Available online at www.sciencedirect.com
ScienceDirect
Vaccine 25 (2007) 7313–7321
Vaccine
www.elsevier.com/locate/vaccine

Effectiveness of MF59™-adjuvanted subunit influenza vaccine in preventing hospitalisations for cardiovascular disease, cerebrovascular disease and pneumonia in the elderly

Joan Puig-Barberà [a,b,*], Javier Díez-Domingo [b], Ángel Belenguer Varea [a], Germán Schwarz Chavarri [a], José A. Lluch Rodrigo [b], Santiago Pérez Hoyos [c], David González Vidal [d]

[a] *Fundación de la Sociedad Valenciana de Medicina de Familia y Comunitaria, Valencia, Spain*
[b] *Centro Superior de Investigación en Salud Pública, Valencia, Spain*
[c] *Escuela Valenciana de Estudios para la Salud (EVES), Valencia, Spain*
[d] *ESTEVE, Departamento Médico, Barcelona, Spain*

Received 18 July 2007; accepted 19 August 2007
Available online 4 September 2007

Abstract

Annual circulation of influenza virus coincides with a peak in cardiovascular and pneumonia mortality/morbidity. This study aimed to determine the effectiveness of MF59™-adjuvanted subunit influenza vaccine in preventing hospitalisation due to acute coronary syndrome (ACS), cerebrovascular accident (CVA) and pneumonia in the elderly. Three case–control studies were performed during the 2004–2005 influenza season in three health districts in Valencia, Spain (total elderly [>64 years of age] population: $n = 105{,}454$). Controls were patients admitted for an acute surgical process or trauma within 10 days of case admission. In total, 159 patients were hospitalised for ACS, 148 for CVA and 242 for pneumonia. The risk of hospitalisation after the start of the influenza season was significantly lower in vaccinated patients compared with non-vaccinated patients (adjusted odds ratios: 0.13 [$P = 0.013$] for ACS; 0.07 [$P = 0.007$] for CVA; 0.31 [$P = 0.005$] for pneumonia). During peak virus circulation, vaccination with MF59™-adjuvanted subunit influenza vaccine was associated with an 87% relative risk reduction in hospitalisation for ACS, 93% for CVA, and 69% for pneumonia.

Keywords: MF59™-adjuvanted subunit influenza vaccine; Cardiovascular diseases; Cerebrovascular disorders; Pneumonia; Elderly; Influenza

1. Introduction

Annual circulation of the influenza virus coincides with a significant seasonal increase in morbidity and mortality, resulting from both the symptoms of influenza itself and from other associated illnesses. For example, one study has estimated a rate of 115 hospitalisations per 100,000 person-years for circulatory and respiratory illness associated with influenza [1]. This rate rose dramatically with age, ranging from 230 in patients aged 65–69 years to 1669 in the ≥85 years of age group. Other studies have shown that mortality from ischaemic heart disease, acute myocardial infarction, cerebrovascular disease, diabetes, cardiorespiratory disease and chronic obstructive pulmonary disease (COPD) was associated with influenza [2–4]. Again, mortality was substantially higher in the elderly [2,4]. Such observations have led some authors to suggest that influenza is the singular cause of the increase in seasonal morbidity and mortality [2].

Despite the elderly being at increased risk of developing influenza-related complications, a considerable percentage remains unvaccinated [5], and the effectiveness of conventional influenza vaccines is substantially lower in this age group compared with young adults [6]. Furthermore, the abil-

* Corresponding author at: Centro de Salud Pública de Castellón, Avda del Mar, 12, 12003 Castellón, Spain. Tel.: +34 96435 8303; fax: +34 96423 9491.
E-mail address: jpuigb@terra.es (J. Puig-Barberà).

0264-410X/$ – see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.vaccine.2007.08.039

ity to mount an effective immune response against infection gradually wanes with age [7]. Adjuvanted influenza vaccines are currently being developed, which aim to improve the effectiveness of influenza vaccines in the elderly.

MF59^{TM}, a novel adjuvant, was first approved for human use in 1997 [8]; the MF59^{TM} adjuvant is an oil-in-water emulsion containing the naturally occurring squalene oil, and as such, is a biodegradable and biocompatible adjuvant [8]. It is thought to act by recruiting and activating antigen-presenting cells at the injection site, thus increasing their capacity to capture, transport and process the co-administered antigens [9]. To date, more than 23 million doses of the MF59^{TM}-adjuvanted subunit influenza vaccine have been distributed [9].

Vaccination with MF59^{TM}-adjuvanted subunit influenza vaccine results in an enhanced immune response in the elderly and in subjects with underlying chronic disease, compared with a non-adjuvanted vaccine [10,11]. Furthermore, a heterotypic immune response is observed, demonstrating the vaccine's ability to confer protection against a broader range of influenza virus strains [12–14]. The MF59^{TM}-adjuvanted subunit influenza vaccine has shown higher clinical efficacy compared with conventional vaccines [15] and has been associated with a reduced risk of hospitalisation for pneumonia in non-institutionalised elderly subjects [16].

The MF59^{TM}-adjuvanted subunit influenza vaccine was used by the public health service of the Valencia Autonomous Region to vaccinate the elderly during the 2004–2005 influenza season. In this study, we have estimated the vaccine's effectiveness in reducing the risk of hospitalisation for acute coronary syndrome (ACS), cerebrovascular accident (CVA) and pneumonia associated with the seasonal increase in influenza virus circulation.

