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Assessment of efficacy and safety of pandemic A/H1N1/2009 influenza vaccine in a group of health care workers

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Key words

Pandemic influenza vaccine; health care workers; A/H1N1/2009 virus

Parole chiave

Vaccino anti virus pandemico; operatori sanitari; virus A/H1N1/2009

Summary

Introduction: *The development in an extremely short time of an efficacious and safe vaccine against the pandemic A/H1N1 virus was a challenge that involved the entire scientific community.* **Aims:** *To assess the immunological and clinical efficacy of the new H1N1v monovalent influenza vaccine (Focetria® Novartis Vaccines, Siena, Italy) in a group of health care workers (HCWs).* **Methods:** *A total of 148 volunteer HCWs were enrolled between Mid-Novembre 2009 and December 2009. After measuring antibody titers, a single intramuscular dose of 7.5 μg of Focetria® monovalent vaccine against A/H1N1/2009 influenza virus with MF59C.1 adjuvant was administered.* **Results:** *Antibody titers (median value) before and after a single dose of vaccine, measured by means of standard heamagglutination inhibition test (HAI), increased from 32 to 256 ($p<0.001$). After vaccination, 79.7% of the subjects showed antibody seroconversion, and in 97.3% seroprotecion was achieved. The ratio between the geometric means of antibody titers (GMTR) was 6.69. For the 3 subjects who reported symptoms of ILI (Influenza-like illness), a regular nasal-pharyngeal swab sample was taken to identify the virus type by RT-PCR, the laboratory results of tests performed on these samples were negative for pandemic A/H1N1/2009 virus. During the entire follow-up period of 6 months no severe adverse events occurred.* **Conclusions:** *The vaccine against pandemic A/H1N1/2009 virus provided protection against the virus and not only contributed to a significant immunization (according to EMEA criteria), but kept all 148 subjects under study free from A/H1N1/2009 influenza illness.*

Riassunto

«Valutazione dell'efficacia e della sicurezza del vaccino anti virus pandemico A/H1N1/2009 in un gruppo di lavoratori della sanità». **Introduzione:** *La messa a punto in tempi strettissimi di un vaccino efficace e sicuro contro il virus Pandemico A/H1N1/2009 ha impegnato la comunità scientifica internazionale.* **Scopo:** *Verificare*

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l'efficacia immunologica e clinica del nuovo vaccino antinfluenzale monovalente H1N1v (Focetria® Novartis Vaccines, Siena, Italy) in un gruppo di lavoratori della sanità. **Metodi:** *Sono stati arruolati tutti gli operatori sanitari (fino ad un massimo di 148 soggetti) che si sono presentati su base volontaria alla vaccinazione tra la metà del mese di Novembre 2009 e Dicembre 2009. Dopo titolazione anticorpale, è stata somministrata una singola dose intramuscolare di 7,5 μg di vaccino monovalente Focetria® anti virus influenzale A/H1N1/2009 adiuvato con MF59C.1.* **Risultati:** *La titolazione anticorpale (valore mediano) prima e dopo la singola dose di vaccino, misurata con test standard di inibizione dell'emoagglutinazione (HAI), è passata da 32 a 256 (p<0,001). Dopo la vaccinazione, il 79,7% di soggetti ha mostrato sieroconversione anticorpale, mentre per il 97,3% si è raggiunta la sieroprotezione. Il rapporto fra le medie geometriche delle titolazioni anticorpali (GMTR) è risultato pari a 6,69. Per i 3 soggetti che avevano accusato una sintomatologia ILI (Influenza-like illness), per la quale è stato regolarmente eseguito un prelievo tramite tampone naso-faringeo per la tipizzazione del virus mediante RT-PCR, i risultati di laboratorio sui tamponi prelevati hanno dato esito negativo per il virus pandemico A/H1N1/2009. Durante tutto il periodo di follow up di 6 mesi non si sono verificati eventi avversi di tipo grave.* **Conclusioni:** *Il vaccino contro il virus pandemico A/H1N1/2009 si è rivelato, in questo studio, protettivo nei confronti del virus e non solo ha contribuito ad una significativa immunizzazione (in accordo con i criteri dell'EMEA), ma ha mantenuto liberi da patologia influenzale (virus A/H1N1/2009 correlata) la totalità dei 148 soggetti in esame.*

Introduction

The emergency of the A/H_1N_1/2009 virus was an unprecedented event in the history of modern virology. The virus contains a combination of genetic segments that had never been encountered before in "human" and "swine" influenza viruses. Genetic analysis of this viral strain showed that it contains 6 genetic segments of the well known triple-recombining swine influenza virus and 2 genetic segments (which encode for neuroaminidase and matrix proteins) that are closely linked to genes of the Eurasia swine influenza virus (7, 11). It was because of these characteristics that it was initially described as "quadruple-recombining".

