus A (H1N1)pdm2009 and B was recorded during the season (*Figure 1*).

Among the 285 sentinel physicians invited, 89 (33%) from 14/21 (67%) districts accepted to participate in the study. Among these, 70 (78.6%) sentinel physicians recruited at least one case and 66 (74%) recruited at least one case that was included in the analysis.

A total of 307 ILI patients were enrolled in the study between week 46/2010 and week 15/2011, following the respiratory infections' incidence of the season. After excluding the patients not meeting the EU case definition (seven patients), those with the symptom onset after week 52/2010 when the first case was confirmed in the study (44 patients), swabbed in more than seven days from the symptom onset (one patient), 255 patients (83.1%) were included in the analysis: 154 influenza confirmed cases and 101 test-negative controls.

Among cases, 66 (42.9%) were positive for A(H1N1)pdm2009 virus, 86 (55.8 %) for B and two (1.3%) for A(H3N2) virus.

The proportion of individuals presenting fever, headache, malaise and myalgia was higher among cases than among controls (*Table 1*). Compared to cases, a higher proportion of controls had at least one chronic condition and at least one GP visit in the previous year (*Table 1*).

Seven (4.5%) cases (four influenza A(H1N1)pdm09 and three influenza B cases) and 13 (12.9%) controls were vaccinated, all of them with Romanian non-adjuvanted influenza seasonal 2010-2011 vaccine. There were not significant

Table 1. Characteristics of medically attended Influenza like illnesses laboratory confirmed (cases, n=154) and test-negative controls (n=101), Romania, season 2010-2011

Characteristic	Cases n (%)	Controls n (%)	p value
Sudden onset	154 (100)	101 (100)	-
Fever	149 (96.7)	90 (89.1)	**0.014**
Headache	131 (85.1)	69 (68.3)	**0.001**
Malaise	106 (68.8)	54 (53.5)	**0.013**
Myalgia	114 (74.0)	49 (48.5)	**<0.0001**
Cough	142 (92.2)	86 (85.2)	0.073
Sore throat	125 (81.2)	74 (73.3)	0.136
Shortness of breath	27 (17.5)	18 (17.8)	0.953
Mean age (± Standard Deviation)	22.6 ±17.7	26.6±24.1	0.054
Sex: male	58 (37.7)	41 (40.1)	0.638
Residence: urban	108 (70.1)	60 (59.4)	0.077
At least one hospitalization in the previous year	6 (3.92)	8 (8.25)	0.147
More than one GP visit in the previous year	34 (22.2)	40 (41.2)	**0.001**
Any chronic condition	17 (11.0)	24 (23.8)	**0.007**
Poor functional status	2 (1.30)	5 (4.9)	0.117
Smoking	12 (7.8)	3 (3.0)	0.109
Eligible for vaccination	35 (22.7)	34 (33.7)	0.055
Seasonal vaccination 2010/11	7 (4.6)	13 (12.9)	**0.016**
Pandemic vaccination A(H1N1)pdm2009 in the season 2009/10	10 (6.5)	8 (8.0)	0.648
Any seasonal influenza vaccination in the previous three seasons	24 (15.6)	20 (20.8)	0.289

differences between cases and controls related to monovalent pandemic vaccination 2009-2010 and previous influenza seasonal vaccination (*Table 1*).

Overall, the crude IVE against medically attended any influenza infection (n=255) was 68% (95% CI: 9, 89). The adjusted IVE for age, chronic conditions, pandemic vaccination, previous seasonal vaccination, number of GPs visits, hospitalisations in the previous years and week of swabbing was 83% (95% CI: 23, 96). The crude IVE for influenza A(H1N1)pdm2009 infection (n=167) was 56% (95% CI: - 50, 90) and the adjusted IVE for the same covariates was 70% (95% CI: -54, 94). The crude IVE for B infection (n= 187) was 76% (95% CI: 6, 95) and the adjusted IVE for age, chronic conditions, previous seasonal vaccination, number of GPs visits in the previous year, smoking and week of swabbing was 95% (95% CI: 37, 100).

Restricting the analysis for the patients eligible for vaccination (n= 69), the crude IVE was 66% (95% CI: -33, 91) and the adjusted IVE for age, GPs visits, pandemic vaccination, previous seasonal vaccination, number of hospitalisation in the previous year and week of swabbing was 78% (95% CI: -119, 98).

Laboratory findings

Until the end of the season at national level, 1657 specimens from sentinel and non-

sentinel sources were tested, 634 (38.3%) being positive for influenza viruses: 320 (50.5%) A(H1N1)pdm2009, two (0.3%) A(H3N2), and 312 (49.2%) B viruses.

From the total number of specimens positive by RT-PCR, 35 strains were isolated: three belonging to subtype A(H3N2) – A/Victoria/210/09 – like; eighteen stains were A(H1N1) – A/California/7/09 like and fourteen isolated strains were type B – B/Brisbane/60/08 – like. All strains were sensitive to oseltamivir. A total of 53 samples were sequenced at national level and nine of them from study subjects (the two A(H3N2) samples, five A(H1N1)pdm2009 and two type B). None of the sequenced strains had the D222G substitution. The most frequent substitutions in HA gene of subtype H1 were: P83S; S203T (found in 100%); D97N and S185T (81% and 71.4% respectively). No specific mutation for neuraminidase resistance in NA gene was observed.