2. Methods

Three case–control studies were performed in the elderly (>64 years of age) population from three health districts in the Valencia Autonomous Region, Spain (total number of elderly residents in these districts: $n = 105{,}454$ at 31 December 2004), where MF59^{TM}-adjuvanted subunit influenza vaccine was used. Subjects not using the public health service may have been vaccinated with a different influenza vaccine. The risk of hospitalisation for ACS, CVA or pneumonia was evaluated for patients who had received MF59^{TM}-adjuvanted subunit influenza vaccine (FLUAD®/Chiromas®, Novartis Vaccines) and for those who had not been vaccinated against influenza. The protocol was approved by the research ethics committees of the participating hospitals.

2.1. Inclusion criteria

Incident cases for each disease were identified from all consecutive emergency hospitalisations following their admission between 15 November 2004 and 31 March 2005 [17]. Diagnoses were made according to the International Classification of Diseases, 9th version, Clinical Modification for ACS (410–411.89 and 413), CVA (431–436) or pneumonia (480–487). Only non-institutionalised patients who were >64 years of age, had lived in the hospital catchment area for the previous 6 months, were able to give informed consent, and remained in hospital for at least 72 h were included in the study.

Each case was paired with one or two controls, matched for hospital and gender. Controls were recruited according to the same inclusion criteria as cases, following emergency hospitalisations for an acute surgical process or trauma. The admission date for controls was matched to the case admission date, preferably being the same day, and with a maximum interval of 10 days.

2.2. Exclusion criteria

Exclusion criteria were: inability to communicate and give consent; unwillingness to participate; being under routine care in a private facility; or having a known allergy to egg protein.

2.3. Data collection

Once written consent was obtained, data were collected by trained field researchers through a review of emergency records, clinical history, face-to-face closed question interviews and consultation of population registers.

2.3.1. Vaccination status

Patients were considered vaccinated if they could remember the month and year of vaccination and the nurse who administered it, and if the period since vaccination was more than 15 days. The information was validated by comparison with the population vaccination register; in the case of discrepancy, details provided by the vaccination register were taken as valid.

2.3.2. Study variables (potential confounding factors)

The following variables were recorded: presence of cardiovascular disease, COPD, asthma, diabetes mellitus, renal impairment, liver disease or neoplasia; regular treatment with hypertensives, antiplatelet agents, anticoagulants, hypolipidaemic agents or insulin prior to admission; blood pressure; smoking habits; level of social interaction; number of hospital admissions in the year prior to study inclusion; number of health centre or home visits in the 3 months prior to study inclusion; whether the patient lived with other people; and whether their usual caregiver had been vaccinated against influenza. Patients were also asked whether they had received pneumococcal vaccine, with the information validated as for influenza vaccination. Dependence was evaluated using the Barthel index which measures how well a person functions independently in their daily life.

2.3.3. Influenza virus circulation and circulating strains

Evolution of the influenza outbreak in Valencia during the 2004–2005 influenza season was evaluated by epidemiological week, from the rate of influenza-like illness (ILI) per 100,000 residents [18]. The relevant circulating influenza virus strains and their isolation frequency were those described by the European Influenza Surveillance Scheme [19].

2.4. Data analysis

A multivariate conditional logistic regression model was used to estimate the odds ratio (OR) of becoming a case after vaccination compared with non-vaccination. Potential confounding factors were included in the model; the model also accounted for indication bias whereby some subjects have a higher probability than others of being vaccinated.

All analyses were performed with Version 9.1 of the STATA statistical programme (Stata Corp., College Station, Texas, USA).

2.4.1. Distribution of study variables (potential confounding factors)

Numerous variables were identified as potential confounding factors (Section 2.3.2), and the frequency distribution of each variable was calculated for cases and controls. The distribution homogeneity was compared with the strength and direction of each variable in each population by fitting a conditional bivariate logistic regression model to obtain the OR. The 95% confidence interval (CI) for the OR was calculated from the standard error estimated by the model. Where the CI does not include 1, a statistically significant association exists between the variable and the likelihood of a person being a case; values >1 signify that the variable is more likely to occur in a case.

2.4.2. Indication bias—calculation of propensity scores

Indication bias is a potential weakness in the analysis of vaccination effectiveness; individuals with certain risk factors can have a higher or lower probability of being vaccinated, which can confound the results of the analysis. Inclusion of a propensity score in the analysis of effectiveness is a well-established method to control for this potential bias [20,21]. The propensity score gives an estimate of the probability that a person would be vaccinated, given their risk factor profile.

In these studies, propensity scores were obtained by means of a logistic regression model which included variables that were statistically associated with vaccination, previously identified using bivariate analysis. The model was then adjusted using the stepwise method that eliminated all variables with a P-value of ≥ 0.10. The Hosmer–Lemeshow test was used to assess the propensity score goodness of fit, and its ability to discriminate was analysed by calculation of the area under the receiver operating characteristics (ROC) curve. Equal distribution among cases and controls was verified using the Kruskal–Wallis non-parametric test.

2.4.3. MF59™-adjuvanted subunit influenza vaccine effectiveness

The OR of becoming a case after vaccination with MF59™-adjuvanted subunit influenza vaccine compared with non-vaccination was estimated for each study using conditional multivariate logistic regression models; an OR <1 signifies that a person was less likely to become a case if they had been vaccinated.