Previous evidence showed that current, trivalent, vaccines against seasonal influenza were not effective in preventing infection by the new A/H1N1 influenza virus, which was further proof of the substantial degree of genetic difference of this virus and of the lack of specific antibodies against this new viral strain (2).

On 11 June 2009, with the declaration by the WHO of the beginning of a new influenza pandemic, the whole world was called to address for the need to produce and distribute as rapidly as possible new monovalent vaccines specific for the new influenza (3).

HCWs are considered to be a population at risk for exposure to both infected materials and sick persons with respiratory diseases and influenza (9). The risk of infection is therefore considerably higher as compared with the general population. In addition, HCW may themselves become a potential source of infection for patients in their care, especially those who are more susceptible because of chronic illnesses or immunodeficiency diseases. Furthermore, a possible massive absenteeism due to the illness would create serious staff organization problems in hospitals, with consequent disruption of services, exactly at a time when there is a parallel increase in the general rate of hospitalization due to spreading infection (13). For these reasons, even in the initial period of limited availability, these workers were given priority for vaccination (9).

A number of studies have shown that vaccination against influenza stimulates the production of antibody titers (measured using standard haemagglutination inhibition assay (HAI)) that were higher than or equal to 40 and, therefore, considered predictive of protection against infection (1, 4). However, these studies have not assessed the protective efficacy of the antibody titers against the pandemic 2009 pandemic influenza A virus infection. In this regard, the diagnosis of influenza is defined by a number of symptoms and described as

Influenza-like illness (ILI). In order to confirm that ILI is caused by the influenza virus, it is necessary to detect the virus through RT-PCR or virus isolation test in nasal fluids within 2-3 days from the onset of the symptoms.

For this reason, it was deemed to be of fundamental importance that this study assessed both the immunological and clinical efficacy of this new vaccine in high risk populations such as HCWs.

Materials and methods

This multicentric study included the participation of the Clinical Unit of Occupational Health, Desio Hospital (Monza-Brianza), Italy, the Unit of Preventive Medicine and the Viral Pathogens and Biosafety Unit of San Raffaele Scientific Institute, Milan, Italy.

The study was approved by the Local Ethical Committee and the subjects enrolled gave their written informed consent after receiving full and complete information on the research protocol and the aims of the study.

All health care workers (up to a maximum of 148) who asked for vaccination on a voluntary basis between mid-November 2009 and December 2009 were enrolled. The population consisted of healthy individuals of both sexes, aged between 19 and 65 years, who did not present any signs of fever at the time of vaccination and had a negative history of ILI in the preceding weeks. The main criteria for exclusion were serious health problems and allergy to any component of the vaccine or eggs, in addition to pregnancy. However, a history of local side effects after previous vaccinations was not deemed a contraindication. Individuals participated voluntarily in the study and none of the subjects refused vaccination.

Anti-pandemic A/H1N1/2009 influenza vaccine

The vaccine administered was Focetria® monovalent vaccine (Novartis Vaccines, Siena, Italy) against the new pandemic A/H1N1/2009 influenza supplied by the local health authorities (ASL), following the World Health Organization (WHO) guidelines. For the 2009-2010 season, the pandemic vaccine preparation was derived from the strain A/California/7/2009(H1N1)v like strain (X-179A) (7.5 microgrammes of haemagglutinin antigen per dose of 0.5 mL) adjuvanted with MF59C.1 (oil/water emulsion containing 9.75 mg of squalene, 1.175 mg di polysorbate 80 and 1.175 mg of sorbitan trioleate in a citrate buffer. This vaccine had been previously approved by the EMEA and authorized by the European Commission on 30 September 2009.

Procedure for collecting blood samples and administration of vaccine

After the subjects had read and signed the informed consent, blood samples were taken (7 mL) at time T0, i.e., shortly before administration of vaccine, and at time T1, i.e., 3-5 weeks after vaccination. The mean ± standard deviation of the number of days after vaccination when the blood sample was taken was 28.3±5.4. Serum was separated from whole blood samples and then collected in cryovials (Nunc Cryo Tubes 1.8 mL, InterMed) and stored at -80°C.