Discussion

Our study suggest a good effectiveness of the Romanian vaccine brand against medically attended laboratory confirmed influenza during the season 2010-2011, consistent with the good matching between the circulating and vaccine strains.

Comparing our results with those reported in the I-MOVE network (11), the overall IVE and the VE against both A(H1N1)pdm2009 and B infections were higher, and also higher than those expected for a seasonal influenza vaccine in a non-pandemic season (12). In addition, comparing the IVE estimates against the A(H1N1)pdm2009 infection with those of the monovalent pandemic VE in the previous season 2009-2010, using laboratory data, the IVE was also higher in the 2010-2011 season. This also differs than preliminary reports in the season 2010-2011 (13-15) or by the I-MOVE network (11) which reported a lower vaccine effectiveness for the A(H1N1)pdm2009 strain.

These differences between our results and those presented by other authors could be explained by factors related to different vaccine brand used, circulating strain, natural immunity or to residual confounding that we fail to control for. We discuss these aspects below.

The Romanian vaccine is a split virion vaccine, non-adjuvanted comprising 15 micrograms of antigen, similar with other brands.

The vaccination uptake at national level was low in both seasons when influenza AH1N1)pdm2009 predominantly circulated (2009-2010 and 2010-2011); 1,205,917 people were vaccinated during the 2010-2011 season, representing around 6% from all Romanian population, similar with that of the previous season (16). However, persons vaccinated in the occupational settings or those who bought the vaccine from pharmacies were not reported, unless the GPs were aware of this.

The investigated strains of predominately circulating A(H1N1)pdm2009 and B viruses matched very well the vaccine strains and point mutation was observed neither in the study cases investigated nor the virological surveillance, consistent with a good VE. However the number of strains investigated in the study and surveillance system was lower than the recommended (10% strains investigated over the seasons). In other studies, the hemagglutinin (HA) D222G substitution was reported, but this is more related to severity of disease since it could cause a shift from α2,6 receptors to the mixed α2,3/α2,6 receptors specificity which might increase binding to α2,3 receptors (17).

Natural immunity gained by asymptomatic infection during the two seasons when the influenza A(H1N1)pdm2009 circulated could have biased the VE results against the pandemic strain infections if this was differential between vaccinated and unvaccinated. Indeed, if a proportion of vaccinated controls were protected due to previous asymptomatic infection and not to vaccination or the unvaccinated controls did not become cases because of natural immunity, the VE would have been overestimated. However, this cannot justify the high VE also found for influenza B that did not circulate in the previous season.

Different bias could have also influenced the results and determining an overestimation of VE estimates. Firstly, the selection bias by preferentially swabbing the vaccinated ILI cases cannot be excluded because the proportion of vaccinated among the negative controls (12%) was higher than in the general population (6%). However, participating GPs swabbed all ILI cases during the season and they did not know the outcome at the moment of swabbing. On the other hand, the low number of GPs participating in the study might have influenced the results if these GPs were more likely to recommend vaccination in their catchment's area. However, the general population it is not the source population of the cases presenting to the GP with ILI (18).

Information bias was also considered. Positive cases presented more general symptoms than controls. If ILI negative patients presented milder disease than cases due to vaccination, the VE could have been overestimated. As mentioned above, healthy adults could also have been vaccinated in an occupational setting and GP might not be aware of vaccination if a medical document was not provided. In this scenario, some positive cases might have been misclassified as non-vaccinated. However, when restricting the analysis to ILI patients targeted for vaccination, always vaccinated by GPs, we obtained a similar high point estimate of the overall influenza VE.

All analyses suggest an important negative confounding factor, because the crude estimate was lower than the adjusted one. From the way we built the regression models, we adjusted more for negative confounding (GP visits, hospitalisations, chronic conditions) and we cannot exclude that the control group to be different that the population given rise to the cases.

Another factor that could have influenced the results, is related to the co-circulation A(H1N1)2009 and B influenza viruses with different geographical distribution. Most of the B cases were recruited in some sentinel districts, where outbreaks occurred in schools.

Conclusion

Our results suggest a good protection of the 2010-2011 seasonal influenza vaccine against medically-attended laboratory confirmed influenza in Romania, taking into account the discussed limitations. This was the third season of the Romanian component of the I-MOVE case-control multicenter study. For the first time we could perform the analysis by type/serotype and in the target groups for vaccination. This was only possible due to improved GPs participation compared to previous seasons and increased recruitment of ILI cases.

The participation in the I-MOVE project allowed estimating the IVE of the vaccine produced in Romania using a sound methodology. Repeating the study in the further seasons will give us the opportunity to investigate other aspects of influenza vaccination in Romania.

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