Chapter 10

Antibody Responses after Influenza Vaccination in Elderly People: Useful Information from a 27-Year Study (from 1988–1989 to 2014–2015)

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Additional information is available at the end of the chapter

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Abstract

Elderly people are more likely than younger people to get flu complications and respond suboptimally to influenza vaccination because of the presence of comorbidities and immunosenescence. In order to collect information about this issue, we evaluated data obtained in 27 winters of study, from 1988–1989 to 2014–2015, in frail elderly institutionalized people (≥60 years) vaccinated with commercially available seasonal trivalent inactivated influenza vaccines. The antibody response was examined comparing hemagglutination inhibition antibody titers in sera collected from 4461 volunteers before and 30 days after vaccination. Examining the results as crude mean responses, we evidenced the ability of influenza vaccines to induce significant increases in antibody titers against all the three vaccine antigens satisfying at least one of the three criteria of the Committee for Medical Products for Human Use (CHMP). Higher responses were found against A/H3N2 vaccine components and, examining different subgroups, in volunteers receiving 45 μg vaccine as compared with 30 μg and in female as compared with male subjects. Very elderly people (>75 years) gave better responses than younger elderly (≤75 years) at least against A/H1N1 strain and the last licensed potentiated vaccines (MF59-adjuvanted and intradermal) were more immunogenic than traditional vaccines (whole, subunit, and split).

Keywords: influenza vaccination, vaccine immunogenicity, HI antibody titers, CHMP criteria, elderly institutionalized people

1. Introduction

Influenza virus infections can affect all age groups, and older individuals are particularly at risk for influenza since, despite having no higher attack rate than younger adults, most influenza-related deaths and severe complications occur in this age group. Although influenza vaccination remains the mainstay in prevention, nonetheless, uncertainties regarding the effectiveness of the influenza vaccines in elderly adults are persistent [1, 2].

The higher rate of flu complications and the reduced vaccine efficacy are generally attributed to both concomitant comorbidities and immunosenescence, i.e., the age-related weakening of the immune system [3, 4].

As reported by Lambert et al. [5], the measurement of vaccine efficacy against influenza illness is a difficult task especially in older adults. Although influenza vaccine effectiveness depends not only on vaccine-induced immune response but also on annual variations in influenza incidence, circulating strain virulence, and the quality of the vaccine-to-circulating strain match [6], previous studies have established that a high serum antibody level can prevent infection at least in children and young adults [7–9], and serological studies based on the evaluation of influenza-specific antibody titers have been widely accepted and used as a surrogate marker for protection against influenza and vaccine efficacy.

Chronic underlying diseases, particularly cardiac and respiratory diseases, were shown to negatively influence the immune response after influenza vaccination in old people [10].

Three previous reviews on serological responses to inactivated seasonal vaccines in elderly people did not consider the possible role of chronic underlying illnesses, because there was not the possibility of controlling for the presence of serious illnesses [11] or because the elderly population was carefully selected to exclude any chronic diseases so that the results would reflect the effect of ageing on comparison with young people [12, 13].

In comparison with community-dwelling elderly people, residents of nursing homes are considered to be at a higher risk of serious influenza-related complications, because they are generally older, more debilitated, and more exposed to influenza infection once the virus is introduced because of the close environment in which they live [14]. However, evaluating vaccine immunogenicity, results reported in the review of Goodwin et al. [12] and results previously obtained in our laboratory [15] suggested that institutionalized elderly responded better when compared with community-dwelling elderly.

The aim of this chapter of the book is to examine the phenomenon of the decreased immunogenicity and efficacy of influenza vaccines in older persons from available data. We examined the data obtained by our research group in 27 winter seasons, from 1988–1989 to 2014–2015, of vaccine immunogenicity in a considerable number (4461) of elderly people (≥60 years of age), most of them with underlying medical conditions, vaccinated with commercially available seasonal trivalent inactivated influenza vaccines. Although some of the results obtained in the different winters were previously published, in the present report the results we obtained are cumulatively examined for the first time.

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2. Materials and methods

2.1. Study design and vaccination

The volunteers initially enrolled in the prospective study of antibody response to influenza vaccination, conducted over a period of 27 consecutive winters, were 4461 elderly people, aged ≥60 years (mean age 80.5 year, range 60–106 years). Eighty-six percent of them were living in nursing homes in Central Italy.

After providing informed consent, all the subjects received one dose of trivalent inactivated influenza vaccine intramuscularly, in the deltoid, or intradermally. The vaccines used were commercially available inactivated trivalent vaccines for the winters from 1988–1989 to 2014–2015 produced by propagation of the virus in embryonated hens' eggs. Each dose of vaccine consisted of 10 μg (from 1988–1989 to 1991–1992) or 15 μg of hemagglutinin (HA) in a 0.5 ml dose (for vaccines administered intramuscularly) or in a 0.1 ml dose (for vaccines administered intradermally) for each of the three influenza strain antigens (A/H3N2, A/H1N1, and B influenza viruses). At the time of recruitment of this study, demographic data, health status, and history of influenza vaccination over the preceding year were obtained from each subject. Serum samples were obtained from the same subject before and 1 month after vaccination. Subjects were included in this study if they did not have a history of immediate hypersensitivity to eggs components. Subjects suffering from specific illnesses or chronic condition were not excluded. The study was conducted according to the Declaration of Helsinki and Good Clinical Practices. Since vaccines were assigned by local health authorities within the annual influenza campaign and sera were leftover sera from samples collected for clinical routine controls, the study did not need to be registered as a formal trial.

2.2. Determination of hemagglutination-inhibiting (HI) antibody titers and measurement results

HI antibody titers were determined using a standard microtiter method [16] with 0.5% chicken (from 1988–1989 to 1996–1997) or turkey erythrocytes (after 1996–1997). Antigens were prepared from the allantoic fluids of embryonated hens' eggs inoculated 3 days earlier with influenza virus. All sera were heat-inactivated at 56°C for 30 min and treated with potassium periodate and trypsin (from 1988 to 1994) or with receptor-destroying enzyme (RDE) of *Vibrio cholerae* (after 1994) to remove nonspecific inhibitors. The first dilution for antibody titration was 1:10. Pre- and postvaccination sera from each of the vaccines were frozen at −30°C until used and tested simultaneously for HI antibody titers using the same antigens as those in the vaccine. To eliminate any subjective bias, HI titers determinations were carried on in a blind fashion, i.e., with the tester unaware of which treatment the donor had received.

2.3. Criteria used for evaluating vaccines immunogenicity

HI antibody titers obtained by following the procedure indicated in the previous section were reported as protection rate (percentage of volunteers showing HI titers ≥40, considered to be associated with protection from influenza infection) [9], geometric mean titers (GMT;

any HI antibody titer <10 was considered equal to 5 for GMT calculation), ratio of postvaccination to prevaccination GMT values (GMTR), and seroconversion rate (percentage of subjects with a fourfold or greater increase in titer and with a postvaccination titer at least equal to 40 in seronegative volunteers). The antibody titers measured 1 month after vaccination were also evaluated according to the criteria of the Committee for Medicinal Products for Human Use (CHMP) for approval of influenza vaccines, which require that for individuals aged ≥60 years at least one of the following values must be met: seroprotection rate ≥60%, GMTR ≥2, or seroconversion rate ≥30% [17].