After the collection of the first blood sample, a single intramuscular (deltoid) dose of 7.5 μg of Focetria® monovalent A/H1N1/2009 influenza vaccine was administered.

Nasal-pharyngeal swab (UTM KIT)

Naso-pharyngeal swabs were collected within 48 hours from ILI symptom onset using the 1 mL Copan Universal Transport Medium (UTM-RT) System. A cotton swab was supplied with a test tube containing the viral transport medium, VTM (5% tryptose phosphate broth, 0.5% bovine albumin serum and 0.001% gentamycin phosphate buffer solution) (Copan Italia, Brescia, Italy). These clinical samples remain stable at room temperature for at least 24 hours, which makes for easy transport until they can be stored at -80°C.

Evaluation of immunogenic efficacy

Immunological efficacy was studied via measurement of the specific antibody responses in

each individual. Antibodies against the new swine virus were measured using the HAI test performed on the stored sera (14). The sera, which were previously defrosted, were initially treated with receptor-destroying enzyme (RDE II; Denka Seiken Co., LTD, Tokyo, Japan) at 37°C for 16 hours, in order to eliminate aspecific reactions, after which, 1:2 serial dilutions were performed with Phosphate Buffered Saline (PBS) in duplicate in a 96-well round bottomed plate. Then, 4 HAI units of antigen (vaccine preparation) were added to each well and the plate was placed in incubation at 35°C. After 30 minutes of incubation, turkey erythrocytes in 0.5% PBS solution (Charles River, Calco, Italy) were added and mixed in each well. Each plate was then left at room temperature to allow the red blood cells to sediment. The haemoagglutination inhibition HAI titer was examined one hour later. The HAI titer corresponds to the last dilution capable of producing a complete haemoagglutination inhibition reaction, i.e., when the erythrocytes sediment at the bottom of the well. All samples were tested in duplicates and blindly and the lowest serum dilution that was possible to test was 1:4. The HAI titer was defined as the reciprocal of the highest serum dilution that completely inhibits haemagglutination.

Evaluation of clinical efficacy

To assess the clinical efficacy, a telephone contact number was supplied operating 24 h a day, so that each participant could report any acute state of ILI. Each participant received a leaflet, that was duly explained, listing in a clear and concise manner the symptoms of influenza-like illness (ILI) that warranted telephone reporting of any state of illness. Such symptoms were fever ≥38°C accompanied by at least one of the following symtoms: headache, general malaise, sensation of fever (sweating, shivers) or asthenia and by at least one of the following respiratory symptoms: cough, painful pharyngitis or nasal congestion. Collection of swab samples was carried out within 3 days of reporting ILI either with the subject reporting directly to the hospital department or by requesting a member of our team to collect the sample at the subject's home.

Laboratory confirmation of Influenza A (H1N1) cases by PCR

Viral RNA was extracted from swabs using the QIAamp Viral RNA Mini Kit (Qiagen, Italy) according to manufacturer's instructions. For amplification of viral RNA, CDC's protocol for real-time RT-PCR for Influenza A (H1N1) was applied (version 2009, revision 2, 6 October 2009). Briefly, RNA was amplified on an ABI7700 thermocycler using the SuperScript™ III One-Step RT-PCR System with Platinum® Taq High Fidelity (Invitrogen) kit according to manufacturer's instructions. Three sets of primer pair and probe were used to distinguish between type A influenza viruses and swine influenza A viruses from swine H1 influenza (http://www.who.int/csr/resources/publications/swineflu/realtimeptpcr/en/index.html).

Evaluation of safety of the new vaccine

Considering that adverse effects of slight to moderate degree are also commonly encountered during regular seasonal vaccination campaigns, this study monitored continuously for 6 months the possible development of severe adverse effects (i.e. effects that could warrant hospitalization of the patient, that could cause persistent or serious disability or lead to life-threatening medical conditions or extreme situations of fatalities (10). Information on such serious adverse effects was collected via the permanently operating telephone lines and via data from the hospital department where participants in the study reported periodically.

Statistical analysis

Data were collected in Microsoft Excel 2000™ calculation sheets and processed with the same programme.

