

ORIGINAL ARTICLE

Comparison between a conventional subunit vaccine and the MF59-adjuvanted subunit influenza vaccine in the elderly: an evaluation of the safety, tolerability and immunogenicity

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Key words

Influenza • Vaccine • MF-59 • Adjuvants

Summary

The objective of this study was to evaluate and compare the safety, tolerability and immunogenicity for two seasonal influenza subunit vaccines, one with MF59 adjuvant (Fluad®) and one without an adjuvant (Arippal®).

A total of 195 subjects aged ≥ 65 years were enrolled to receive one dose of vaccine intramuscularly, 96 were vaccinated with Fluad®, 99 received Arippal®.

Blood samples were taken from all subjects in order to assess their antibody titre by the haemagglutination inhibition assay (HI), before (Time 0) and after (Time 1: 28 ± 7 days) vaccination, against the A/H3N2 (A/Moscow/10/99), A/H1N1 (A/New Caledonia/20/99) and B/Shandong/7/97 antigens contained in the influenza vaccine in the 2002/2003 influenza season for the northern hemisphere.

A good humoral antibody response was detected for both vaccines, meeting all the criteria of EMEA. The number of subjects

in whom a ≥ 4 -fold increase in antibody titre was recorded, in comparison with the pre-vaccination value, proved to be lower in the group vaccinated with Arippal® than in those vaccinated with the adjuvanted preparation. Fluad® exhibited better immunogenicity than Arippal®. This difference was probably linked to the potentiated immune stimulation exerted by the adjuvant molecules.

These results take on a particular importance if we consider that the immune system is weaker in the elderly; the administration of an adjuvanted vaccine in such subjects is clearly preferable in that it provides greater and more prolonged protection.

Both vaccines were generally well tolerated; no severe adverse events occurred in any of the subjects vaccinated, confirming the excellent safety profile of Fluad® and Arippal®.

Introduction

Although some molecules, such as zanamivir and oseltamivir, can be used to combat influenza viruses [1-5], it has long been recognized that vaccines are the most efficacious weapon against influenza. This conviction has guided experimental research since the virus was first isolated in 1933 [6].

Today, the vaccines most frequently used in mass vaccination are “split” vaccines, “subunit” vaccines and “viroosomal” vaccines. In elderly subjects these vaccines, administered intramuscularly (deltoid muscle), did not exhibit sufficient immunogenicity, so adjuvants as MF59® have been introduced [7].

Until such time as different formulations become available, molecules of a different nature (adjuvants) are used to improve the immunological response; when associated to specific antigens, these molecules are able to induce a heightened immune response [8-10]. One of the best-known adjuvants currently used in Italy is MF59® (Novartis Vaccines and Diagnostics Srl, Italy) [11], the safety, tolerability and immunogenicity of which have been the subject of numerous clinical and experimental studies [12-17].

The aim of the present study was to evaluate the immunogenicity of an MF59-adjuvanted subunit influenza vaccine in comparison with that of a conventional subunit vaccine. Furthermore we evaluated the safety and tolerability of both vaccines.

Materials and methods

The study was approved by the local ethics committee.

STUDY POPULATION

We enrolled 195 subjects aged ≥ 65 years, who were randomly selected from among residents in social community facilities during the 2002/2003 influenza season in Messina (South Italy).

All subjects were given clear, complete, written information regarding the experimental treatment, in order to obtain a fully informed consent signed; whenever necessary, explanations were also provided by medical staff. The study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki and good clinical practice.

Tab. I. Demographic features of the subjects enrolled in the groups vaccinated with AGRIPPAL® and FLUAD®.

Demographic variable	AGRIPPAL®	FLUAD®
Females	74	70
Males	25	26
Mean age (years) ± SD	80.29 ± 7.78	79.04 ± 8.29

Using a computer generated list, subjects were randomized to receive a MF59-adjuvated (Fluad®; N= 96), or a non-adjuvated subunit influenza vaccine (Agrippal®; N = 99).

After thorough medical examination, blood samples were taken from all subjects in order to assess their antibody titres, before (Time 0) and after (Time 1: 28 ± 7 days) vaccination, against A and B antigens contained in the influenza vaccine in the 2002/2003 season.