The data were adjusted for the likelihood of vaccination (propensity score) and other previously defined confounding factors. The following criteria were applied to fit the models: (a) vaccination status was forced to be present in all adjusted models; (b) propensity score was initially included but when the propensity score did not fit the data and therefore did not contribute to improving estimates it was eliminated from the model (c) when the propensity score remained in the model, only variables not included in the estimation of the propensity score were considered for addition to the model; (d) if the propensity score did not fit the data, variables were sequentially included to improve the estimate of vaccine effectiveness, with a P-value of <0.10 or a change of 10% or more in the estimated effect of vaccination considered as an improvement; (e) estimated OR were plotted against the epidemiological period and the best fits, with the smallest CI range, were obtained; (f) plausibility and parsimony were taken into account.

2.5. Prevention of cases with MF59™-adjuvanted subunit influenza vaccine

As the sample was of incident cases and the frequency of each disease in the population was <10%, the OR can be accepted as a non-biased relative risk estimator [22]. This enables the impact of vaccination to be estimated as a fraction of the illness prevented by the vaccine, through the expression 1–OR [23]. The absolute reduction in the number of admissions for each outcome as a result of vaccine exposure is the difference between the incidence in exposed patients (p1) minus the incidence in non-exposed patients (p2). Both parameters were estimated from the incidence rate in the general population during the study period (p), with the numerator being all cases detected in the non-institutionalised population, and the denominator being the census of elderly subjects. The vaccine coverage rate (e) for the general population was assumed to be equal to that of the controls. Thus p2 and p1 are calculated according to the following equations: $p2 = p/[rt^{*}e + (1 - e)]$ and $p1 = rt^{*}p2$ [24] where rt is the OR. The inverse of the difference between the two rates gives the number of people needed to vaccinate in order to prevent a single case.

Table 1
Age and influenza vaccination status of patients hospitalised for acute coronary syndrome, cerebrovascular accident or pneumonia and their controls, and influenza vaccination status of their caregivers

	Cases	Controls	*P*
Acute coronary syndrome (cases, $n=144$; controls, $n=258$)			
Mean age, years (S.D.)	75.7 (6.8)	78.8 (7.6)	<0.001
Influenza-vaccinated, *n* (%)	114 (79.2)	181 (70.2)	0.05
Usual caregiver influenza-vaccinated, *n* (%)	73 (50.7)	101 (39.2)	0.025
Cerebrovascular accident (cases, $n=134$; controls, $n=246$)			
Mean age, years (S.D.)	76.9 (6.7)	79.4 (7.4)	0.002
Influenza-vaccinated, *n* (%)	91 (67.9)	184 (74.8)	0.320
Usual caregiver influenza-vaccinated, *n* (%)	59 (44.0)	83 (33.7)	0.037
Pneumonia (cases, $n=198$; controls, $n=321$)			
Mean age, years (S.D.)	78.5 (7.3)	78.5 (7.4)	0.928
Influenza-vaccinated, *n* (%)	150 (76.1)	251 (78.2)	0.580
Usual caregiver influenza-vaccinated, *n* (%)	99 (50.2)	138 (43.0)	0.141

CI = confidence interval; S.D. = standard deviation.

3. Results

3.1. Patients included

During the study period there were 159 hospitalisations for ACS, 148 for CVA and 242 for pneumonia that met the inclusion criteria. After consideration of the exclusion criteria, 144 (90.6%) cases admitted for ACS, 134 (90.5%) for CVA, and 198 (81.8%) for pneumonia were included in the study. The main reasons for exclusion were a lack of consent or suitable controls. Cases were unique for each study.

A total of 75.2% and 78.1% of vaccinated cases and controls, respectively ($P=0.314$), were vaccinated and on the population register. Of these, all cases and 99.73% of controls had received MF59^{TM}-adjuvanted subunit influenza vaccine. As the type of vaccine used cannot be determined for those subjects who were not registered, we have taken these proportions to represent the entire data set as the MF59^{TM}-adjuvanted subunit influenza vaccine was the vaccine used by the Valencia Autonomous Region during the study period.

3.2. Distribution of study variables (potential confounding factors)

Details of the distribution of study variables for all three indications are given in the supporting tables and Table 1. For ACS and CVA, there were some statistically significant differences between cases and controls with respect to age and vaccination status of the patient and caregiver; no such differences were observed for pneumonia (Table 1). Details of which variables were included in each analysis are given below.

3.3. MF59-adjuvanted influenza vaccination effectiveness: ACS

The calculated propensity score showed a good fit to the data (Hosmer–Lemeshow test, $P=0.6$; area under ROC curve = 0.86) and had a significantly different distribution between cases and controls ($P=0.008$). The propensity score was therefore included in the multivariate analysis, as was the number of cardiovascular risk factors. While other factors differed between cases and controls, they did not contribute to a better fit of the model and were therefore not included.