2.4. Statistical analyses

Statistical analyses and subanalyses considered in this work were applied to populations with a relatively large number of people, as a consequence both GMT and rate statistics were well approximated by a log normal and normal distributions, respectively. Moreover, since rates values were not close to 0 or 100%, thus significant differences between mean values of the groups were analyzed by Student's *t*-test. Both estimated mean values with their corresponding 95% confidence intervals (CI) the p-value of the *t*-statistic have been reported in the paper. In particular, *p*-values <0.01 were considered highly statistically significant, whereas *p*-values <0.05 were regarded as marginally statistically significant. Values of postvaccination GMT observed against different antigens and in different years were examined as such and also corrected for prevaccination status according to Beyer et al. [18] in order to verify that significant differences in the postvaccination status were independent on the prevaccination HI titers. Vaccine response was evaluated also according to the dosage of vaccine antigens (30 and 45 μg), gender, and age (≤75 and >75). For each antigen, significant differences between subpopulations means were evaluated and the corresponding statistical significance was indicated.

A multiple comparison test between groups of vaccine type and between antigens was executed by using one-way analysis of variance (ANOVA). Paired comparison values were presented only when one-way ANOVA comparison identified potentially significant differences. All statistical analyses were carried out using MATLAB® of MathWorks Inc. release 2014b.

3. Results

3.1. Study population and demographic characteristics

Table 1 reports the baseline characteristics of the 4461 elderly volunteers, aged ≥60 years (range 60–106) vaccinated with commercially available seasonal trivalent inactivated influenza vaccines for each year of the 27 consecutive winters (from 1988–1989 to 2014–2015) studied. The number of volunteers examined each year varied from 64 to 372. The mean age was lower in the first years studied (from 1988–1989 to 1998–1999) when a mixed population of community-dwelling and institutionalized elderly was examined (60–80 years) than in the other seasons when volunteers were totally recruited from nursing homes (82–86 years). The

majority of elderly subjects has been previously vaccinated (61–100%). Although not reported in **Table 1**, percentage of volunteers, ≥80%, presented underlying diseases or risk factors for influenza and as a consequence used chronic drugs. The most frequent chronic diseases were cardiovascular, respiratory diseases, and diabetes. The most frequent drugs used were antihypertensive/inotropic drugs and benzodiazepines.

					Type of seasonal vaccine used[a]					
Season	**No. of subjects**	**Mean age (range)**	**Living situation[b]**	**Vaccination status prior to study[c]**	**Whole**	**Sub-u**	**Split**	**MF59**	**ID**	**Vaccine dosage**
1988–1989	282	73 (61–93)	M	na	232	50	–	–	–	30 μg
1989–1990	82	69 (60–83)	M	88%	–	59	23	–	–	30 μg
1990–1991	372	66 (60–87)	M	69%	159	213	–	–	–	30 μg
1991–1992	124	69 (60–93)	M	61%	108	–	16	–	–	30 μg
1992–1993	270	nd (>60)	M	96%	245	8	17	–	–	45 μg
1993–1994	298	76 (60–99)	M	na	51	–	247	–	–	45 μg
1994–1995	235	78 (60–100)	M	90%	32	–	203	–	–	45 μg
1995–1996	213	77 (60–100)	M	90%	–	213	–	–	–	45 μg
1996–1997	173	80 (60–99)	M	96%	–	173	–	–	–	45 μg
1997–1998	176	na (>60)	M	85%	36	140	–	–	–	45 μg
1998–1999	116	74 (60–102)	M	94%	–	110	6	–	–	45 μg
1999–2000	139	83 (60–103)	I	96%	–	46	78	15	–	45 μg
2000–2001	128	83 (60–103)	I	100%	–	82	46	–	–	45 μg
2001–2002	96	82 (60–104)	I	98%	–	–	96	–	–	45 μg
2002–2003	107	82 (60–105)	I	100%	–	–	107	–	–	45 μg
2003–2004	125	83 (60–101)	I	100%	–	–	33	92	–	45 μg
2004–2005	158	82 (60–99)	I	98%	–	–	36	122	–	45 μg
2005–2006	105	83 (60–99)	I	100%	–	–	40	65	–	45 μg
2006–2007	88	83 (60–98)	I	98%	–	–	21	67	–	45 μg
2007–2008	66	84 (61–102)	I	100%	–	–	–	66	–	45 μg
2008–2009	114	83 (60–103)	I	98%	–	–	–	114	–	45 μg

					Type of seasonal vaccine used[a]					
Season	**No. of subjects**	**Mean age (range)**	**Living situation[b]**	**Vaccination status prior to study[c]**	**Whole**	**Sub-u**	**Split**	**MF59**	**ID**	**Vaccine dosage**
2009–2010	64	83 (65–98)	I	100%	–	–	–	64	–	45 μg
2010–2011	112	85 (64–101)	I	100%	–	–	–	112	–	45 μg
2011–2012	151	84 (65–102)	I	98%	–	–	–	103	48	45 μg
2012–2013	252	85 (60–103)	I	100%	–	–	26	137	89	45 μg
2013–2014	204	86 (60–106)	I	100%	–	–	–	183	21	45 μg
2014–2015	211	84 (60–104)	I	100%	–	–	1	203	7	45 μg
Total	4461	85 (60–106)			863	1094	996	1343	165	

[a]Whole: whole-virus vaccine; Sub-u: sub-unit vaccine; Split: split-virus vaccine; MF59: subunit MF59-adjuvanted vaccine; ID: Intradermal subunit vaccine.
[b]I: Institutionalized elderly; M: mixed, both institutionalized and community living elderly.
[c]Percent of elderly having received influenza vaccination in the previous year.
na: not available

Table 1. Characteristics of studied population and type of influenza vaccines in the 27 winter seasons studied (from 1988/1989 to 2014/2015).

3.2. Vaccines

As reported in **Table 1**, different formulations such as whole, split (composed by viruses disrupted, by a detergent, and containing the internal and external component of the virus), and subunit (composed of just the purified surface glycoproteins of the virus, i.e., hemagglutinin (HA) and neuraminidase) of trivalent inactivated vaccines were used in the different years or in the same year. In the first four studied years (from 1988–1989 to 1991–1992), the HA concentration for each strain was lower (10 μg for each antigen) as compared with the concentration (15 μg for each antigen) of the vaccines used in all the years after the winter season 1991–1992. Whole and subunit formulations were administered respectively to 863 and 1094 volunteers in the first 13 years of the study (from 1988–1989 to 2001–2002). Nine hundred ninety-six elderly people were vaccinated with split vaccine in many years studied and, starting from the 1999–2000 season, 1343 volunteers received a subunit vaccine potentiated with MF59 adjuvant. In the last period of the study, a limited number of elderly people was vaccinated with vaccine administered intradermally (165 volunteers from 2011–2012 to 2014–2015). The percentages of previously influenza-vaccinated people were high and ranged from 88 to 100%, not considering 3 years (1990–1991, 69%; 1991–1992, 61%, and 1993–1994, data not available).

The antigenic composition of the vaccines used is reported in **Figure 1** and each year was formulated according to the recommendations of both "Ministero della Salute (Italy)" and

WHO (Northern Hemisphere) for the corresponding studied winter. During the 27-year period covered by our study (1988–2014), the WHO recommended 15 A/H3N2, 7 A/H1N1, and 12 B new influenza strains for inclusion in seasonal vaccines.