VACCINES

Each subject received a single 0.5mL intramuscular dose of the assigned vaccine, preferably into the deltoid region.

Both vaccines contained the surface antigens of the strains indicated by the World Health Organization (WHO) for the northern hemisphere (NH) during the 2002/03 influenza season:

- A/H3N2 (A/Moscow/10/99);
- A/N1N1 (A/New Caledonia/20/99);
- B/Shandong/7/97.

The adjuvated vaccine (Fluad®) was formulated as follows: 15 µg of each of the surface antigens, obtained from virions cultivated in egg and adjuvated with MF59C.1. The MF59C.1 adjuvant is composed of 9.75 mg of squalene; 1.175 mg of polysorbate 80; 1.175 mg of sorbitan trioleate; 0.66 mg of sodium citrate; 0.04 mg of citric acid and water for injectable preparations.

The non-adjuvated vaccine (Agrippal®) was made up of 15 µg of each of the surface antigens, obtained from virions cultivated in egg and inactivated in formaldehyde and suspended in a solution of sodium chloride, potassium chloride, monobasic potassium phosphate, bi-hydrated dibasic sodium phosphate, magnesium chloride, calcium chloride and water for injectable preparations.

IMMUNOGENICITY

In order to assess the short-term immunogenic and protective efficacy of the two types of vaccine, blood samples were taken from all patients 28 ± 7 days after vaccination.

The laboratory tests recommended by the European Medicine Agency (EMA) to assess immunogenic efficacy are haemagglutination inhibition (HI) and single radial haemolysis (SRH).

Our laboratory carried out the HI test according to the method of Couch [18]. When the HI test is used, the criteria for evaluating vaccine immunogenicity in subjects above the age of 60 years, as established by the Committee for Proprietary Medicinal Products (CPMP/BWP/214/96), are the following:

- a number of seroconversions (pre-vaccination titre < 1:10, post-vaccination titre ≥ 1:40) or a significant increase in the antibody titre (equal to or greater than 4 times the initial value) in more than 30% of the subjects vaccinated;
- the increase in antibody titre, expressed as the geometric mean (Geometric Mean Titre GMT), must be at least doubled;
- the percentage of subjects with an antibody titre ≥ 1:40 must be greater than 60%.

Thus, for each viral antigen, we calculated the Geometric Mean Titre (GMT) of the sera pre- and post-vaccination and the ratio of the GMT (GMR – Geometric Mean Ratio).

Tab. II. Numbers and percentages of subjects meeting the EMA (CPMP/BWP/214/96) criteria.

Vaccine	A/H3N2 (A/MOSCOW/10/99)		A/H1N1 (A/NEW CALEDONIA/20/99)		B (B/SHANDONG/7/97)	
	AGRIPPAL®	FLUAD®	AGRIPPAL®	FLUAD®	AGRIPPAL®	FLUAD®
	Protected before vaccination	83 84%	74 77%	70 70.7%	70 72.9%	67 67.6%
Protected after vaccination	98 98.9%	95 98.9%	95 96%	92 95.8%	97 98%	93 96.6%
Sera-Conversions	1 1%	4 4.2%	10 9.9%	4 4.2%	2 2%	11 11.5%
≥ 4 titre upgrade conversions included	41 41.4%	59 61.4%	42 42.4%	53 55.2%	45 45.4%	67 69.7%
GMT						
T0	92.02	80	58.38	65.35	49.35	30.40
T1	256.98	377.81	185.34	255.82	170.41	160
GMR						
T1/T0	2.76	4.72	3.17	3.91	3.45	5.26

Fig. 1. GMT and 95% CI values observed in the non-adjuvated vaccine group at times T0 and T1.

