

Background

In influenza vaccine efficacy studies, virus identification using real-time polymerase chain reaction (rtPCR) is considered the ideal end point [1]. This approach, especially if performed in large populations, could be difficult to carry out and the results could depend on the level of influenza viruses circulation. This is why serological studies are often used as surrogate methods.

Serological studies are able to measure the antibodies that most people develop in response to a natural infection. This antibody response may persist at a detectable level for months. Influenza serological assays, such as haemagglutination inhibition (HI) or microneutralisation, can quantify these virus-specific antibodies as an indicator of infection [2].

Objectives

In order to understand if serological data are able to determine the vaccine efficacy, here we analyze the antibody response of 181 elderly volunteers (aged ≥ 65 years) to influenza vaccine. In particular we examined:

1. the HI titer of volunteers that had or hadn't a serologically evidenced influenza infection after vaccination;
2. the correlation between post-vaccination antibody titres and presence of symptoms in the infected volunteers.

Materials & Methods

After obtaining informed consent, 181 volunteers aged ≥ 65 years living in nursing homes of Umbria (a small region of central Italy) were immunized in with trivalent inactivated influenza seasonal vaccines available for the 2014-15 influenza season. Serum samples, obtained before, 1 and 6 months after vaccination, were analyzed by HI assay [3] using as antigens the three strains included in the vaccine (A/California/7/2009 (H1N1); A/Texas/50/2012 (H3N2); B/Massachusetts/2/2012).

The serological results observed 1 month after vaccination were evaluated according to the Committee for Medicinal Products for Human Use (CHMP) criteria for approval of influenza vaccines in the elderly, which require that at least one of the following criteria be met: seroprotection rate (HI ≥ 40) $\geq 60\%$, GMTR (ratio between post- and pre-vaccination geometric mean titer) ≥ 2 and seroconversion rate $\geq 30\%$ [4].

A serological evidence of recent influenza infection was made on comparing HI titers found in sera collected 6 and 1 months after vaccination. Vaccinated volunteers were considered positive if they had a seroconversion (a fourfold or greater increase in HI titer in seropositive subjects or from <10 to ≥ 40 in seronegative volunteers).

In the infected group, the presence and the severity of influenza-like illness (ILI) were documented by the physicians of the volunteers studied.

References

1. Petrie JG. et al. JID 2011:203
2. Chen MI. et al. PLoS One 2010; 5:e12474
3. Harmon MW: Influenza viruses. In Laboratory diagnosis of viral infections. 2nd edition. Edited by Lenette H. New York: Dekker; 1992: 515-34.
4. Committee for Proprietary Medicinal Products (CPMP): CPMP/BWP/214/96. 1997:1-18.

Results

1. Overall response to influenza vaccine components (Figure 1)

The results obtained in the overall population for each of the 3 vaccine antigens are shown in Figure 1. One month after vaccination, statistically significant increases were found in the percentage of seroprotected volunteers and in the values of their corresponding GMT against all the 3 vaccine antigens. At least 1 of the 3 CHMP requirements were satisfied against all the vaccine components.

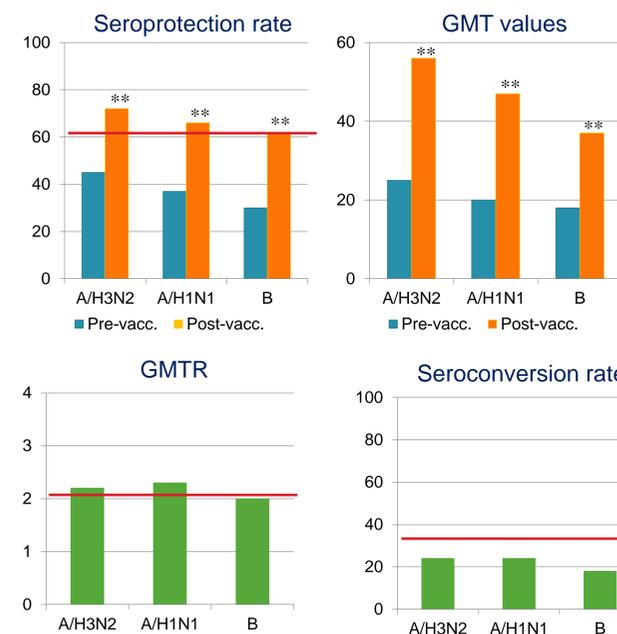
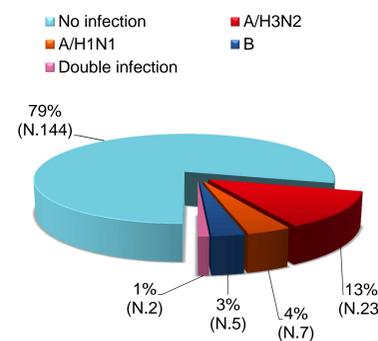


