Assessment of seasonal influenza vaccine effectiveness in patients from a central Italy reference hospital: pitfalls and intricacies from a pilot case-control study

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ABSTRACT

BACKGROUND: Influenza vaccination protects high-risk populations from severe outcomes.

METHODS: During the 2011-2012 flu season at "Gemelli Hospital" in Rome, all hospitalised patients who developed suspected influenza-like illness within 14 days were recruited to assess the feasibility of testing the influenza vaccine effectiveness against laboratory-confirmed influenza requiring hospitalisation.

RESULTS: Sixty-two patients were recruited; among them, 18 were laboratory confirmed for influenza A or B. In the target group for vaccination (N=47), the prevalence of vaccinated subjects was less than expected (38%).

CONCLUSIONS: No difference in vaccine status between patients with positive and negative laboratory tests was found due to the small sample size. Larger numbers are required to explore influenza vaccine effectiveness.

Key words: influenza vaccine effectiveness, hospitalisation, influenza-like illness, pilot case-control study.

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INTRODUCTION

Influenza vaccines have been used to reduce the complications of influenza (mortality and hospitalisation for pneumonia or other respiratory disorders) [1-3]. Each year, there is a strong effort made to produce that year's vaccine and deliver it to the appropriate sections of the population, especially elderly individuals [3]. The main discussion is focused on the use of inactivated vaccines for seasonal influenza and the evidence for their efficacy and safety. Since

the 2008/2009 season, several efforts have been made to measure seasonal influenza vaccine effectiveness against laboratory-confirmed influenza-like illness (ILI) [4-6]. However, this system does not allow the assessment of vaccine effectiveness against severe influenza outcomes such as those requiring hospitalisation.

To provide additional information for public health strategies, this study was designed to assess the feasibility of testing seasonal influenza vaccine effectiveness against hospitalisation with laboratory-confirmed influenza and several other clinical outcomes in patients requiring hospitalisation for respiratory conditions.

METHODS

This study is based on the protocol of the multicentre case-control study using the test-negative design [7-8] in 21 hospitals located in France, Italy and Spain [9].

Recruitment began on December 5th, 2011 (week 49/2011), at the "Gemelli" Hospital in Rome, which has a catchment area of more than 400,000 inhabitants. The hospital is a 1,700-bed tertiary care centre with approximately 60,000 patient admissions/year, mostly from Central and Southern Italy. The hospital has medical and surgical specialties, as well as Intensive Care Units. Moreover, a 40-bed Unit for infectious disease patients is operating. We expected to identify approximately 200 cases hospitalised for respiratory disease.

The study was approved by the local Ethics Committee, and informed consent was provided by all the participants (or by her/his legal guardian).

Study population. From December 2011 to April 2012, children (aged >6 months) and non-institutionalised adults (≥18 years) with respiratory syndrome who were hospitalised for at least 24 hours through the Emergency Ward were invited to participate.

Inclusion criteria were as follows: 1) no contraindication for influenza vaccination; 2) onset of ILI symptoms within 14 days prior to nasal-pharyngeal swabbing; 3) no previous ILI in the 2011-12 season.

ILI has been defined as the presence of at least one systemic symptom (fever, malaise, headache, myalgia) and at least one respiratory symptom (cough, sore throat, shortness of breath).

During the study period (from week 49/2011 to week 15/2012), the weekly average number of admissions at the Emergency Ward for any medical cause was 630. The reported number of patients screened for respiratory disorders was 360. Among these, we considered 104 patients who spent at least 24 hours in the hospital. The average number of patients included in the study was 5 patients per week. For each patient included, the operator spent a mean of 30 minutes for the interview and 15 minutes to perform and collect the swab sample.

Up to April 14th, 2012, 104 patients were invited to participate. Two patients refused. Twelve patients could not be recruited because of the severity of their health conditions.

Finally, 92 probable ILI cases gave informed consent; after checking for compliance with the common set of inclusion criteria and considering only complete data collection, 62 patients were selected. Thirty patients were excluded because seven were children aged <6 months and 12 had no ILI diagnosis; for seven patients, the delay from the onset of symptoms to swab was greater than 14 days, two had a previous ILI diagnosis in the season, and two were not eligible for influenza vaccination.

Data sources and collection. Data sources included hospital medical records, interviews of the patient, the patient's family and patient's general practitioners (GP), vaccination registries and the laboratory database. Vaccination status was ascertained by interviewing the patient's GP.

In particular, information on the ILI episode (date of symptom onset), presence of chronic diseases, number of hospital admissions in the past 12 months, number of GP consultations in the previous three months, smoking habits, vaccination against influenza in 2011-12 and in the last two seasons and functional status were collected.

Individuals belonged to the target group for vaccination if they corresponded to the Italian specific recommendations for vaccination [7].