In the period prior to the influenza season (epidemiological weeks 47–52, 2004), subjects vaccinated with the MF59^{TM}-adjuvanted subunit influenza vaccine showed a major, but not significant, risk of hospitalisation with ACS compared with non-vaccinated subjects (OR 2.61; 95% CI 0.63–10.76). However, during epidemiological weeks 7–14, 2005, following the peak of influenza circulation, the risk of hospitalisation with ACS was reduced in subjects vaccinated with the MF59^{TM}-adjuvanted subunit influenza vaccine com-

Table 2
Risk (odds ratio and adjusted odds ratio) of hospitalisation for acute coronary syndrome, cerebrovascular accident and pneumonia in subjects who had received MF59^{TM}-adjuvanted subunit influenza vaccine

	Epidemiological week, 2005	Odds ratio (95% CI)	*P*	Adjusted odds ratio (95% CI)	*P*
ACS	7–14	0.89 (0.37–2.08)	0.786	0.13 (0.03–0.65)	0.013
CVA	3–10	0.66 (0.31–1.40)	0.276	0.07 (0.01–0.48)	0.007
Pneumonia	2–12	0.73 (0.40–1.35)	0.324	0.31 (0.14–0.71)	0.005

ACS = acute coronary syndrome; CVA = cerebrovascular accident; CI = confidence interval. The analysis was adjusted for the following variables: ACS: propensity score, ≥3 cardiovascular risk factors. CVA: age, smoking habits, COPD, systolic blood pressure ≥160 mmHg, transient ischaemic attacks, treatment with a hyperlipidaemic agent and Barthel index. Pneumonia: age, COPD status, presence of cardiopathy, diabetes mellitus, smoking habits, pneumococcus vaccination, Barthel index, number of home visits and vaccination status of the caregiver.

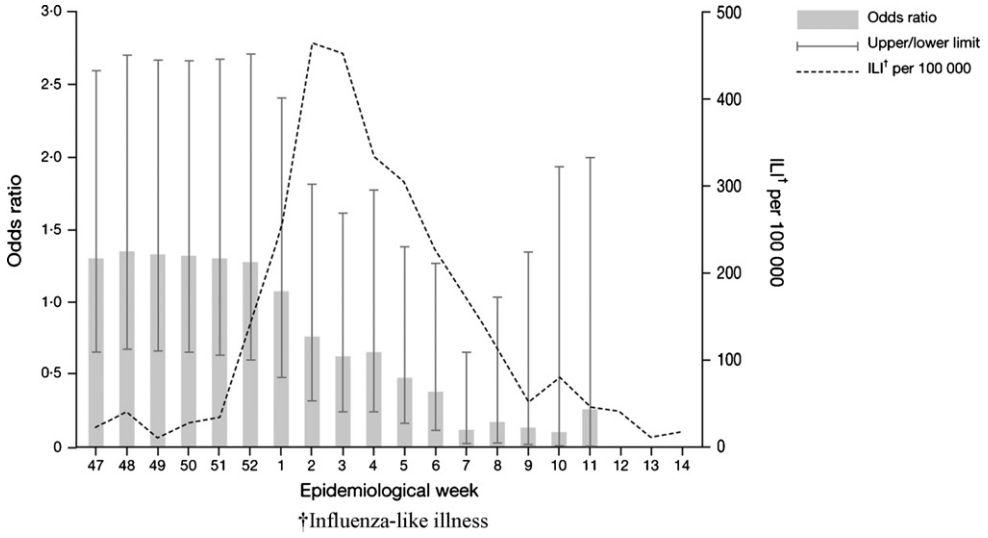

Fig. 1. Evolution of the risk (adjusted odds ratio for each week estimated from the model described in the text and in Table 2) of admission for acute coronary syndrome accounting for vaccination with the corresponding incidence of influenza-like illness (ILI) in the 2004–2005 season.

pared with non-vaccinated subjects (adjusted OR 0.13; 95% CI 0.03–0.65) (Table 2). Fig. 1 shows the evolving cumulative effect of influenza vaccination, by epidemiological week, in relation to influenza virus circulation, with the adjusted OR declining as the rate of ILI increases.

3.4. *MF59*^{TM}*-adjuvanted influenza vaccination effectiveness: CVA*

The calculated propensity score showed a good fit to the data (Hosmer–Lemeshow test, $P = 0.682$; area under ROC curve = 0.85), however, its distribution did not differ significantly between cases and controls. Therefore, it was not included in the multivariate analysis; age, smoking habits, COPD, systolic blood pressure ≥160 mmHg, transient ischaemic attacks, treatment with a hyperlipidaemic agent and Barthel index were included in the model.

After adjustment for these variables, influenza vaccination did not alter the risk of hospitalisation with CVA when influenza virus circulation was at a low level (epidemiological weeks 47–51, 2004). However, beyond epidemiological week 52 in 2004, a trend towards a reduced risk was observed, as shown by the evolving cumulative effect of influenza vaccination shown in Fig. 2. The reduced risk of CVA in subjects vaccinated with MF59^{TM}-adjuvanted subunit influenza vaccine (adjusted OR 0.07; 95% CI 0.01–0.48) coincided with

