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Influenza vaccine effectiveness against laboratory confirmed influenza in Greece during the 2013–2014 season: A test-negative study

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ABSTRACT

Background: In 2013–2014 Greece experienced a resurgence of severe influenza cases, coincidental with a shift to H1N1pdm09 predominance. We sought to estimate Vaccine Effectiveness (VE) for this season using available surveillance data from hospitals (including both inpatients and outpatients).
Methods: Swab samples were sent by hospital physicians to one of three laboratories, covering the entire country, to be tested for influenza using RT-PCR. The test-negative design was employed, with patients testing positive serving as cases and those testing negative serving as controls. VE was estimated using logistic regression, adjusted for age group, sex, region and calendar time, with further adjustment for unknown vaccination status using inverse response propensity weights. Additional age group stratified estimates and subgroup estimates of VE against H1N1pdm09 and H3N2 were calculated.
Results: Out of 1310 patients with known vaccination status, 124 (9.5%) were vaccinated, and 543 patients (41.5%) tested positive for influenza. Adjusted VE was 34.5% (95% CI: 4.1–55.3%) against any influenza, and 56.7% (95% CI: 22.8–75.7%) against H1N1pdm09. VE estimates appeared to be higher for people aged 60 and older, while in those under 60 there was limited evidence of effectiveness. Isolated circulating strains were genetically close to the vaccine strain, with limited evidence of antigenic drift.
Conclusions: These results suggest a moderate protective effect of the 2013–2014 influenza vaccine, mainly against H1N1pdm09 and in people aged 60 and over. Vaccine coverage was very low in Greece, even among groups targeted for vaccination, and substantial efforts should be made to improve it. VE can and should be routinely monitored, and the results taken into account when deciding on influenza vaccine composition for next season.

1. Introduction

Seasonal influenza vaccination is recommended every year, because of the antigenic change in circulating influenza viruses and the short duration of the immunity induced. The vaccine provides usually moderate protection, which can vary substantially from year to year [1]. Factors responsible for this variation include the closeness of the antigenic match between vaccine and circulating strains, and the timing of the seasonal influenza outbreak relative to vaccination [2]; other relevant, non-vaccine-related factors include the degree of influenza virus circulation and co-circulation of other respiratory pathogens.

Therefore, many countries are regularly performing observational studies to evaluate influenza Vaccine Effectiveness (VE) [2–7]. Such studies usually employ the test-negative design, a variant of the case-control design in which, from a given population seeking medical care for influenza-like illness (ILI), those who test positive for influenza serve as cases and those who test negative serve as controls. The test-negative design is simple and convenient, and controls for the different healthcare-seeking behaviour between vaccinated and unvaccinated participants [8].

Abbreviations: ICU, Intensive care unit; ILI, Influenza-like illness; HA, hemagglutinin; HCDCP, Hellenic Centre for Disease Control and Prevention; NIRL, National Influenza Reference Laboratory; RT-PCR, Reverse-Transcriptase Polymerase Chain Reaction; VE, Vaccine Effectiveness.

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Recommendations on which individuals should be vaccinated against influenza vary between countries [9]. In Greece, influenza vaccination is recommended for all persons aged 60 and over, patients with certain chronic conditions, pregnant women, healthcare workers and carers of infants or elderly people (http://goo.gl/wp4AIG).

After a relatively mild 2012–2013 influenza season during which both H3N2 and H1N1pdm09 strains were in roughly equal circulation, in 2013–2014 Greece experienced a resurgence in severe influenza cases coincidental with a shift to H1N1pdm09 predominance. Among cases with laboratory-confirmed influenza reported up to May 22nd, 2014 there were 145 deaths and 338 intensive-care unit (ICU) admissions, compared to 49 deaths and 108 ICU admissions in the previous season [10]. Thus the 2013–2014 influenza season was comparable in severity to the first post-pandemic season 2010–2011 [11], and was featured prominently in many media reports regarding the high death rate and the reported low uptake of vaccination [12].

Against this background, we decided to utilize the available surveillance data to estimate the effectiveness of influenza vaccination for the 2013–2014 season, using a test-negative design. As secondary objectives, we sought to determine how early in the season we could have a reliable VE estimate, explore any differences in VE among age groups and by type of influenza strain, and describe the genetic features of representative strains that circulated in Greece during the season.

2. Materials and methods

As part of influenza surveillance activities in Greece, nasopharygeal swabs taken by hospital physicians were sent for Real-Time Reverse-Transcriptase Polymerase Chain Reaction (RT-PCR) testing to one of three laboratories, which cover the entire country: (1) the National Influenza Reference Laboratory (NIRL) of Southern Greece (Hellenic Pasteur Institute, Athens), (2) the NIRL of Northern Greece (2nd Laboratory of Microbiology, Medical School, Aristotle University of Thessaloniki), and (3) the Department of Microbiology, Medical School, National and Kapodistrian University of Athens. Physicians from all public and private hospitals could send at their discretion samples from both inpatients and outpatients, provided the patient's clinical presentation was suggestive of ILI.