3.3. Overall response to influenza vaccination

The ability of licensed influenza vaccines to elicit an antibody response against vaccine antigens was examined comparing HI antibody titers in blood samples collected from the 4461 volunteers before and 1 month after vaccination with commercially available seasonal trivalent inactivated influenza vaccines in 27 consecutive winters (from 1988–1989 to 2014–2015).

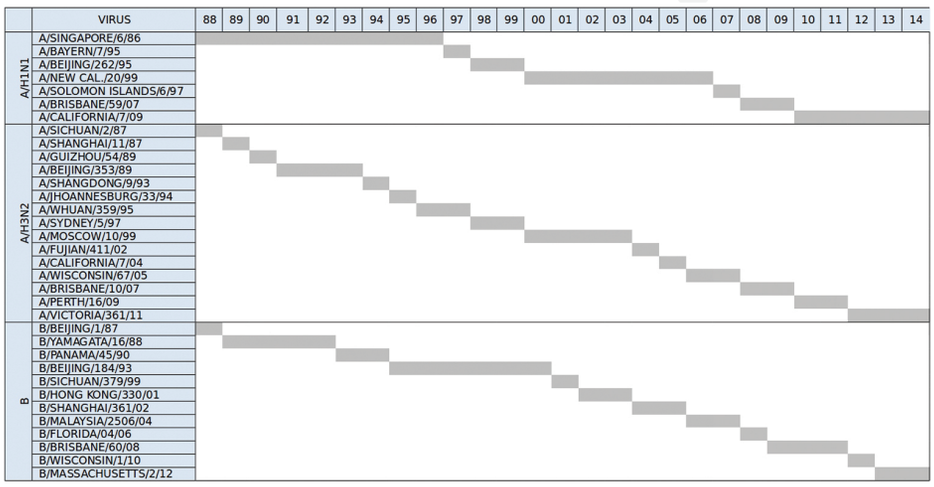

Figure 1. Recommended viruses for influenza vaccines by World Health Organization between 1988 and 2014.

Vaccine component (*N* = 4461)	Seroprotection rate (95% CI)		Seroconversion rate (95% CI)	GMT (95% CI)		Number of reached CHMP criteria/3
	Prevacc.	Postvacc.		Prevacc.	Postvacc. [GMTR]	
A/H3N2	35.1 [A,B]	65.7** [A,B]	30.0** [A,B]	20.9 [A,B]	54.6** [A,B] [2.6]	3/3
	(33.7–36.5)	(64.3–67.1)	(28.5–31.2)	(20.2–21.6)	(52.5–56.8)	
A/H1N1	23.5	52.6**	25.1	14.2	35.3 ** [2.5]	1/3
	(22.2–24.7)	(51.1–54.1)	(23.8–26.0)	(13.7–14.6)	(34.0–36.7)	
B	23.3	54.5**	25.6	14.5	35.7 ** [2.5]	1/3
	(22.1–24.5)	(53.0–55.9)	(24.3–27.1)	(14.1–14.9)	(34.5–37.0)	

**: *p*-value < 0.01 comparing pre- and postvaccination values.
A: *p*-value <0.01 comparing A/H3N2 and A/H1N1 antigens.
B: *p*-value <0.01 comparing A/H3N2 and B antigens.

Table 2. Mean values of the HI antibody responses observed in the 27-years study of the total population to the three influenza vaccine antigens and reachment CHMP criteria.

The HI antibody response after one dose of influenza vaccine was evaluated for each antigen (A/H1N1, A/H3N2, and B) and data obtained were processed in order to calculate, for each population considered in the paper, pre- and postvaccination seroprotection rate, seroconversion rate, pre- and postvaccination GMT, and GMTR together to their corresponding 95% confidence intervals. For each antigen, the values of these parameters referred to the overall population are reported in **Table 2**. 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 three different vaccine antigens. The three CHMP requirements were satisfied 1 month after vaccination against the A/H3N2 vaccine component, whereas only the requested value of GMTR was reached against the A/H1N1 and B antigens.

Table 3 reports the results obtained examining the reachment of the CHMP criteria for each studied year against the three vaccine antigens. The seroprotection rate (HI titer ≥40) was higher than the requested 60% in 20 years against A/H3N2 (74%), 16 years against A/H1N1 (59%), and 14 years against B antigen (52%) of the 27 years studied. Values of GMTR satisfying the requested value ≥2 were found in 22 (81%), 25 (93%), and 21 (78%) years against A/H3N2, A/H1N1, and B vaccine components, respectively. The lower positive results were found for seroconversion requested to be ≥30%. This value was reached in 13 years against A/H3N2 (48%), 10 years against A/H1N1 (37%), and 8 years against B virus (30%). In some years none of the three CHMP criteria was satisfied, i.e., in 3 years against A/H3N2 (11%), 2 years against A/H1N1 (7%), and in 7 years against the B antigen (26%). Years with responses satisfying all the three CHMP criteria ranged between 22% (B antigen) and 48% (A/H3N2 antigen). Because the use of a vaccine featuring a novel antigen might affect the antibody response, considering data reported about vaccine antigenic composition in **Figure 1**, we identified the presence or absence of a novel vaccine component in each year studied, but we could not evidence any obvious association between vaccine HI antibody response and the presence of a new vaccine component.

Vaccine component	**N. of years (%) [95% CI]**			
	Seroprotection ≥60%	**Seroconversion ≥30%**	**GMTR ≥2**	**Reachment of three CHMP criteria**
A/H3N2	20 (74%) [55–93]	13 (48%) [29–67]	22 (81%) [67–96]	13 (48%)
A/H1N1	16 (59%) [41–78]	10 (37%) [18–56]	25 (93%) [78–107]	8 (30%)
B	14 (52%) [33–71]	8 (30%) [11–48]	21 (78%) [64–92]	6 (22%)

Table 3. Reachment of the CHMP criteria in the total population in the 27 years examined.

The data reported in **Table 2** evidenced differences in the values of the HI antibody titers against the three different vaccine antigens. HI antibody values against A/H3N2 antigen were in most instances significantly higher before and after vaccination as compared with those found both against A/H1N1 and B vaccine components.

Since the baseline serological status is considered to be important in evaluating immunogenicity of influenza vaccines and is regarded as capable of affecting the serological outcomes, in order to reduce the heterogeneity among the responses found against the three vaccine antigens, we examined the GMT values of the overall population correcting the postvaccination titers for the prevaccination status according to Beyer (**Figure 2**) [18].