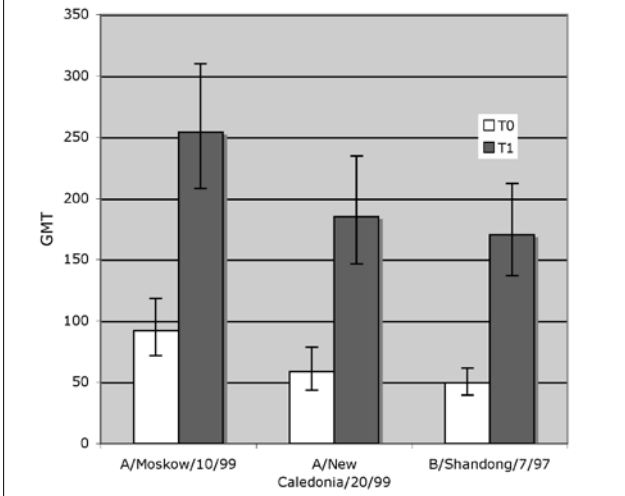


Fig. 2. GMT and 95% CI values observed in the adjuvated vaccine group at times T0 and T1.



SAFETY AND TOLERABILITY

We assessed any adverse reactions, both at the injection site and at the systemic level and any Serious Adverse Event (SAE) reported during the trial; the time course and intensity of expression were considered. Observation for solicited local and system reactions was continued for 7 days after vaccination using a clinical diary. SAE were monitored by the investigators for 1 month.

STATISTICAL ANALYSES

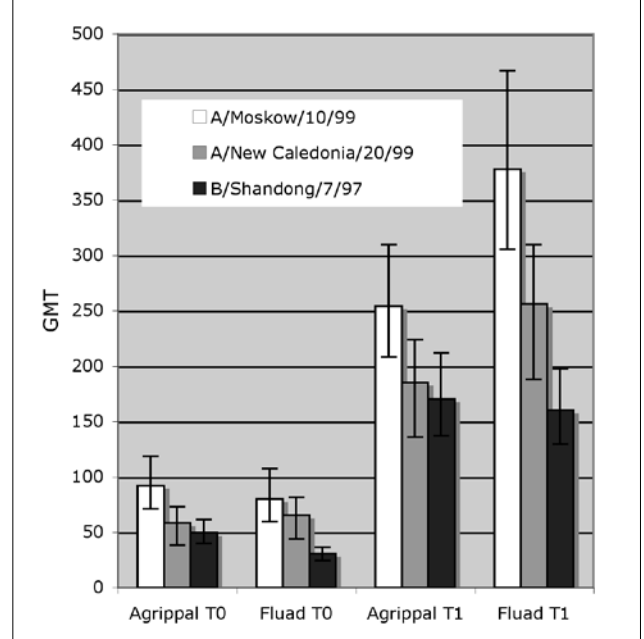
Immunogenicity

Statistical analysis of the data was performed by calculating the GMT, with a Confidence Interval of 95% (95% CI), and the GMR, by means of Excel (Microsoft) software.

Safety and tolerability

The χ^2 test was applied by means of the PEPI 4.0 software (J.H. Abramson e P.M. Gahlinger, Sagenbrush,

Fig. 3. GMT and 95% CI values observed for the single viruses at times T0 and T1 in both study groups.

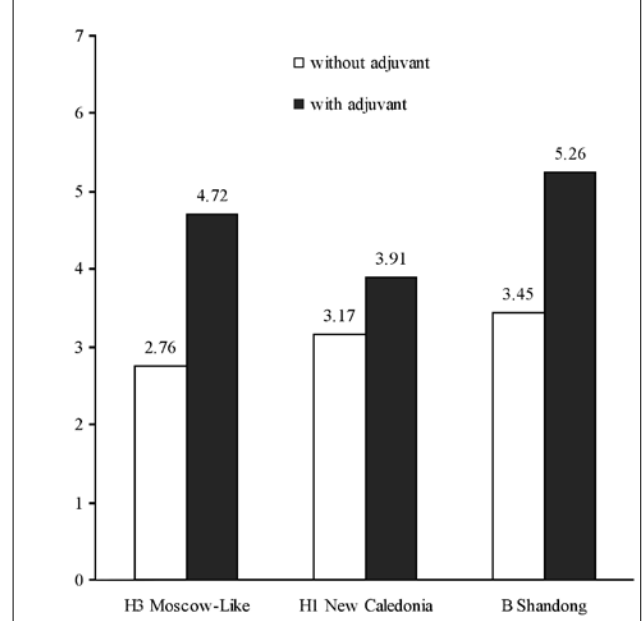


London, UK); a value of $p \leq 0.05$ was taken to be statistically significant.

Results

Table I shows the main demographic features of the subjects enrolled in the study. No significant differences were recorded between the two study groups.