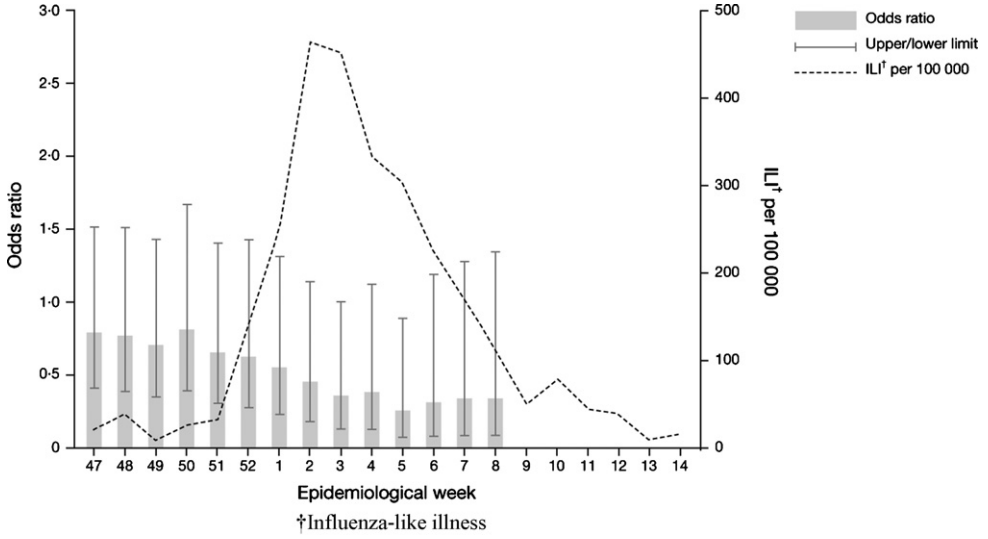

Fig. 2. Evolution of the risk (adjusted odds ratio for each week estimated from the model described in the text and in Table 2) of admission for cerebrovascular accident accounting for vaccination with the corresponding incidence of influenza-like illness (ILI) in the 2004–2005 season.

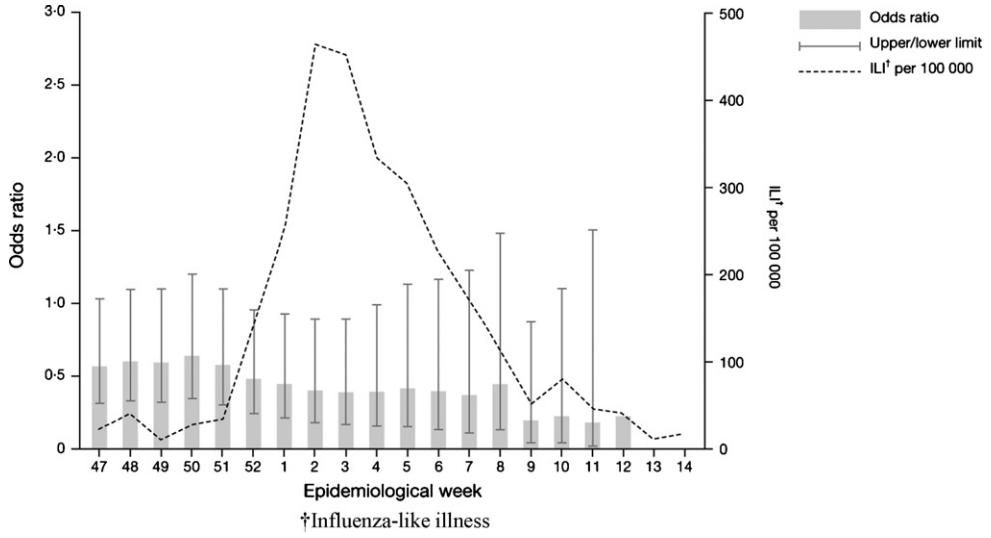

Fig. 3. Evolution of the risk (adjusted odds ratio for each week estimated from the model described in the text and in Table 2) of admission for pneumonia accounting for vaccination with the corresponding incidence of influenza-like illness (ILI) in the 2004–2005 season.

the peak intensity of influenza virus circulation in 2004–2005 during epidemiological weeks 3–10, 2005 (Fig. 2; Table 2).

3.5. MF59^{TM}-adjuvanted influenza vaccination effectiveness: pneumonia

The propensity score did not show a good fit to the data (Hosmer–Lemeshow test, $P = 0.039$) and its distribution was similar between cases and controls ($P = 0.63$); it was therefore not included in the multivariate analysis. The analysis was adjusted for age, COPD status, presence of cardiopathy, diabetes mellitus, smoking history, pneumococcus vaccination, Barthel index, number of home visits and vaccination status of the caregiver.

Prior to the onset of the influenza season, the adjusted OR of being hospitalised for pneumonia associated with influenza vaccination was not significant (OR 0.88; 95% CI 0.55–1.39) (Fig. 3). However, during peak influenza virus circulation (epidemiological weeks 2–12, 2005), receipt of influenza vaccine reduced the risk of hospitalisation with pneumonia (OR 0.31; 95% CI 0.14–0.71) (Table 2). Fig. 3 shows the evolving cumulative effect of influenza vaccination, by epidemiological week, in relation to influenza virus circulation, with the adjusted OR declining as the rate of ILI increases.

3.6. Effectiveness of MF59^{TM}-adjuvanted subunit influenza vaccine in preventing cases of ACS, CVA and pneumonia

During the period of influenza virus circulation, the estimated effectiveness of the MF59^{TM}-adjuvanted subunit influenza vaccine in preventing emergency hospitalisations for ACS was 87% (95% CI 35–97%); for CVA, 93% (95% CI 52–99%); and for pneumonia, 69% (95% CI 29–86%).

For all three diseases, the hospitalisation rates were higher for elderly people who had not received the MF59^{TM}-adjuvanted subunit influenza vaccine (Table 3). Finally, in order to prevent one emergency admission for ACS, CVA and pneumonia, the number of elderly people who needed to be vaccinated was 1073, 639 and 567, respectively (Table 3).