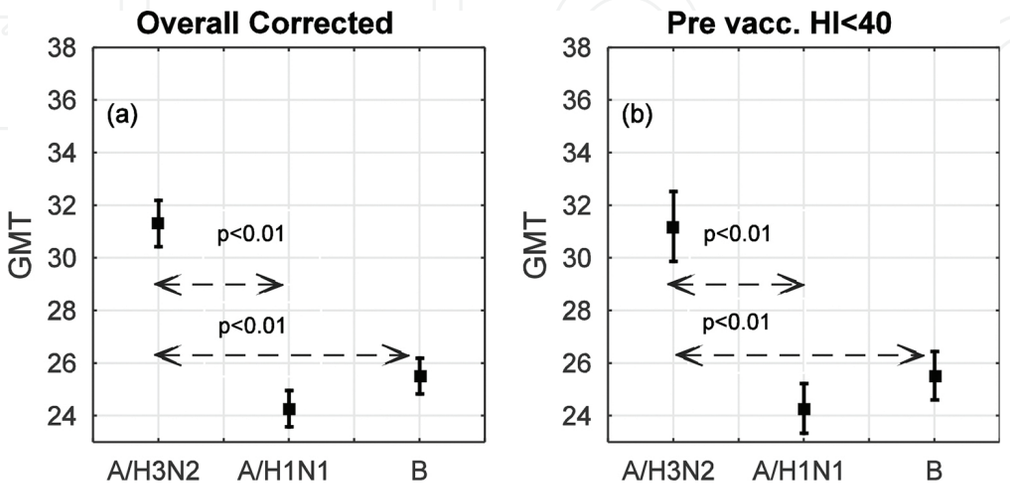

Figure 2. Postvaccination GMT values of: (a) the overall population corrected for the average prevaccination status according to Beyer; and (b) subjects unprotected before vaccination. Comparison of antigens is also shown when differences are significant. The bars indicate the ranges of the 95% confidence limits.

For comparison purposes, also the postvaccination GMT values of the prevaccination unprotected volunteers (HI < 40) are shown in **Figure 2** as indicated by the corresponding labels. The data reported confirmed that the responses against the A/H3N2 antigen were higher as compared with those against A/H1N1 and B antigens.

3.4. Factors associated with vaccine response

Since different factors may have an impact on vaccine response, we controlled for a number of variables for which we could obtain data. We did not consider the health status of the study participants, previous vaccination histories, and living situation, since a high percentage of the subjects had chronic underlying disease, was previously vaccinated, and was living in a nursing home.

3.4.1. Subanalysis according to different influenza vaccine dosages

In Italy, as in most European countries, seasonal trivalent influenza vaccines containing 10 μg HA for each antigen (30 μg) has been used until 1991. From 1992 onwards European influenza vaccines contain 15 μg HA per strain (45 μg), according to the European Harmonization of Requirements for Influenza Vaccines [17]. As a consequence, in the first 4 years of the 27-year period examined in our study, we used 30 μg and, after the winter 1991–1992, 45 μg vaccines.

Since previous observations suggested that increase in influenza vaccine dosage might be associated with an increase in antibody titers, at least against some of the vaccine strains [19, 20], we compared HI immune response following vaccination with 30 or 45 μg vaccines. As reported in **Table 1**, 860 (19%) and 3601 (81%) of the 4461 elderly subjects received respectively a 30 or a 45 μg trivalent influenza vaccine. **Table 4** reports the results obtained studying the induced HI antibody response. Significant increases were observed against all the three vaccine antigens comparing pre- and postvaccination data against all the three different vaccine antigens examining the percentages of seroprotected people and GMT values both after 30 and 45 μg vaccine administration.

Vaccine component	Vaccine dose (N)	Seroprotection rate		Seroconversion rate (95% CI)	GMT (95% CI)		
		Prevacc.	Postvacc.	(95% CI)	Prevacc.	Postvacc. [GMTR]	CHMP criteria satisfied
A/H3N2	30 μg	14.1 [A]	39.3**[A]	16.9 [A]	13.2 [A]	26.3**[A] [2.0]	1/3
	(860)	(11.7–16.4)	(36.0–42.6)	(14.5–19.4)	(12.4–14.0)	(24.6–28.0)	
	45 μg	40.1	72.0**	32.9	23.3	65.1** [2.8]	3/3
	(3601)	(38.5–41.7)	(70.5–73.5)	(31.4–34.4)	(22.4–24.3)	(62.3–68.0)	
A/H1N1	30 μg	10.2	35.7**[A]	20.8 [A]	9.5 [A]	22.5**[A] [2.4]	1/3
	(860)	(8.2–12.3)	(32.5–38.9)	(18.1–23.5)	(8.9–10.0)	(21.0–24.2)	
	45 μg	26.7	56.6**	26.1	15.6	39.4** [2.5]	1/3
	(3601)	(25.2–28.1)	(55.0–58.3)	(24.6–27.6)	(15.0–16.2)	(37.7–41.1)	
B	30 μg	5.1	30.0**[A]	21.2 [A]	8.1 [A]	19.9**[A] [2.5]	1/3
	(860)	(3.6–6.6)	(26.7–33.1)	(18.4–24.1)	(7.7–8.5)	(18.5–21.3)	
	45 μg	27.6	60.3**	26.6	16.6	41.1** [2.5]	2/3
	(3601)	(26.2–29.1)	(58.7–61.9)	(25.2–28.0)	(16.1–17.2)	(39.6–42.7)	

**: *p*-value <0.01 comparing pre- and postvaccination values.
A: *p*-value <0.01 comparing response between vaccine dosages (30 and 45 μg).

Table 4. HI antibody response in volunteers divided according to the vaccine dosage (30 or 45 μg).

At least one of the three CHMP requirements, i.e., the value of GMTR (≥2), was always reached using vaccine containing 30 μg of antigen but following 45 μg vaccine administration all the three parameters were satisfied against A/H3N2 antigen and two of them against the B antigen.

Postvaccination results observed after 45 μg vaccine administration were always significantly higher as compared with those after 30 μg vaccine.

However, comparing values found in the two groups of people before vaccination, we observed that the two groups were poorly comparable since there were differences in the prevaccination

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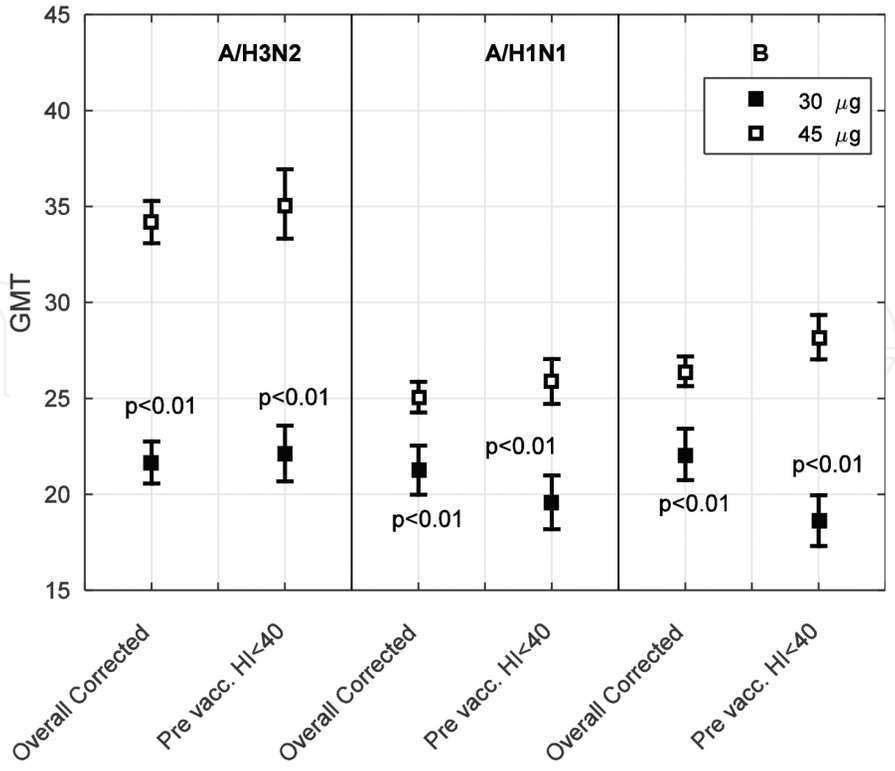