The efficacy of the vaccine was verified using the HAI test according to criteria established by the European Committee for Proprietary Medici-

nal Products (EMEA, 1997) for an adult population aged 18-60 years (6).

According to these criteria, at least one of the following requirements had to be verified:

- number of seroconversions or significant increase (≥4-fold) of the antibody titer in more than 40% of vaccinated subjects;
- >2.5-fold post-vaccination increase in the geometric mean of antibody titers;
- the number of subjects achieving a post-vaccination antibody titer ≥40 exceeding 70%.

To compare groups the statistical analysis used parametric tests (T-Test), non- parametric tests (Wilcoxon Signed Rank Test, Mann-Whitney Rank Sum Test) in case of failure of normality test and ratio between proportions (χ^2 Test) for comparison between antibody response before and after vaccination. A p value <0.05 was considered significant.

Data were processed using the Sigma-Stat™ statistical programme.

Table 1 - General characteristics of population under study

General characteristics	HCW* (N=148)
Females [N (%)]	78 (52.7)
Males [N (%)]	70 (47.3)
Age (years)	
Mean (SD)	39.4 (12.3)
Median	41
Range	19-65
Vaccine against 2009 seasonal influenza [N (%)]**	11/148 (7.4)
Influenza vaccine previous years [N (%)]	93/143 (65)
Number of days between vaccination and antibody titer measurement T1 (mean ± SD)	28.3±5.4

* HCW=Health Care Workers; ** The 2009 seasonal vaccine contained 15 μg of haemagglutinin antigen of each of the following strains: A/Brisbane/59/2007 (H1N1), A/Brisbane/10/2007 (H3N2), B/Brisbane/60/2008

Results

General characteristics of the study population

The general characteristics of the population under study are reported in table 1.

The number of males was similar to that of females and the mean age was 39.4±12.3 years. No significant differences were present related with gender (p=0.323). Only a small number of subjects were over 60 years of age and were not considered separately in the study. A small proportion of individuals (7.4%) received the 2009 seasonal vaccine prior to the pandemic vaccine, however, a large proportion of subjects (65%) received the seasonal vaccine in previous years.

Pre- and post-vaccination antibody titers

In order to determine the antibody response to the vaccine strain, the HAI assay was determined in sera collected prior to and 28 days post-vaccination. The mean antibody titer was 51.1±45 prior to vaccination and 519.8±886.9 after vaccination (table 2 and figure 1).

Thus, a single dose of vaccine elicited a significant antibody response (p<0.001) in the HCWs. No significant differences were observed between males and females (p=0.323). Indeed, as shown in the reverse cumulative distribution curves, one third of the subjects had antibody titers higher than 40 before vaccination (T0), however, more than 97% of the total study population showed an increase in antibody titers to higher than 40 after vaccination T1) (figure 2).

We next analysed the seroprotection (SP) before and after the administration of a single dose of vaccine against pandemic A/H1N1/2009 virus in the population under study. SP was defined by HAI titers ≥1:40. Table 3 shows that 35% of the subjects already had antibody titers considered as seroprotective against the new virus before administration of the vaccine. After vaccination 79.7% of the subjects showed antibody seroconverion (SC), whereas 97.3% of the population was seroprotected. Seroconversion is deemed to have occurred when the pre-vaccination HAI antibody titer increases from a value of <10 to a post-vaccination value of ≥40,

Table 2 - Specific antibody response before and after administration of single dose of vaccine against pandemic A/H1N1/2009 virus in population under study

	Pre-vaccination	Post-vaccination
Total subjects (number)	**148**	**148**
Ab titers		
Mean (SD)	51.1 (45.0)	519.8 (886.9)
Median	32*	256*
Range	4-256	32-8192
Geometric mean	41.2	275.7
Increase in Ab titer (number of times)		
Mean (SD)		*11.9 (16.2)*
Median		*8*
Range		*1-128*
GMTR		6.69
Males (number)	**70**	**70**
Ab titers		
Mean (SD)	41.8 (32.8)	331.9 (343.2)
Median	32*	256*
Range	16-256	32-2048
Geometric mean	36.4	218.5
Increase in Ab titer (number of times)		
Mean (SD)		*10.0 (11.4)*
Median		*6*
Range		*1-64*
GMTR		6.0
Females (number)	**78**	**78**
Ab titers		
Mean (SD)	59.4 (52.5)	688.4 (1155.5)
Median	32*	256*
Range	4-256	32-8192
Geometric mean	46.1	340.2
Increase in Ab titer (number of times)		
Mean (SD)		*13.6 (19.4)*
Median		*8*
Range		*1-128*
GMTR		7.4

Ab titers are expressed as reciprocals of dilutions. GMTR: geometric mean titer ratios=ratio between geometric mean of antibody titers post - and pre- vaccination. *$p<0.001$ Wilcoxon Signed Rank Test

or, when the pre-vaccination titer is ≥10, this increases must be at least 4-fold after vaccination.