Fig. 4. GMR values observed at times T0 and T1 in both study groups.



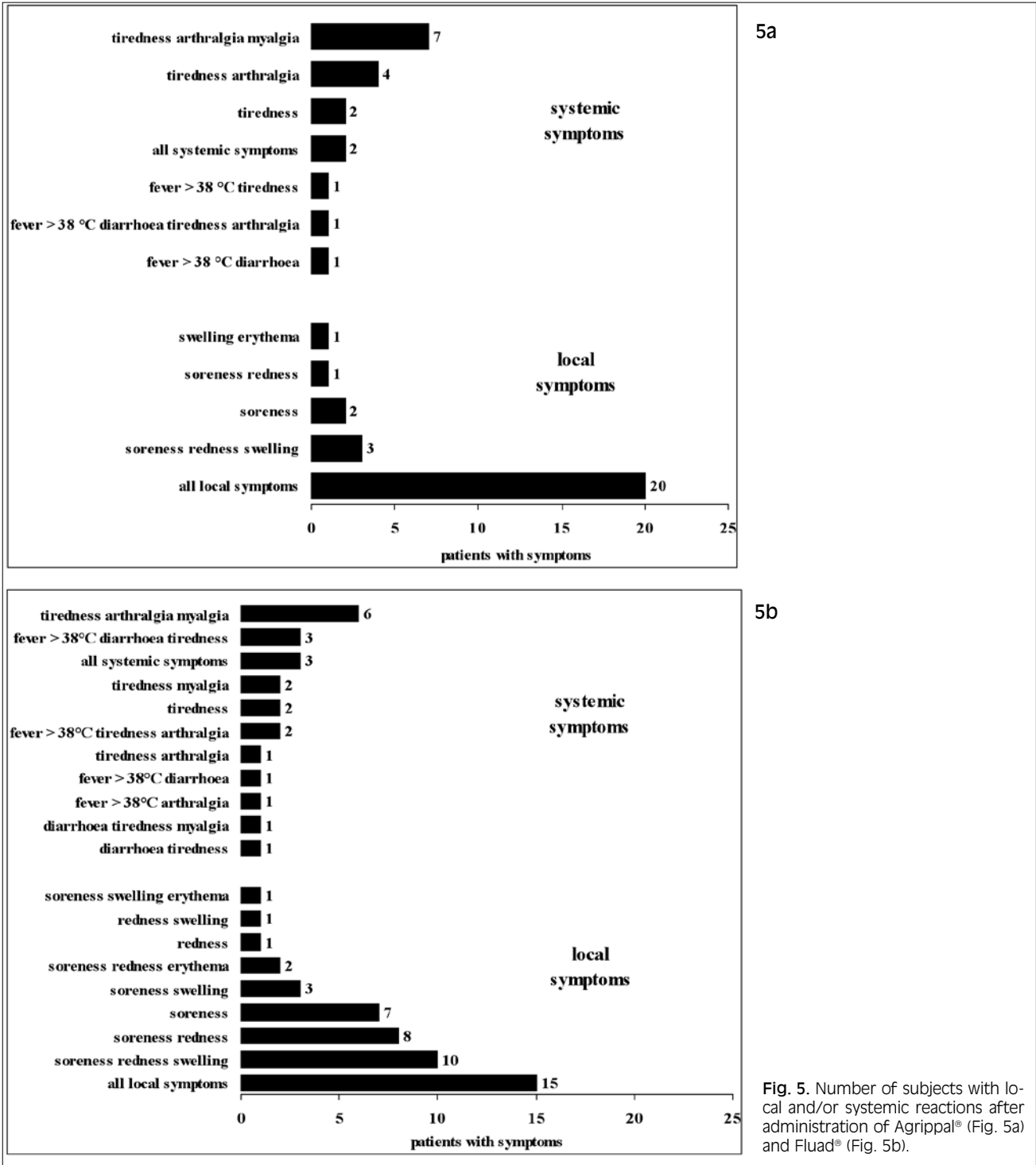


Fig. 5. Number of subjects with local and/or systemic reactions after administration of Agrippal® (Fig. 5a) and Fluvad® (Fig. 5b).