Figure 3. Postvaccination GMT values of populations divided according to the vaccine dosage (30 or 45 μg)), as indicated by legend labels. Postvaccination GMT values calculated on the overall population have been corrected for the average prevaccination status according to Beyer. For comparison purposes, post-GMT values of subjects unprotected before vaccination are also shown. The bars indicate the ranges of the 95% confidence limits.

status. Volunteers vaccinated with 45 μg vaccine showed prevaccination HI titers in most instances significantly higher as compared with the 30 μg volunteers. In order to have more homogeneous and comparable data, we examined vaccine immunogenicity both correcting the titers for prevaccination status of overall population [17], and considering only prevaccination unprotected volunteers (HI titers < 40). As shown in **Figure 3**, GMT corrected for prevaccination status confirmed that the increasing of the antigen dosage increments the response to the vaccine antigens. Postvaccination values found considering only people nonseroprotected before vaccination again evidenced a statistically significant higher response induced by 45 μg vaccine as compared with 30 μg.

3.4.2. Subanalysis of immunogenicity within the elderly groups, i.e., younger elderly (≤75 years) and very elderly (>75 years)

In a recent meta-analysis about the effect of age on the influenza vaccine–induced immune response based on studies from the past 20 years, Goodwin at al. [12] concluded that aged individual (>65 years) had a significantly reduced antibody response to vaccination. The studied elderly were categorized into two age groups, above or below 75 years. Antibody responses among the very elderly (≥75 years of age) were especially impaired with seroconversion levels at 32%, 46%, and 29% to A/H1N1, A/H3N2, and influenza B, respectively, compared with 42%, 51%, and 35% observed in people aged <75 to >65 years of age [12].

In order to have additive information we considered the immune responses found in volunteers of our study aged ≤75 or >75 years. The exact age was available for only 2712 people (61%) of the 4461 participants and 658 (24%) were aged ≤75 years and 2054 (76%) were >75 years. The results obtained are reported in **Table 5** and show that in both groups the vaccine administration induced significant increases in HI titers evaluated as percentage of seroprotected people (HI ≥ 40) and as GMT values. CHMP criteria were always satisfied for GMTR parameter (≥2) against all the three vaccine antigens. All the three requested values were reached in both groups against A/H3N2 antigen and only in >75 year group against A/H1N1 antigen. Against the B antigen, the requested value for seroconversion (≥30%) was not reached in both groups and the value for seroprotection (≥60%) was satisfied only in >75-year group.

Vaccine component	Group (*N*)	Seroprotection rate (95% CI)		Seroconversion rate (95% CI)	GMT (95% CI)		
		Prevacc.	Postvacc.		Prevacc.	Postvacc. [GMTR]	CHMP criteria satisfied
A/H3N2	≤75	28.5 [A]	60.7 **[A]	31.8	16.5 [A]	46.9 **[A] [2.8]	3/3
	(658)	(24.1–30.9)	(28.2–35.4)	(56.9–64.4)	(15.1–17.9)	(42.5–51.7)	
	Age >75	40.1	71.4 **	34.4	23.5	66.7 ** [2.8]	3/3
	(2054)	(37.9–42.2)	(69.4–73.3)	(32.4–36.4)	(22.3–24.8)	(62.7–70.9)	
A/H1N1	≤75	24.6	52.6 **[A]	24.5 [A]	14.2 [a]	35.6 **[A] [2.5]	1/3
	(658)	(21.3–27.9)	(48.7–56.4)	(21.2–27.8)	(13.0–15.4)	(32.3–39.3)	
	Age >75	26.9	60.3 **	29.8	15.7	42.5 ** [2.7]	3/3
	(2054)	(25.0–28.8)	(58.2–62.4)	(27.8–31.8)	(15.0–16.5)	(40.1–44.9)	
B	≤75	254.6[A]	54.3 **[A]	26.7	14.3 [A]	36.8 **[A] [2.6]	1/3
	(658)	(21.3–27.9)	(50.4–58.0)	(23.4–30.0)	(13.2–15.5)	(33.4–40.5)	
	Age >75	30.6	62.8 **	26.4	18.0	42.6 ** [2.4]	2/3
	(2054)	(28.6–32.6)	(60.7–64.9)	(24.5–28.3)	(17.2–18.8)	(40.5–44.9)	

**: *p*-value <0.01 comparing pre- and post-vaccination values.
A: *p*-value <0.01 comparing response between age groups.
a: *p*-value <0.05 comparing response between age groups.

Table 5. HI antibody response of populations divided according to the age (younger elderly, ≤75 years, and very elderly, >75 years).

Comparing results obtained in the two groups, the responses observed in the oldest group (>75) were in most instances higher than those observed in the younger elderly (≤75). However, since the prevaccination status of these two groups were not fully comparable, we evaluated the values of GMT corrected for prevaccination status and GMT in people unprotected (HI < 40) before vaccination. Again the values were higher in the very elderly as compared with the

younger against A/H1N1 for GMT corrected and against A/H1N1 and B for the GMT unprotected people (**Figure 4**).

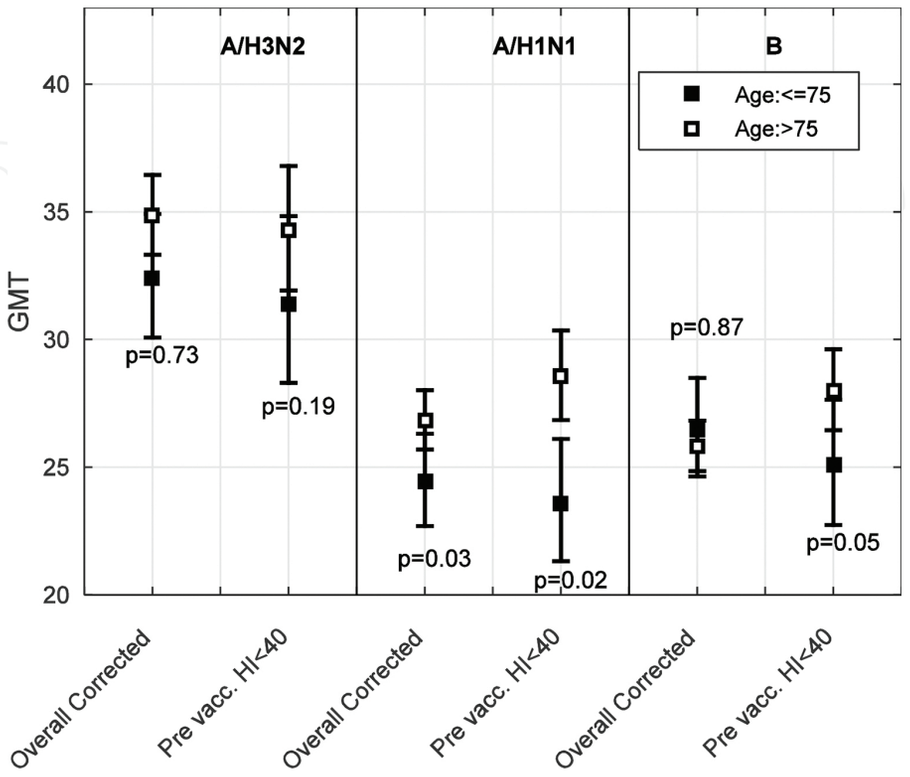

Figure 4. Postvaccination GMT values of populations divided according to the age class (younger elderly, ≤75 years, and very elderly, >75 years), as indicated in legend labels. Postvaccination GMT values calculated on the overall population have been corrected for the average prevaccination status according to Beyer. For comparison purposes, post-GMT values of subjects unprotected before vaccination are also shown. The bars indicate the ranges of the 95% confidence limits.