The GMTR (geometric mean of antibody titers) was higher than 2.5, thus verifying the EMEA criteria for assessing the efficacy of the vaccine (GMTR=6.69).

As age was previously negatively associated with potency of the antibody response to the seasonal vaccine (12), the study population was further divided into age groups (≤30 years, 31-40 years, 41-50 years and >50 years) to better assess the percentage variations of seroprotection and serocon-

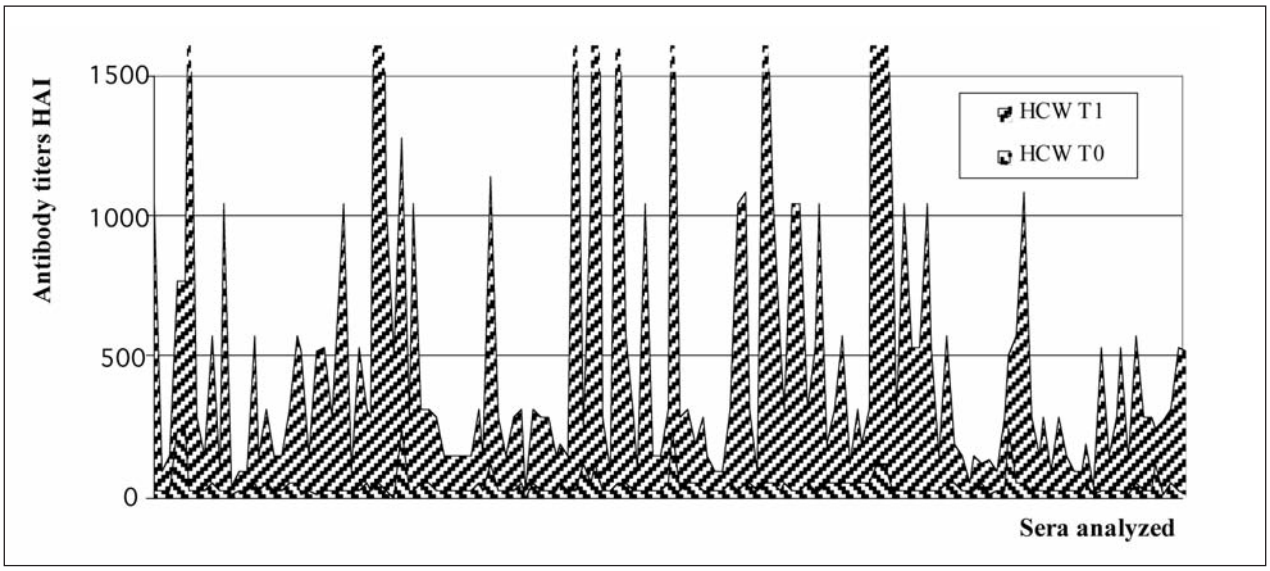

Figure 1 - Antibody titers before (T0) and after (T1) a single dose of vaccine against pandemic A/H1N1/2009 virus

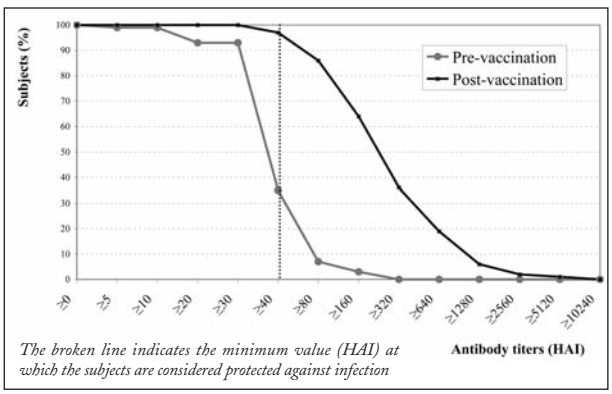

Figure 2 - Inverse cumulative distribution of antibody titers in serum before and after vaccination in HCW