4. Discussion

In these case–control studies, receipt of an MF59^{TM}-adjuvanted subunit influenza vaccine was associated with a reduced risk of emergency admission for ACS, CVA or pneumonia in the elderly (>64 years of age).

The findings of this study are consistent with other recently published studies regarding influenza vaccine effectiveness in preventing episodes of cardiac arrest, acute myocardial infarction and cerebrovascular accident [25–30]. Inconclusive results of other studies have been attributed to insufficient sample size [31], or because of exclusion of patients in peri-

Table 3
Rate of emergency hospitalisations per 1000 elderly people (>64 years of age) who were either vaccinated or not vaccinated with MF59^{TM}-adjuvanted subunit influenza vaccine, and number needed to vaccinate to prevent one emergency admission for acute coronary syndrome (ACS), cerebrovascular accident (CVA) or pneumonia

	Emergency hospitalisation rate per 1000 elderly people (>64 years of age)		Number needed to vaccinate to prevent one emergency admission
	Non-vaccinated	Vaccinated	
ACS	1.07	0.14	1073
CVA	1.68	0.12	693
Pneumonia	2.55	0.79	567

ods of increased risk, which weakens the vaccination effect [32].

In this study, data were obtained from a broad, general population and the estimates were adjusted to the epidemic wave, resulting in improved accuracy [33]. The lack of effect in periods prior to influenza virus circulation supports a potentially protective effect of the MF59^{TM}-adjuvanted subunit influenza vaccine in preventing hospitalisations for cardiovascular and cerebrovascular events and pneumonia, and supports the positive findings of previous studies.

However, the possibility of selection, classification, confounding and indication bias [17,20] must be taken into account, as well as the epidemiological and biological plausibility of the results; these factors are discussed below.

A sample of incident cases was created in which cases and controls were identified through the daily revision of admission lists, without previous knowledge of vaccination status. The cases and controls met the same inclusion and exclusion criteria, and were matched according to geographical area of residence, hospital and case admission date. These factors ensured that the probability of being detected and included in the study was comparable for cases and controls and made selection bias less likely [17].

Information on admission diagnosis, comorbidity, antecedents and risk factors was obtained in the same manner for both cases and controls, and validated from clinical records and follow-up of the diagnostic episode. The presence of recall bias can be rejected as both cases and controls were hospitalised for acute processes and were not aware of the purpose of the study. However, the presence of residual confounding bias due to the generic definition of comorbidity must be assumed. Multivariate analysis was performed in an attempt to reduce this bias, together with an exploration of other factors directly or indirectly associated with comorbidity such as age, vaccination status of the caregiver, smoking habits, the Barthel index and the use of health services.

Vaccination history was verified blindly using a population vaccination register. During this process, data were obtained on the date, batch and type of vaccine administered. Registration frequency was similar in vaccinated cases and controls 63% and 64%, respectively. The percentage of all included cases and controls that were actually vaccinated and were also registered was 75.21% and 78.05% ($P = 0.314$), respectively. Of these, 100% of cases and 99.73% of controls were vaccinated with the MF59^{TM}-adjuvanted subunit influenza vaccine. The type of vaccine received was certified by data, including batch, and commercial name, in the population-wide vaccination register. Thus, we can conclude that the same proportions can be imputed to the remaining non-registered subjects, as the fact of being registered was wholly independent of the study goals or methods, and of the inclusion criteria. Any classification bias due to the use of non-adjuvanted vaccine was therefore either nil, or would act against the effectiveness of the MF59^{TM}-adjuvanted vaccine.

As discussed earlier, indication bias can be associated with estimates of vaccination effectiveness [20]. Patients with risk factors associated with selected pathologies may have a higher or lower probability of being vaccinated, which would confound the vaccination effect. In order to correct this, an adjusted analysis was performed using conditional logistic regression, the first stage of which explored the contribution of the propensity score to improving the fit of the model. The propensity score assigned each subject with a conditional probability of having been vaccinated, according to their characteristics. The fit of the propensity score to the data was verified and its calibration and discrimination capacity evaluated. The propensity score was used as a categorical variable in the multivariate model, thereby guaranteeing the robustness of the results [21]. When the propensity score did not fit the data and therefore did not contribute to improving estimates, an adjustment was made according to comorbidity variables and the variables relating to residual confounding bias.

After taking confounding factors into account, a protective effect of the MF59^{TM}-adjuvanted subunit influenza vaccine on hospitalisations for cardiovascular and cerebrovascular events and pneumonia was observed. The effect was significant during periods of peak influenza virus circulation and was not observed outside this period. Various authors have reported the coincidence and temporal association of the peak intensity of influenza virus circulation with excess mortality, morbidity and hospitalisations for cardiovascular disease and pneumonia [2,34]. The association of the MF59^{TM}-adjuvanted subunit influenza vaccine with a reduction in hospitalisations during this period lends epidemiological plausibility to our findings [33].