3.4.3. Subanalysis according to responses found in females and males

Previous data indicated that receipt of trivalent inactivated influenza vaccines results in significantly higher HI antibody titers among females than males, both in adults and elderly people [21].

In our study, sex data were available for about all the people studied (4457/4461) and the volunteers were prevalently females (70%). We examined the vaccine immunogenicity in females and males and the results are reported in **Table 6**. Postvaccination increases found against all the three vaccine antigens were statistically significant in both groups. All the three CHMP criteria were satisfied against A/H3N2 antigen in female subjects, whereas only the GMTR requirement was satisfied in males against A/H3N2 and both in males and females against A/H1N1 and B antigens. Comparison of postvaccination values evidenced statistically higher values in the female compared with male group. However, since differences were found also in the prevaccination values we compared the GMT corrected for the prevaccination status and examined the GMT found considering only volunteers not seroprotected before vaccina-

tion. The female responses were again higher than those of male against all the three vaccine antigens (**Figure 5**).

Vaccine components	Group (*N*)	Seroprotection rate (95% CI)		Seroconversion rate (95% CI)	GMT (95% CI)		EMA criteria satisfied
		Prevacc.	Postvacc.		Prevacc.	Postvacc. [GMTR]	
A/H3N2	F	36.9 [A]	68.7 **[A]	32.7 [A]	21.7 [A]	60.0 **[A] [2.8]	3/3
	(3142)	(35.2–38.5)	(67.1–70.3)	(31.0–34.4)	(20.8–22.6)	(57.3–62.9)	
	M	30.9	58.6 **	23.1	19.1	43.8 ** [2.3]	1/3
	(1315)	(28.4–33.4)	(55.9–61.3)	(20.8–25.4)	(17.9–20.4)	(40.9–46.9)	
A/H1N1	F	24.1	55.3 **[A]	27.7 A	14.4	38.0 **[A] [2.6]	1/3
	(3142)	(22.6–25.6)	(53.6–57.1)	(26.2–29.2)	(13.9–15.0)	(36.3–39.8)	
	M	22.1	46.2 **	18.7	13.6	29.8 ** [2.2]	1/3
	(1315)	(19.8–24.3)	(43.5–48.9)	(16.5–20.9)	(12.8–14.4)	(27.8–31.8)	
B	F	24.9 [A]	56.9 **[A]	26.7 [A]	15.2 [A]	38.2 **[A] [2.5]	1/3
	(3142)	(23.4–26.5)	(55.2–58.6)	(25.2–28.2)	(14.6–15.7)	(36.6–39.9)	
	M	19.3	48.6 **	22.7	13.0	30.5 ** [2.4]	1/3
	(1315)	(17.2–21.5)	(45.8–51.3)	(20.5–25.4)	(12.3–13.7)	(28.7–32.4)	

**: *p*-value <0.01 comparing pre- and post-vaccination values.
A: *p*-value <0.01 comparing response between M and F.

Table 6. HI antibody response of populations divided according to gender (male: M; female: F).

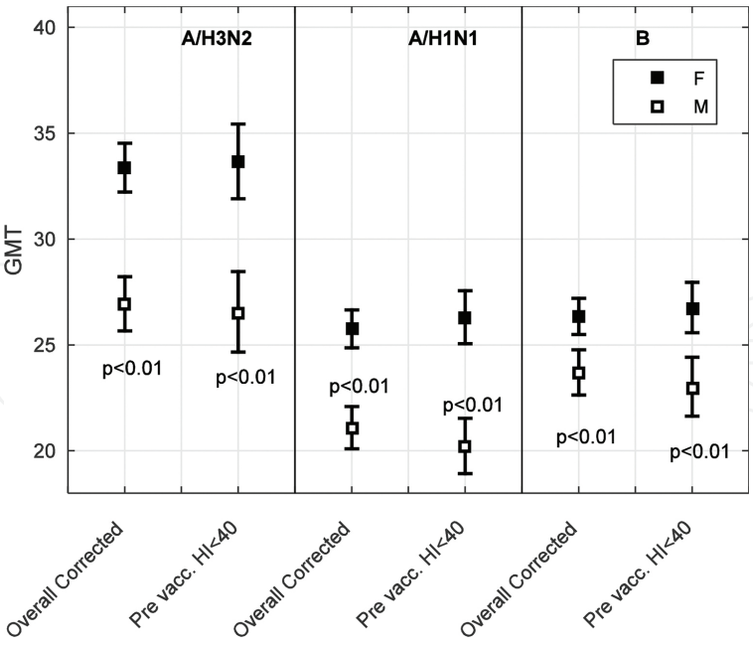

Figure 5. Postvaccination GMT values of populations divided according to gender (male: M and female: F) as indicated in legend labels. Postvaccination GMT values calculated on the overall population have been corrected for the average prevaccination status according to Beyer. For comparison purposes, post-GMT values of subjects unprotected before vaccination are also shown. The bars indicate the ranges of the 95% confidence limits.

3.4.4. Evaluation of vaccine immunogenicity in "strong responder"

Examining the antibody response after influenza vaccination, McElhaney et al. [22] considered as a vaccination efficiency-related parameter the HI antibody titer ratio between day 30 and day 0 and identified as weak/nonresponder people with a ratio 1– < 4 and as strong responders those with a ratio ≥4, i.e., people who seroconverted after vaccination. Using the same parameter we decided to evaluate in the groups identified as strong responders the induction of HI antibody response evaluated as GMT values against the three vaccine antigens.

The data obtained comparing results found in people who seroconverted after vaccination are reported in **Table 7**, and in most instances confirmed the results obtained examining the overall population of subgroups vaccinated with vaccine containing different dosages of antigens or subdivided in male and female. The responses induced by a 45 μg vaccine or in female were in most instances statistically higher than those induced by a 30 μg vaccine or in male volunteers, respectively. Moreover, the immune responses evaluated in volunteers with an age ≤ or >75 years were similar against A/H1N1 and B antigens and higher against the A/H3N2 antigen in people aged >75 years as compared with response in those ≤75 years.