Table 3 - Seroprotection (SP) and Seroconversion (SC) before and after administration of single dose of vaccine against pandemic A/H1N1/2009 virus in the two populations under study

Immunogenicity	HCW (N=148)	
	Pre- vaccination	Post- vaccination
GMT	41.2	275.7
T1/T0 GMTR		6.69
Number of SP (%)	52 (35.1)	144 (97.3)
Number of SC (%)		118 (79.7)

GMT: geometric mean titer=geometric mean of antibody titers; GMTR: geometric mean titer ratios=ratio between geometric mean of antibody titers post- and pre-vaccination

Table 4 - Percentages of SP and SC for different age groups within the population under study

Immunogenicity	HCW (N=148)	
	Pre- vaccination	Post- vaccination
SP≤30 years (%)	19/46 (41.3%)	46/46 (100%)
SP 31-40 years (%)	14/27 (51.9%)	27/27 (100%)
SP 41-50 years (%)	10/44 (22.7%)	43/44 (97.7%)
SP>50 years (%)	9/31 (29.0%)	28/31 (90.3%)
SC≤30 years (%)		42/46 (91.3%)
SC 31-40 years (%)		22/27 (81.5%)
SC 41-50 years (%)		33/44 (75.0%)
SC>50 years (%)		21/31 (67.7%)

SP = seroprotection; SC = seroconversion

version within each age group. As shown in table 4, the older age groups were less protected against the virus than the younger population (below 40 years of age). Given the genetic and antigenetic characteristics of the new virus, it could in fact be assumed that the younger age groups (under 40 years) would be those least protected against infection, but the results obtained disprove this assumption. The percentages of seroconversion appear to be negatively associated with increasing age, even though no significant linear correlation could be identified between age and increase in antibody titer (figure 3).

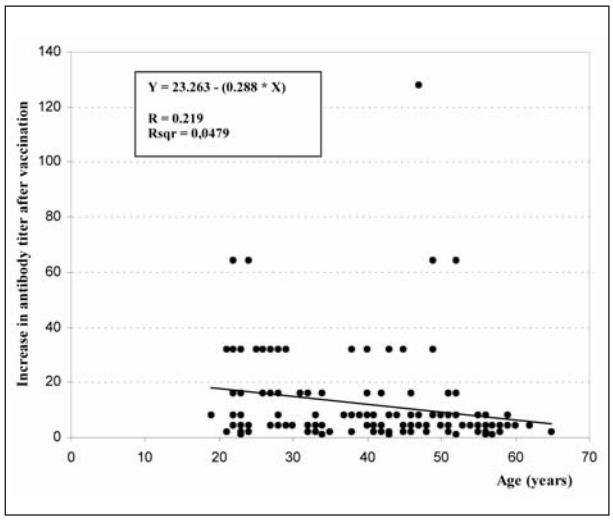

Figure 3 - Simple regression age/seroconversion after vaccination in HCW

Clinical efficacy of the new vaccine

The clinical efficacy of the new vaccine, intended as absence of development of influenza illness associated with the pandemic A/H1N1/2009 virus, may be considered significant in the case of the subjects under study. In fact, of the 148 subjects enrolled in the study period (November 2009-May 2010), only 3 reported symptoms of ILI. In these cases, a nasal-pharyngeal swab sample was obtained in order to identify the virus type (RT-PCR) and for all 3 the laboratory results on the swab sample were negative for the pandemic A/H1N1/2009 virus.

Safety of the new vaccine

In the safety assessment of the vaccine, during the entire period of 6 months (active and passive follow-up) no serious adverse events occurred and for this reason the vaccine was deemed safe. As previously explained, evaluation of slight or moderate effects was deliberately not considered since such manifestations are extremely common in all vaccination campaigns, including seasonal campaigns, and do not pose a valid limitation on the use of the vaccine.

Discussion and conclusion

The primary significant result is undoubtedly the high percentage of seroprotection that the subjects under study acquired following administration of the vaccine. In fact, after 28 days the percentage of HCW who were seroprotected was 97.3%, therefore the new vaccine, in spite of being prepared in an extremely short space of time, showed an elevated capacity of immunization against the pandemic A/H1N1/2009 virus. Vaccination with a single dose is therefore a guarantee of seroprotection against influenza illness.