Nasal and/or pharyngeal swabs and laboratory procedures. According to the study protocol, nasal and/or pharyngeal swabs (Copan Diagnostic) from patients with suspected ILI were collected and sent to the reference laboratory at the Microbiology Department of "Gemelli" Hospital in Rome.

The isolates underwent a molecular analysis for the currently circulating influenza

A (subtypes H3 and H1) and B viruses.

Each patient was swabbed twice, and tests were performed on both swabs to increase the sensitivity.

An internal control for RNAseP and Applied Biosystems enzymes were systematically included to check for the presence of genetic material in the samples.

The identification and characterisation of influenza in the samples using real-time PCR included a first test of positivity for influenza A or B:

1. If influenza B was detected, no further tests were performed.
2. If influenza A was detected, H1N1v was investigated using CDC primers (Applied Biosystems), and H3N2 and H1N1 seasonal variants were investigated using ISS primers (as part of the Influnet protocol).

We defined a case as a patient with a nasopharyngeal sample positive for influenza A or B. The controls were patients with negative samples for any influenza virus.

Descriptive statistical analysis. The study participants were described by their baseline characteristics. The ILI clinical manifestations of the cases and controls were compared using the chi-square test, Fisher's exact test, (univariate model) and the CATMOD procedure (model adjusted for age and sex).

The analyses were performed using SAS software (version 9.1.3 for Windows, SAS Institute, Cary, North Carolina). For comparative analyses, we excluded the controls with onset of symptoms prior to the first laboratory confirmed case or after the week of the last laboratory confirmed case.

RESULTS

Table 1 lists the main characteristics of the ILI patients.

Abbreviation: BMI: body mass index; CVD: cardiovascular disease; SD: standard deviation.

Fifty-seven percent of the patients were men, and the mean patient age was 48.2±24.7 years: 16% were aged ≤5 years, and 23% were aged ≥65 years. Seventy-six percent belonged to a target group recommended for vaccination. Among the entire sample (N=62) and the target group (N=47), only 29% and 38%, respectively, had been vaccinated against influenza.

Table 1 shows also the prevalence of co-morbidities (respiratory disease, cardiovascular disease, cancer, diabetes).

The first and the last laboratory-confirmed cases were detected on January 2nd (week 1/2012) and March 20th (week 12/2012) of 2012, respectively.

For the entire sample, there were 18 laboratory-confirmed cases for influenza, with 16 of virus A and 2 of virus B. Of the 16 influenza A cases, 13 were subtype H3N2, one was subtype H1N1v, and two were not identified.

In this study, patients swabbed after 7 days from the onset of flu symptoms were included. There were four positive tests with a delay from symptom onset to swab of more than 7 days.

Table 2 shows the difference in the ILI clinical manifestations and vaccine status between lab test-positive and test-negative patients (excluding controls with onset of symptoms prior to the first laboratory confirmed case or after the week of the last laboratory confirmed case, N=16).

The patients more frequently had sore throat than the controls (p=0.008 and p=0.053 respectively). Moreover, a higher non-statically significant prevalence of fever and rhinorrhoea were found in the cases.

There were no differences in the vaccine status between the cases and controls, considering the entire sample and the target group (N=33).

DISCUSSION

This study investigates the feasibility of and the difficulties in testing seasonal influenza vaccine effectiveness against major influenza complications, such as hospitalisation for respiratory disorders in laboratory-confirmed influenza.

In 2011, the first Italian cases of ILI were reported in week 42/2011, and the epidemic period started in the week 51/2011; however, no significant signs of increased influenza activity were detected until the middle of January 2012. The epidemic curve reached its peak at week 5/2012, with an incidence of

TABLE 1

CHARACTERISTICS OF HOSPITALISED PATIENTS WITH ILI DIAGNOSIS AT ADMISSION (N=62)	
DEMOGRAPHIC CHARACTERISTICS	
Men, N (%)	35 (56.4)
Age, years mean ±SD	48.2 ±24.7
Age ≤5 years, N (%)	10 (16.1)
Age ≥ 65 years, N (%)	14 (22.6)
TARGET GROUP FOR VACCINATION	
Belongs to target group recommended for vaccination, N (%)	47 (75.8)
Elderly ≥65 years, N (%)	14 (29.8)
High-risk group but aged < 65 years, N (%)	31 (65.9)
Health operator, N (%)	2 (4.2)
VACCINATION STATUS AND HISTORY	
Seasonal flu vaccination 2011-12, N (%)	18 (29.0)
Seasonal flu vaccination 2011-12 in target group for vaccination, N (%)	18 (38.3)
Seasonal flu vaccination 2010-11, N (%) (N 60)	17 (28.3)
CONFOUNDING FACTORS	
Antiviral treatment between ILI symptom onset and swabbing	10 (16.1)
CHRONIC DISEASE IN PATIENTS AGED ≥18 YEARS (N=52);	
Respiratory disease, N (%)	13 (25.0)
CVD, N (%)	10 (19.2)
Cancer, N (%)	29 (55.8)
Diabetes, N (%)	11 (21.1)
Immunodeficiency, N (%)	11 (21.1)
Dementia, N (%)	1 (1.9)
Endocrine disease, N (%)	6 (11.5)
BMI ≥ 30, N (%)	9 (17.3)
Smoking habits in patients ≥ 18 years Yes No Ex	 15 (28.8) 33 (63.5) 4 (7.7)

Abbreviation: BMI: body mass index; CVD: cardiovascular disease; SD: standard deviation.