IMMUNOGENICITY

Before vaccination, no significant difference was recorded between the two study groups in terms of the number of subjects who displayed protective levels with regard to the three vaccine strains considered (A/Moscow/10/99, $p = 0.3126$; A/New Caledonia/20/99, $p = 0.8543$; B Shandong/7/97, $p = 0.2549$). In the subjects vaccinated with Agrippal®, we observed an increase in antibody titre (≥ 4 times the initial value) in 40 subjects (40.4%, IC 95% 30.7-50.1) with regard

to A/Moscow/10/99, in 32 (32.3%, IC 95% 23.1-41.5) for A/New Caledonia/20/99 and in 43 (43.4%, IC 95% 33.6-53.1) for B Shandong/7/97 (Tab. II). In the group vaccinated with Fluvad®, an increase in antibody titre was recorded in 55 subjects (57.3%, IC 95% 47.4-67.2) for A/Moscow/10/99, in 49 (51%, IC 95% 40.9-61.0) for A/New Caledonia/20/99 and in 56 (58.3%, IC 95% 48.4-68.1) for B Shandong/7/97 (Tab. II). Seroconversion after administration of the non-adjuvated vaccine (Agrippal®) was recorded in 1 subject

(1%, IC 95% 0-2.96) for A/Moscow/10/99, in 10 (10.1%, IC 95% 4.1-16.1) for A/New Caledonia/20/99 and in 2 (2.2%, IC 95% 0-5.1) for B Shandong/7/97. In the subjects vaccinated with the adjuvated vaccine (Fluad®) seroconversion occurred in 4 subjects (4.2%, IC 95% 0.2-8.2) for A/Moscow/10/99, in 4 (4.2%, IC 95% 0.2-8.2) for A/New Caledonia/20/99 and in 11 (11.5%, IC 95% 5.1-17.8) for B Shandong/7/97 (Tab. II).

In the non-adjuvated vaccine group, administration of the vaccine increased the number of protected subjects as follows: for the virus A/Moscow/10/99 from 83 (84%, IC 95% 76.7-91.22) to 98 (98.9%, IC 95% 96.84-100); for A/New Caledonia/20/99 from 70 (70.7%, IC 95% 61.73-79.6) to 95 (96%, IC 95% 92.13-99.86); and for B/Shandong/7/97 from 67 (67.6%, IC 95% 57.73-76.26) to 97 (98%, IC 95% 95.2-100).

In the group vaccinated with the adjuvated vaccine, administration increased the number of protected subjects as follows: for the virus A/Moscow/10/99 from 74 (77%, IC 95% 68.6-85.4) to 95 (98.9%, IC 95% 96.8-100); for A/New Caledonia/20/99 from 70 (72.9%, IC 95% 64.00-81.8) to 92 (95.8%, IC 95% 96.8-100); and for B/Shandong/7/97 from 73 (76%, IC 95% 67.4-84.5) to 93 (96.8%, IC 95% 93.2-100).

Figure 1 shows the GMT values observed before and after vaccination in the subjects who received the non-adjuvated vaccine, together with the 95% CI values.

Figure 2 shows the GMT values observed before and after vaccination in the subjects who received the adjuvated vaccine, together with the 95% CI values.

Figure 3 shows the GMT values observed for the single virus at times T0 and T1 in the 2 study groups. As will be seen, the GMT values recorded in the subjects vaccinated with Fluad® proved to be higher.

At 3 weeks post-vaccination, significantly higher GMTs were reported for both vaccine groups versus baseline as IC show (Figs. 1 and 2). In the Fluad® group post-vaccination GMTs against both A strains were significantly higher compared with the Agrippal® group (A/H1N1: 255.82 versus 185.34 respectively; A/H3N2: 377.81 versus 256.98, respectively). Against the B strain post-vaccination HI titers were 170.41 in the Agrippal® group and 160 in the Fluad® group.