In addition, these results agree with data previously published by the Authors who tested the efficacy of the vaccine in populations from geographical areas where the pandemic first spread.

An interesting finding was that nearly 35% of the subjects already possessed antibody titers considered to be seroprotective (HAI ≥40) before administration of the vaccine. It should, however, be taken into account that the vaccine was not available in Italy until November 2009, and that the period November-December 2009 coincided with the maximum diffusion of pandemic 2009 influenza AH1N1 virus infection recorded in Italy (46th week) (8). We cannot, therefore, exclude that some of the participants may have been previously exposed to similar viral antigens or were infected with the new virus in the days immediately preceding the sampling at time T0 and, for this reason, they showed high baseline antibody titers at time T0.

The percentage of individuals with seroconversion was also high, reaching 79.7%. No significant differences as regards sex were observed.

The post-vaccination geometric mean of antibody titers (GMT) of the subjects was 275.7, confirming a good antibody increase, while the ratio between geometric means of antibody titers (GMTR) before and after vaccination was 6.69.

The percentages of seroprotection and seroconversion were also evaluated by dividing both populations by age group (≤30 years, 31-40 years, 41-50 years e >50 years). Given the characteristics of the new pandemic virus, it would have been expected that the subjects in the oldest age group would be

the most protected (due to development of antibody cross-protection resulting from previous exposure to influenza viruses) (15). However, it was observed that in fact the subjects aged ≥40 years benefited most from vaccination since we found a lower percentage of subjects already protected against infection in exactly this age group. This finding suggests that vaccination should be extended to all age groups, without exception, especially for populations at risk like those under study. Increase in age appeared to be negatively associated with antibody response, even though no significant linear correlation was observed between the two variables (R=0.219).

In parallel to measuring seroprotection and seroconversion rates, clinical efficacy of the vaccine was also assessed, verifying whether also the immunogenicity of the antibodies produced was such as to keep the subjects under study free of A/H1N1/2009 influenza illness. This was possible via active and passive follow-up for ILI symptoms and analysis of the sample taken with nasal-pharyngeal swab in those subjects who became ill. Consistently with the low incidence of pandemic influenza A infection during winter 2010, three out of 148 study participants reported ILI symptoms. However, none of the three patients tested positive for the A/H1N1/2009 virus. Although we cannot exclude that ILI cases were missed by lack of reporting, we can conclude that the new vaccine proved likely to be 100% efficacious in maintaining the population under study free from illness.

As regards the safety of the new vaccine, no serious adverse effects were observed during the 6 months of follow-up, thus confirming the safety of the vaccine in the population under study.

Although the majority of infections caused by A/H1N1/2009 virus were acute, not severe and often self-limiting, nevertheless this low disease severity should not lower our attention in respect of other possible future pandemic viruses with characteristics of high inter-human transmission potential and high risk of spreading between countries, as is the case of the present virus, as the degree of pathogenicity of such viruses unfortunately can never be estimated in advance. The term "pandemic" should therefore sound like an alarm bell so that we will not be unprepared if the virus has a high degree of pathogenicity.

Conclusions

1) In this study, the new vaccine against the pandemic A/H1N1/2009 virus proved to be protective against the virus and not only contributed to achieving a significant immunization (according to EMEA criteria), but also kept all the 148 subjects under study free from A/H1N1/2009 influenza illness.

2) No age group should be excluded *a priori* from receiving vaccination since it cannot be predicted with sufficient accuracy which age groups are less protected and therefore more at risk.

3) The vaccination method, as further confirmation of the numerous studies carried out over the years, proved in this study too to be safe and free of severe adverse effects.

4) Vaccination is the sole real weapon capable of preventing influenza illness and no negative finding emerged from this study that contraindicates its use. Once again the benefits obtained from the possibility of preventing influenza infection exceeded any possible risks, and this is particularly true for categories of subjects at risk. Regarding HCW, moreover, in reference to Art. 25 of Law 81/08, the active role of the occupational health physician in the management of vaccination campaigns at the workplace should be stressed, which in fact was the case in this study.

5) The influenza pandemic in 2009 caused by A/H1N1 virus was found to have a "mild" degree of pathogenicity and often with self-limiting features, and for this reason no serious disruption occurred, which could have been the case if it had possessed the feared aggressiveness. This, however, should not be cause to lower our guard and all the more so not think that vaccination is superfluous.

No potential conflict of interest relevant to this article was reported

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