9.58 cases per 1,000 served population [10]. In the Lazio Region, the seasonal vaccine was available from October 17th, 2011, and the epidemic period started on week 52/2011 and lasted until week 11/2012; the epidemic curve reached its peak at week 4/2012 [10].

One of the intricacies we observed in our study is related to the time elapsed from symptom onset, which may be rather unclear, until the clear-cut recognition of an ILI syndrome. Thus, choosing to define the time limit within 7 days yielded a lower number of recruited individuals but a "cleaner" population.

The number of recruited hospitalised patients was lower than expected but quite compatible with the curve of the incidence of

TABLE 2

DIFFERENCE IN THE CLINICAL MANIFESTATIONS BETWEEN TEST-POSITIVE (CASES, N=18) AND TEST-NEGATIVE PATIENTS (CONTROLS, N=28)

ILI CLINICAL MANIFESTATIONS	TOTAL N=46	CONTROLS N=28 (60.9)	CASES N=18 (39.1)	P VALUE[a]	P VALUE[b]
FEVER, N (%)	38 (82.6)	21 (75.0)	17(94.4)	0.12	0.11
COUGH, N (%)	40 (87.0)	24 (85.7)	16 (88.9)	0.99	0.80
SHORTNESS OF BREATH, N (%)	32 (69.6)	22 (78.6)	10 (55.6)	0.11	0.17
SORE THROAT, N (%)	19 (41.3)	8 (28.6)	11 (61.1)	0.06	0.05
HEADACHE, N (%)	13 (28.3)	9 (32.1)	4 (22.2)	0.12	0.73
MYALGIA, N (%)	17 (37.0)	13 (46.4)	4 (22.2)	0.02	0.25
MALAISE, N (%)	41 (89.1)	25 (89.3)	16 (88.9)	0.99	0.99
RHINORRHOEA, N (%)	27 (58.7)	14 (50.0)	13 (72.2)	0.22	0.15
DIARRHOEA, N (%)	9 (19.6)	5 (17.9)	4 (22.2)	0.72	0.76
VOMITING, N (%)	11 (24.4)	5 (17.9)	6 (35.3)	0.28	0.26
2011-12 SEASONAL INFLUENZA VACCINATION, N (%)	13 (28.3)	8 (28.6)	5 (27.8)	0.99	0.99
2010-11 SEASONAL INFLUENZA VACCINATION, N (%)	14 (31.8)	10 (35.7)	4 (25.0)	0.52	0.29
	Target group N=33	Controls N=23 (69.7)	Cases N=10 (30.3)	P value[a]	P value[b]
2011-12 SEASONAL INFLUENZA VACCINATION, N (%)	13 (39.4)	8 (28.6)	5 (27.8)	0.46	0.36
2010-11 SEASONAL INFLUENZA VACCINATION, N (%	14 (42.4)	10 (43.5)	4 (40.0)	0.99	0.57

[a]Fisher's Exact Test, [b]age and sex adjusted

ILI in Italy for the 2011-2012 season [11]. In this regard, we must also consider that this study did not aim at assessing the prevalence of influenza per se but its clinical respiratory complications, which are less frequent than the primary disorder. To overcome this limitation, only a larger catchment area or recruitment from more than one hospital should be considered. In addition, only 18 patients were diagnosed with influenza, whereas the remaining part of the study patients had an ILI of unknown origin. With respect to the objective of the study, which was to assess the effectiveness of the seasonal influenza vaccine in a hospital population, this result clearly shows that in our hospital population, ILI might be caused by many different aetiologic agents other than influenza.

In this pilot study, the low number of patients recruited does not allow us to make definitive conclusions on influenza vaccine efficacy. In particular, with regard to severely affected patients, one pitfall of the study was the difficulty of interviewing them within the context of the Intensive Care Unit, a time when family members were not always accessible. Moreover, for the swabbed patients, the prevalence of vaccinated subjects was low, even in the target group, suggesting reluctance to obtain vaccination against influenza. Many patients admitted not to trust vaccination as a useful procedure; in a few cases, they were scared of a worsening their clinical condition after vaccination. A similar observation was reported by our group during the 2009-10 pandemic season concerning an adult cohort in Molise Region [12].

CONCLUSIONS

The experience of this pilot study with a relative low number of patients will allow us

to introduce some general and logistic changes in future years to overcome the observed limitations and to plan a study with a larger number of patients.

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