Studies on the effect of influenza on vascular disease progression support the hypothesis of a relationship between influenza infection and acute activation of atherosclerotic lesions, a precursor to acute thrombotic vascular events. For example, in mice prone to atherosclerosis, influenza infection causes major alterations in atherosclerotic plaques, with infiltration of inflammatory cells [35]. Furthermore, the synergism between influenza infection and a greater susceptibility to *Streptococcus pneumoniae* is well established [36]. Several potential mechanisms for the role of influenza infection in the progression of atherosclerotic disease have been suggested, including: production of auto-antibodies to modified low density lipoprotein and other auto-antigens; direct vessel wall colonisation that may initiate a local autoimmune reaction; and molecular mimicry whereby structural similarity between influenza viral antigens and self-antigens may stimulate an atherogenic autoimmune reaction, leading to initiation, progression or destabilisation of atherogenic plaques [37,38].

Finally, these findings were obtained during a season in which A/H3N2 isolations predominated. The isolated strains were initially similar to A/Wellington/1/2004; however, as the outbreak progressed, the isolates became similar

to A/California/7/2004. Both of these strains were distinguishable from the strain included in the season's vaccine, which was similar to A/Fujian/411/2002 [19]. Hence, these results additionally support the previously described ability of MF59^{TM}-adjuvanted subunit influenza vaccine to stimulate heterotypic immunity [12,13].

5. Conclusions

These results suggest that MF59^{TM}-adjuvanted influenza vaccination is associated with a significant reduction in the risk of hospitalisation for ACS, CVA and pneumonia during the period of influenza virus circulation. Such results are consistent with the findings of other studies in different populations and influenza seasons. The results are both epidemiologically and biologically plausible, and confirm reports that a relationship may exist between influenza virus infection and the development of acute cardiovascular and cerebrovascular events, and pneumonia. Annual vaccination against influenza can contribute to a substantial decrease in cardiovascular, cerebrovascular and pneumonia morbidity associated with influenza virus infection, in a population of people who are at increased risk of infection due to their waning immune system. Given that traditional risk factors fail to identify many patients at risk of suffering cardiovascular and cerebrovascular events [39] and that the elderly are at an increased risk of pneumonia, the findings from our case–control studies support recommendations for the generalised use of MF59^{TM}-adjuvanted subunit influenza vaccine in the elderly (>64 years of age) even when no additional risk factors are present.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.vaccine.2007.08.039.

References

[1] Thompson WW, Shay DK, Weintraub E, Brammer L, Bridges CB, Cox NJ, et al. Influenza-associated hospitalizations in the United States. JAMA 2004;292:1333–40.
[2] Reichert TA, Simonsen L, Sharma A, Pardo SA, Fedson DS, Miller MA. Influenza and the winter increase in mortality in the United States, 1959–1999. Am J Epidemiol 2004;160:492–502.
[3] Madjid M, Miller CC, Zarubaev VV, Marinich IG, Kiselev OI, Lobzin YV, et al. Influenza epidemics and acute respiratory disease activity are associated with a surge in autopsy-confirmed coronary heart disease death: results from 8 years of autopsies in 34892 subjects. Eur Heart J 2007;28:1205–10.
[4] Wong C-M, Chan K-P, Hedley AJ, Peiris JSM. Influenza-associated mortality in Hong Kong. CID 2004;39:1611–7.
[5] Kroneman M, Paget WJ, van Essen GA. Influenza vaccination in Europe: an inventory of strategies to reach target populations and optimise vaccination uptake. Euro Surveill 2003;8:130–8.
[6] Nicholson KG, Wood JM, Zambon M. Influenza. Lancet 2003;362:1733–45.
[7] Gavazzi G, Krause KH. Ageing and infection. Lancet Infect Dis 2002;2:659–66.
[8] Podda A, Del Giudice G, O'Hagan D. MF59: a safe and potent adjuvant for human use. In: Schijns D, O'Hagan D, editors. Immunopotentiators in modern vaccines. London: Elsevier Academic Press; 2006. p. 149–59.
[9] O'Hagan DT. New generation vaccine adjuvants. Encyclopedia of Life Science 2007. Available from http://www.mrw.interscience.wiley.com/emrw/047001590X/home. Accessed 02 July 2007.
[10] Banzhoff A, Nacci P, Podda A. A new MF59-adjuvanted influenza vaccine enhances the immune response in the elderly with chronic diseases: results from an immunogenicity meta-analysis. Gerontology 2003;49:177–84.
[11] Baldo V, Baldovin T, Floreani A, Carraro AM, Trivello R, Family Medicine Group of Pianiga. MF59-adjuvanted influenza vaccine confers superior immunogenicity in adult subjects (18–60 years of age) with chronic diseases who are at risk of post-influenza complications. Vaccine 2007;25:3955–61.
[12] De Donato S, Granoff D, Minutello M, Lecchi G, Faccini M, Agnello M, et al. Safety and immunogenicity of MF59-adjuvanted influenza vaccine in the elderly. Vaccine 1999;17:3094–101.
[13] Del Giudice G, Hilbert AK, Bugarini R, Minutello A, Popova O, Toneatto D, et al. An MF59-adjuvanted inactivated influenza vaccine containing A/Panama/1999 (H3N2) induced broader serological protection against heterovariant influenza virus strain A/Fujian/2002 than a subunit and a split influenza vaccine. Vaccine 2006;24:3063–5.
[14] Baldo V, Baldovin T, Floreani A, Fragapane E, Trivello R, The Family Medicine Group. Response of influenza vaccines against heterovariant influenza virus strains in adults with chronic diseases. J Clin Immunol May 21, 2007 (Epub ahead of print).
[15] Iob A, Brianti G, Zamparo E, Gallo T. Evidence of increased clinical protection of an MF59-adjuvant influenza vaccine compared to a non-adjuvant vaccine among elderly residents of long-term care facilities in Italy. Epidemiol Infect 2005;133:687–93.
[16] Puig-Barberà J, Diez-Domingo J, Hoyos SP, Varea AB, Vidal DG. Effectiveness of the MF59-adjuvanted influenza vaccine in preventing emergency admissions for pneumonia in the elderly over 64 years of age. Vaccine 2004;23:283–9.
[17] Miettinen OS. The "case–control" study: valid selection of subjects. J Chronic Dis 1985;38:543–8.
[18] Direcció General de Salut Pública. Conselleria de Sanitat de la Comunidad Valenciana. Red Centinela Sanitaria. Analysis of the results of the influenza monitoring system in the Valencia Community by means of the Sentinel Physicians' Surveillance Network: 2004–2005 season. http://www.sp.san.gva.es/epidemiologia/Main (accessed July, 2006).
[19] European Influenza Surveillance Scheme. Annual report: 2004–2005 influenza season. Utrecht, the Netherlands. NIVEL, 2006. www.eiss.org (accessed July, 2006).
[20] Hak E, Verheij Th. JM, Grobbee DE, Nichol KL, Hoes AW. Confounding by indication in non-experimental evaluation of vaccine effectiveness: the example of prevention of influenza complications. J Epidemiol Community Health 2002;56:951–5.
[21] Weitzen S, Lapane KL, Toledano AY, Hume AL, Mor V. Principles for modeling propensity scores in medical research: a systematic literature review. Pharmacoepidemiol Drug Saf 2004;13:841–53.
[22] Pearce N. What does the odds ratio estimate in a case–control study? Int J Epidemiol 1993;22:1189–92.
[23] Miettinen OS. Proportion of disease caused or prevented by a given exposure, trait or intervention. Am J Epidemiol 1974;99:325–32.
[24] Schlesselman JJ. Case–control studies. Design, conduct, analysis. Oxford: Oxford University Press; 1983. pp. 40–9.
[25] Siscovick DS, Raghunathan TE, Lin D, Weinmann S, Arbogast P, Lemaitre RN, et al. Influenza vaccination and the risk of primary cardiac arrest. Am J Epidemiol 2000;152:674–7.