Group	A/H3N2			A/H1N1			B		
	N (total)	GMT post [GMTR] (95% CI)	Corrected GMT (95% CI)	*N* (total)	GMT post [GMTR] (95% CI)	Corrected GMT (95% CI)	*N* (total)	GMT post [GMTR] (95% CI)	Corrected GMT (95% CI)
30 μg	146	84.0 [A] [7.6]	82.9 [A]	179	76.8 [A] [10.0]	86.81[A]	182	83.8 [A] [9.7]	94.1 [A]
	(860)	(77.2–91.5)	(74.9–91.86)	(860)	(71.4–82.7)	(80.3–93.85)	(860)	(77.9–90.1)	(86.9–101.9)
45 μg	1185	164.7 [9.0]	133.6	940	127.4 [9.8]	126.1	959	110.2 [8.4]	118.9
	(3601)	(155.3–174.6)	(125.–142.7)	(3601)	(120.0–135.3)	(118.5–134.1)	(3601)	(104.3–116.4)	(112.1–126.3)
≤75	209	127.7 [A] [9.2]	112.9 [A]	161	123.0 [10.0]	121.6	176	113.4 [9.1]	122.7
	(658)	(111.4–146.4)	(99.3–128.3)	(658)	(106.1–142.6)	(104.7–141.2)	(658)	(98.6–130.4)	(105.3–143.1)
>75	707	183.3 [9.2]	146.2	612	127.6 [9.7]	128.35	543	112.8 [8.1]	117.3
	(2054)	(169.6–198.1)	(133.5–159.9)	(2054)	(118.7–137.2)	(119.3–138.2)	(2054)	(104.9–121.2)	(108.6–126.7)
F	1027	162.1 [A] [8.9]	130.3 [a]	872	120.3 [9.9]	121.6 [A]	842	113 [a] [8.9]	120.9 [A]
	(3142)	(152.2–172.5)	(121.7–139.6)	(3142)	(113.3–127.8)	(114.4–129.3)	(3142)	(106.7–119.6)	(113.8–128.4)
M	304	125.9 [8.4]	109.4	246	108.4 [9.3]	100.1	299	86.9 [7.8]	93.9
	(1315)	(113.4–139.7)	(97.1–123.1)	(1315)	(96.9–121.3)	(90.6–110.4)	(1315)	(79.9–94.4)	(86.3–102.2)

**: *p*-value <0.01 comparing pre- and postvaccination values.
A: *p*-value <0.01; a: *p*-value <0.05 comparing response between different groups.

Table 7. HI antibody response of strong responder population divided according to the vaccine dosage (30 or 45 μg), age class (younger elderly, μ75 years, and very elderly, >75 years), and gender (male: M; female: F).

3.4.5. Subanalysis according to the different types of vaccine used

Finally, since different vaccine formulations (whole, subunit, split, MF59-adjuvanted, and intradermally administered) were used in the 27 years studied, we compared the results obtained after administration of the different types of vaccine. Chi-square and one-way analysis of variance (ANOVA) were used for evaluating multiple comparisons among groups vaccinated with the different vaccine types. Estimates and comparison intervals are shown in **Figure 6**. Paired comparison *p*-values resulting from the multicomparison test are reported in **Tables 8** only when one-way ANOVA comparison identified potentially significant differences.

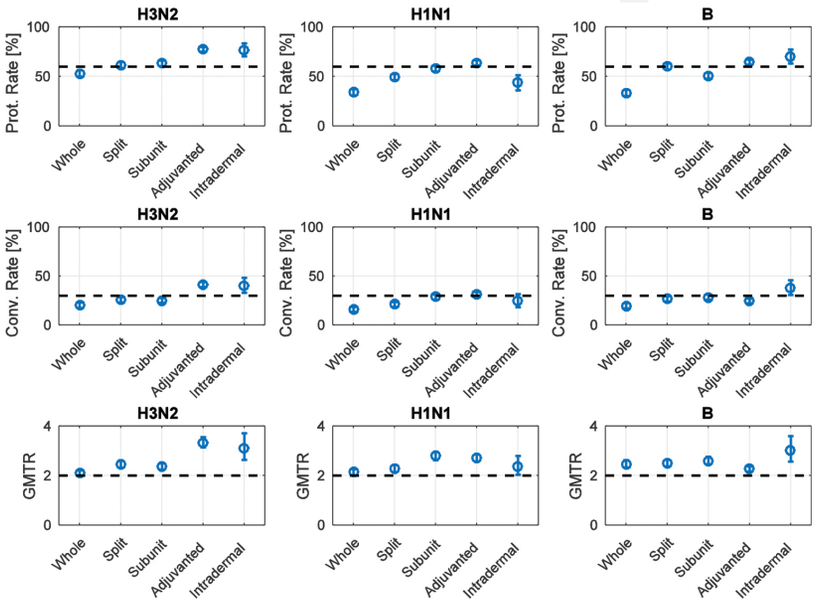

Figure 6. Values of CHMP parameters against the three vaccine antigens following vaccination with whole (*N* = 863), split (*N* = 996), subunit (*N* = 1094), MF-59 adjuvanted (*N* = 1343), and intradermally administered (*N* = 165) influenza vaccines. The black-dashed bold line in each figure represents the CHMP threshold value for the corresponding parameter. The bars indicate the ranges of the 95% confidence limits.

All vaccines used induced HI antibody responses satisfying at least one (prevalently GMTR value ≥2) of the three CHMP criteria. The antibody response induced by whole vaccine was in most instances lower as compared with responses induced by the others vaccines (**Table 8**). However, as reported in **Table 1**, many of the volunteers vaccinated with whole vaccine in the first years of the study received a vaccine with a low dose of antigen (30 μg). The responses induced by split and subunit vaccines against A/H3N2 and B antigens were similar; on the contrary against A/H1N1 antigen, the response induced by split vaccine was significantly lower as compared with subunit.

The two enhanced vaccines, MF59-adjuvanted and intradermal, induced similar and higher responses compared with conventional vaccines against A/H3N2 antigen.

Against A/H1N1, the response induced by MF59-adjuvanted vaccine was in most instances higher than conventional and intradermal vaccines.

Against B antigen, intradermal vaccine induced higher HI response than that induced by conventional and MF59-adjuvanted vaccines. In some cases the differences were statistically significant.

	p-Values (when <0.05)									
Parameter	**Whole /sub-u**	**Whole /split**	**Whole /MF59**	**Whole /ID**	**Split /sub-u**	**Split /MF59**	**Split /ID**	**Sub-u /MF59**	**Sub-u /ID**	**MF59 /ID**
A/H3N2 antigen										
Protection	<0.01	<0.01	<0.01	<0.01	–	<0.01	<0.01	<0.01	<0.01	–
Conversion	–	–	<0.01	<0.01	–	<0.01	<0.01	<0.01	<0.01	–
GMTR	<0.01	–	<0.01	<0.01	–	<0.01	<0.05	<0.01	<0.01	–
A/H1N1 antigen										
Protection	<0.01	<0.01	<0.01	–	<0.01	<0.01	–	<0.05	<0.01	<0.01
Conversion	–	<0.01	<0.01	–	<0.01	<0.01	–	–	–	–
GMTR	–	<0.01	<0.01	–	<0.01	<0.01	–	–	–	–
B antigen										
Protection	<0.01	<0.01	<0.01	<0.01	<0.01	–	–	<0.01	<0.01	–
Conversion	<0.01	<0.01	–	<0.01	–	–	<0.05	–	–	<0.01
GMTR	–	–	–	–	–	–	–	<0.01	–	<0.01

Whole: whole-virus vaccine; Sub-u: sub-unit vaccine; Split: split-virus vaccine; MF59: subunit MF59-adjuvanted vaccine; ID: Intradermal subunit vaccine.

Table 8. Paired comparison of results obtained in volunteers divided in groups according to the type of vaccine used for immunization. *p*-values resulting from the multicomparison test are reported only when one-way ANOVA comparison identified potentially significant differences.

4. Discussion

This study describes the humoral antibody response of 4461 elderly frail institutionalized volunteers prevalently vaccinated in the previous year after vaccination with influenza inactivated trivalent vaccines commercially available for the different years studied during a 27-year period (from winter season 1988–1989 to 2014–2015).