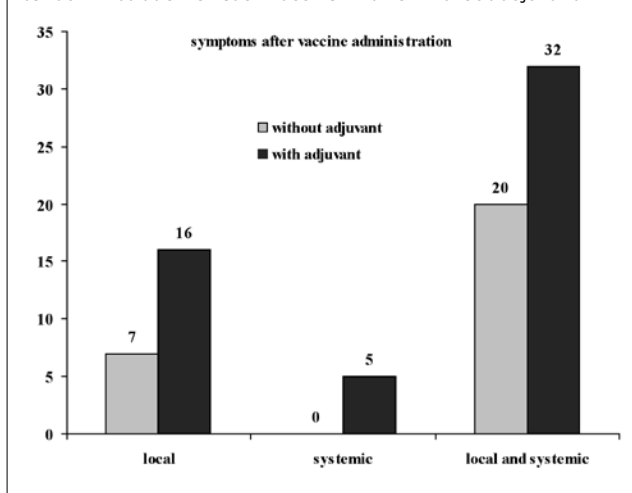
The ratios between the GMT values observed before and after vaccination in both study groups are shown in Figure 4.

SAFETY AND TOLERABILITY

During the entire clinical follow-up, no serious SAE were reported.

Figures 5a and 5b show the solicited local and systemic reactions recorded after administration of both types of vaccine. Following the administration of the non-adjuvated vaccine, 27 subjects (27.27%, IC 95% 18.5-36.1) suffered at least one solicited reactions at the injection site (pain on touching, swelling, erythema). In the same group, 18 subjects (18.2%, IC 95% 10.6-25.8) had sys-

Fig. 6. Number of subjects with local and systemic reactions after administration of each vaccine (with or without adjuvant).



temic symptoms; in particular, 2 subjects had all of the systemic manifestations (Figure 5a).

In the group vaccinated with Fluad®, 48 of the 96 subjects (50%, IC 95% 39.9-60.0) reported at least one solicited reactions at the injection site and 15 had all of the local symptoms (Figure 5b). With regard to solicited systemic symptoms, 23 subjects (23.96%, IC 95% 15.4-32.5) had signs and/or symptoms; in particular, 2 suffered all of the systemic symptoms (Figure 5b). Statistical analysis by means of the χ^2 test revealed a significant difference between the two groups with regard to both general and local symptoms ($p < 0.001$). Reactions reported in both vaccine groups were generally of mild or moderate intensity and short living.

On analysing the concomitance of systemic and local symptoms, it emerged that 7 of the subjects who received the non-adjuvated vaccine presented only local symptoms; none had systemic symptoms alone, and 20 suffered both systemic and local symptoms (Figure 6). In the group of subjects who received the adjuvated vaccine, 16 presented local symptoms alone, 5 had only systemic signs/symptoms, and 32 suffered both systemic and local symptoms (Figure 6). When applied to these data, the χ^2 test revealed a significant difference between the two groups ($p < 0.001$).

Discussion

The results of this study provide a further contribution to the assessment of the immunogenicity of the MF59-adjuvated influenza vaccine (Fluad®) in comparison with the conventional non-adjuvated vaccine (Agrippal®).

Although the high number of subjects with seroprotective titers already at baseline in both vaccine groups, the number of subjects in whom a ≥ 4 -fold increase in antibody titre was recorded, in comparison with the pre-vaccination value, was lower in the group vaccinated with Agrippal® than in those vaccinated with the Fluad®. Moreover, the GMR values were higher in the

group that received the adjuvanted than in the group that received the non-adjuvanted vaccine. These results take on a particular importance if we consider that the immune system is weaker in the elderly. Therefore, the administration of an adjuvanted vaccine in such subjects is clearly preferable in that it provides greater and more prolonged protection.

No serious adverse events occurred in any of the subjects vaccinated, confirming the excellent safety profile.

As reported in previous publications [15, 19-22], Flud[®] induced more solicited local and systemic reactions than Agrippal[®]. Reactions were usually of mild or moderate intensity and transient and did not require any medical intervention.

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Conclusions

The higher immunogenicity of Flud[®] in this trial enables us to conclude that the adjuvanted vaccine Flud[®] is preferable to non-adjuvanted formulations in anti-influenza strategies aimed at the elderly.

The greater antibody expression observed after the administration of the adjuvanted vaccine means that elderly subjects, whose immune reactivity may be compromised, will not be left with an insufficient antibody titre for part of the influenza season.

In agreement with the data reported in the national and international literature [15, 19-22], our results show that both local and systemic reactions are negligible in terms of intensity and duration.

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