[26] Naghavi M, Barlas Z, Siadaty S, Naguib S, Madjid M, Casscells W. Association of influenza vaccination and reduced risk of recurrent myocardial infarction. Circulation 2000;102:3039–45.
[27] Lavallee P, Perchaud V, Gautier-Bertrand M, Grabli D, Amarenco P. Association between influenza vaccination and reduced risk of brain infarction. Stroke 2002;33:513–8.
[28] Grau AJ, Fischer B, Barth C, Ling P, Lichy C, Buggle F. Influenza vaccination is associated with a reduced risk of stroke. Stroke 2005;36:1501–6.
[29] Nichol KL, Nordin J, Mullooly J, Lask R, Fillbrandt K, Iwane M. Influenza vaccination and reduction in hospitalizations for cardiac disease and stroke among the elderly. N Engl J Med 2003;348:1322–32.
[30] Armstrong BG, Mangtani P, Fletcher A, Kovats S, McMichael A, Pattenden S, et al. Effect of influenza vaccination on excess deaths occurring during periods of high circulation of influenza: cohort study in elderly people. BMJ 2004;329:660.
[31] Gurfinkel EP, de la Fuente RL, Mendiz O, Mautner B. Influenza vaccine pilot study in acute coronary syndromes and planned percutaneous coronary interventions: the FLU Vaccination Acute Coronary Syndromes (FLUVACS) Study. Circulation 2002;105:2143–7.
[32] Jackson LA, Yu O, Heckbert SR, Psaty BM, Malais D, Barlow WE, et al. Influenza vaccination is not associated with a reduction in the risk of recurrent coronary events. Am J Epidemiol 2002;156:634–40.
[33] Jackson LA, Jackson ML, Nelson JC, Neuzil KM, Weiss NS. Evidence of bias in estimates of influenza vaccine effectiveness in seniors. Int J Epidemiol 2006;35:337–44.
[34] Spodick DH, Flessas AP, Johnson MM. Association of acute respiratory symptoms with onset of acute myocardial infarction: prospective investigation of 150 consecutive patients and matched control patients. Am J Cardiol 1984;53:481–2.
[35] Naghavi M, Wyde P, Litovsky S, Madjid M, Akhtar A, Naguib S, et al. Influenza infection exerts prominent inflammatory and thrombotic effects on the atherosclerotic plaques of apolipoprotein E-deficient mice. Circulation 2003;107:762–8.
[36] McCullers JA, Bartmess KC. Role of neuraminidase in lethal synergism between influenza virus and *Streptococcus pneumoniae*. J Infect Dis 2003;187:1000–9.
[37] Gurevich VS, Pleskov VM, Levaya MV. Autoimmune nature of influenza atherogenicity. Ann NY Acad Sci 2005;1050:410–6.
[38] Gurfinkel E, Lernoud V. The role of infection and immunity in atherosclerosis. Expert Rev Cardiovasc Ther 2006;4:131–7.
[39] Braunwald E. Shattuck lecture—cardiovascular medicine at the turn of the millennium: triumphs, concerns, and opportunities. N Engl J Med 1997;337:1360–9.