Figure 1. HI antibody response of 181 elderly volunteers vaccinated with 2014-15 trivalent inactivated influenza vaccine. The values of pre- and post-vaccination seroprotection rate, seroconversion rate, pre- and post-vaccination GMT, and GMTR are reported. **: p<0.01 comparing pre- and post-vaccination data

2. Serological confirmed influenza virus infections (Figure 2)



Totally, 37 of the 181 volunteers examined (21%) seroconverted 6 months after vaccination. In particular, as shown in Figure 2, 23 infections were caused by A/H3N2, 7 by A/H1N1, 5 by B influenza viruses. In two volunteers a double infection (H3+H1 and H1+B) was found.

Figure 2. Serological confirmed influenza virus infections.

Conclusions

Although the validity of using serologic confirmation of infection rather than virus identification to determine vaccine efficacy has been questioned, our results, though obtained analyzing a small population, confirm the validity of the serological approach

3. HI antibody response to vaccine antigens in infected and non-infected volunteers (Table 1)

As shown in Table 1, before vaccination the infected group showed lower antibody levels than non-infected volunteers against the A/H3N2 vaccine antigen. One month after vaccination, the values of non-infected group were always higher. In some instances the differences were statistically significant.

In the infected group, none of the 3 CHMP requirements were satisfied.

Table 1. HI antibody response of the studied volunteers divided in infected and non-infected.

In yellow the CHMP criteria satisfied.

Antigen	Group (N)	Seroprotection rate		Seroconv. rate	GMT		GMTR
		Pre-vacc.	1 month		Pre-vacc.	1 month	
A/H3N2	Non infected (144)	49 ^a	79 ^{**A}	28 ^a	28.9	70.9 ^{**A}	2.4
	Infected (37)	27	46 ^{**}	8	14.2	22.8	1.6
A/H1N1	Non infected (144)	36	68	28 ^a	20.1	51.1 ^{**}	2.5
	Infected (37)	41	59	8	21.1	35.7	1.7
B	Non infected (144)	30	64 ^{**}	19	17.8	38.0 ^{**}	2.1
	Infected (37)	32	57 [*]	13	21.1	33.5	1.6

** : p-value <0.01 comparing pre- and post-vaccination values
a : p<0.05; A: p-value <0.01 comparing response between infected and not infected volunteers

4. Seroprotection rate and severity of ILI (Figures 3a,b)

Analyzing the seroprotection rate of volunteers divided according to absence/presence of influenza like illness (ILI) and to the severity of the ILI, the following values were found:

- 80%, 68% and 64% against A/H3N2, A/H1N1 and B antigen, respectively, among non-infected volunteers (40% of them were protected against all vaccine antigens, Figure 3a);
- 50% (7/15) among infected volunteers without ILI;
- 10% (1/9) among volunteers with mild infections;
- 20% (1/5) among volunteers with severe infections (Figure 3b).

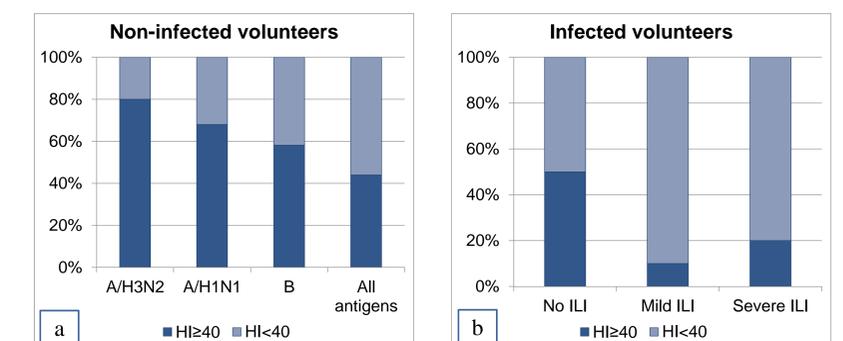


Figure 3. Percentages of seroprotected volunteers among non-infected (a) and infected people (b) sorting by absence or presence of ILI and by severity of ILI.