The first data were obtained by examining the results found in the 27-year period studied as crude mean responses and evidenced the ability of influenza vaccine administration to elicit antibody response in elderly volunteers (**Table 2**). One month after vaccination, significant increases were found against all the three vaccine antigens; however, vaccination induced significantly higher HI antibody titers against A/H3N2 antigen as compared with A/H1N1 and B strains. The higher responses against A/H3N2 strain were substantially confirmed considering the number of years in the 27-year period examined in which the CHMP criteria were

fulfilled (**Table 3**) or comparing GMT values after correction for baseline titers or considering responses in prevaccination unprotected people (**Figure 2**).

In accordance with our results, higher titers after vaccination against A/H3N2 strain were previously found by Sasaki et al. [23] and Ohmit et al. [24], but it was not possible to discriminate between the possibility that A/H3N2 antigen is more immunogenic than A/H1N1 and B antigens or the possibility that the higher GMT and protection rate values might depend from earlier contact with the A/H3N2 virus due to vaccination or natural infection. Since all the volunteers were previously vaccinated, the possibility of the influence of a different circulation of A/H3N2 strains is more acceptable. The A/H3N2 viruses have the highest rate of evolution among the three influenza subtypes currently circulating, with antigenically distinct strains emerging on average 2–5 years and capable of a better diffusion among the population [25].

Further considerations about the results obtained derive from *post hoc* analyses conducted to determine whether vaccine dose, age, sex, and type of vaccine might influence the vaccine-induced humoral immune response.

Although the issue of increase in the antibody titers following increase in influenza vaccine dosage is not completely clarified [19, 20, 26], our data found using vaccines with 30 or 45 μg of antigens for vaccine dose, suggested that the increase in influenza vaccine dosage is generally associated with an increase in the induction of antibody titers. Significant antibody titers increases were observed both administering vaccines with 30 or 45 μg of antigens for vaccine dose against all the three vaccine antigens. However, postvaccination values following vaccination with 45 μg vaccine were in most instances statistically higher as compared with 30 μg both considering mean values for the overall population (**Table 4**) or GMT corrected for prevaccination status or calculated in prevaccination unprotected volunteers (**Figure 3**). In accordance with these observations, recently (December 2009) in the United States, a high-dose (60 μg HA per strain) trivalent inactivated influenza vaccine was licensed for people 65 years of age or older. The high dose vaccine was found to improve in people aged ≥65 years both antibody response and protection against laboratory-confirmed influenza illness [27, 28].

Considering vaccine immunogenicity in younger elderly (≤75 years) or in very elderly (>75 years), vaccine administration induced statistically significant increases in both groups. Comparing the two groups, the values were in many instances slightly higher in the very elderly as compared with younger elderly, and in some instances the differences were statistically significant. However, the differences persisted against A/H1N1 antigen both after correction for prevaccination status or calculation in unprotected volunteers before vaccination, and against B antigen only considering responses in unprotected people (**Figure 4**). The highest response of very elderly people as compared with younger elderly volunteers might be due to the fact that they probably represent a more selected group of elderly people capable of longer surviving and with a possible lower degree of age-associated alteration of the immune system [29].

However, since the differences were particularly evident against the A/H1N1 strain and are in accordance with previous data found in our laboratory showing in two different winter seasons a higher ability to give HI antibody response against A/H1N1 strains of people born between

1903 and 1919 as compared with volunteers born between 1920 and 1957, we cannot exclude the possibility that the differences might be due to cross-reactivity generated from exposure to the 1918 A/H1N1 virus or related A/H1N1 strains [30].

As far as sex could influence the immune response against influenza vaccines, our results confirmed previous data indicating that receipt of trivalent inactivated influenza vaccines results in significantly higher HI titers among females than males, both in adults and elderly people [21]. Significant rises in antibody titers were found after vaccination both in males and females, but the values observed in females were significantly higher as compared with males (**Table 6**) and the differences persisted also considering only GMT of volunteers unprotected before vaccination or GMT corrected for prevaccination status (**Figure 5**).

Sex hormones have been considered to be the most important mediators of sex differences and males with high level of testosterone have been found to have low antibody responses after influenza vaccination [31, 32].

However, since our data were obtained in elderly people, i.e., after the reproductive senescence, they support the hypothesis that the sex hormones are not the only mediator of sex differences in humoral response to influenza vaccination and there is the possibility that genetic differences also might underlie sex-based differences in adaptive immune response to viral vaccines [21, 33].

These results (vaccine dose, age, and sex) were, at least in part, confirmed also considering responses evaluated in strong responder, i.e., in volunteers showing a positive response after vaccination (**Table 7**).

Comparison of the different type of vaccines used in the 27-year period evidenced higher immunogenicity of the new "enhanced vaccines" specially licensed for elderly individuals, i.e., adjuvanted and intradermally administered vaccines, as compared with traditional whole, subunit, and split vaccines (**Table 7**, **Figure 6**) supporting previously published data [34, 35].

Our study had several limitations. The most important are that our observations may apply only to frail seniors living in care facilities and that the subanalysis groups were not fully comparable. However, since institutionalized people represent a significant target group for influenza vaccination, it is important to analyze their response to influenza vaccines. An additional limitation is the lack of data demonstrating clinical efficacy against influenza infection and illness. Although there is substantial evidence that HI antibody titers represent a good correlate of protection from severe illness in young adults, the predictive value of these measurements in older adults might be variable. Although the number of volunteers and of winter seasons we examined was considerable and comparable to the data reported in a review, differently from a review on influenza vaccine immunogenicity, the results obtained in each year were considered cumulatively not taking into account of the different characteristics of the vaccines used through the 27-year period. Indeed, the antigenic composition of influenza vaccines differ, even considerably, from one year to another, since it is updated each year to match the strains circulating in the community and inactivated influenza vaccines are available in different formulations (whole, split, and subunit with or without adjuvants), which are administered intramuscularly or intradermally. Moreover, a further aspect that should be

carefully considered as compared with those of a review on HI antibody titers after influenza vaccine administration is the HI assay itself. HI test is not standardized across laboratory and was found to be highly variable and sensitive to factors such as reagents, erythrocyte source, and virus passage history. The results reported in the present report were all obtained in the same laboratory, although in different years and although, some changes were introduced in the HI test used during the 27-year period as reported in Section 2.

In conclusion, our data evidenced that the use of influenza vaccination appears to be an appropriate strategy to address the challenge of influenza infections of the elderly. However, they underline the need of studies for new improved influenza vaccines, since, as previously found, the vaccine-induced HI antibody responses against the three vaccine antigens were different and resulted not satisfactory against A/H1N1 and B antigen, since the postvaccination values of seroprotected volunteers were lower than the requested 60% (**Table 3**).

Moreover, they underline the necessity to expand researches and approaches to understand immunosenescence and its relationship to vaccine-induced immunity in order to have more valid vaccines. The vaccine-induced stimulation of HI antibody response following vaccination was found not only to be higher against one vaccine component as compared with the other two, but also to be influenced by different factors as vaccine dose, age, sex, and type of vaccine. It is therefore important, as suggested by Lambert et al. [5], both to understand the mechanisms that result in these differences and to use such information to devise more immunogenic influenza vaccine candidates.

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