Swabs were sent to these laboratories along with a standardized paper notification form, filled in by the physician; the form is available at the Hellenic Centre for Disease Control and Prevention (HCDCP) website (http://goo.gl/O30IoU), and contains basic demographic and clinical information including age, sex and influenza vaccination status. Laboratories marked the test result (positive/negative, plus influenza type and subtype for positive samples) on this reporting form, and forwarded it by fax to the Department of Epidemiological Surveillance and Intervention of the HCDCP. This was the data source utilized for this study.

Patients under one year of age were excluded from the analysis, since age was recorded in completed years and the seasonal influenza vaccine is not recommended in infants under 6 months of age according to the Greek National Childhood Immunization Schedule (http://goo.gl/nKmT0E). To avoid bias, the study period was limited to weeks with laboratory-confirmed influenza cases.

The study followed the test-negative design; patients with a positive RT-PCR for influenza were classified as cases and those testing negative as controls. The Odds Ratio (OR) for influenza vaccination was calculated, similar to a case control study, and VE was derived as one minus OR, expressed as a percentage. Because cases and controls are sampled in the same process, a situation conceptually similar to incidence density sampling in case-control studies, exposure OR is an unbiased direct estimator (i.e. not an approximation) of the Incidence Rate Ratio (RR) for the corresponding hypothetical cohort [13]. However, because outcome status and the ratio of cases to controls are not known in advance and do not affect recruitment, a test-negative study is fundamentally different than a traditional case-control study [8,14,15].

For univariate analyses, the Mann–Whitney test was used to compare continuous variables and the Fisher's exact test to compare categorical variables. All p-values are two-sided. In addition to calculating a crude VE, logistic regression was used to estimate the VE adjusted for age group, sex, region (North or South – the latter including samples sent to the University of Athens) and month of sample collection. To further adjust the analysis for participants with unknown (not reported) vaccination status, we used inverse response propensity weights in which "response" was defined as "known vaccination status" and response odds were modeled using logistic regression as a function of age group, sex, region and RT-PCR test result. Additional subgroup VE estimates for H1N1pdm09 and H3N2 influenza subtypes were calculated. The effect of age on VE was examined by including appropriate interaction terms in the models and performing likelihood ratio testing. Furthermore, we calculated "rolling" weekly estimates of VE for the duration of the influenza season, in order to assess how early we could obtain stable estimates. All analyses were performed using the R software environment, version 3.1.0 [16].

Finally, in order to assess vaccine-virus match at the genetic level, a temporally and geographically representative sample of circulating H1N1pdm09 and H3N2 strains was selected from the study patients, and sequencing of the hemagglutinin (HA) gene was performed. The HA1 domain contains the receptor-binding cavity as well as most of the antigenic sites of the HA molecule. HA1 amplification was performed by one-step RT-PCR and the purified products sequenced, as previously described [17]. The obtained sequences were used to build phylogenetic trees, and compare the circulating influenza strains with the vaccine and other reference strains. All sequences were submitted to the GISAID database.

3. Results

From November 2013 to May 2014 there were a total of 1370 unique patient swab samples sent to the three laboratories, for which the reporting form had been forwarded to the HCDCP: 873 from the NIRL South, 472 from the NIRL North and 25 from the University of Athens. Fig. 1 shows the distribution of these patients over time, grouped by lab result, along with the corresponding community ILI rate (as recorded by a sentinel surveillance system operated by the HCDCP). The first confirmed influenza cases occurred in week 01/2014; 21 influenza-negative patients that had been sampled in earlier weeks were excluded from further analysis. 39 samples were from infants under one year old and were also excluded; none of these infants was reported to have been vaccinated against influenza.

Of 1310 patients finally analyzed, 712 (54.4%) were male and 598 (45.6%) female, and in 1090 (83.2%) their vaccination status was known (Table 1). 124/1090 patients (11.4%) were vaccinated against influenza while 966/1090 (88.6%) were not. In those aged 60 and over, vaccination coverage was 18.4% (84/456 patients), while in those aged 18–60 it was 6.3% (40/634 patients). The date of vaccination was reported in just 13/124 patients (10.5%), the majority of whom (8 cases) were vaccinated in October 2013.

A positive RT-PCR test for influenza was found in 543/1310 patients (41.5%); of those 41 (7.6%) were vaccinated, 457 (84.2%) were unvaccinated, and in 45 (8.3%) vaccination status was unknown. The distribution of influenza types and subtypes was not significantly different between vaccinated and unvaccinated