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Short communication

# Immunogenicity, safety and tolerability of monovalent 2009 pandemic influenza A/H1N1 MF59-adjuvanted vaccine in patients with β-thalassemia major

Susanna Esposito[a], Emanuela D'Angelo[a], Cristina Daleno[a], Francesco Peia[a], Alessia Scala[a], Domenico Serra[a], Nadia Mirra[a], Carlotta Galeone[b], Nicola Principi[a,*]

[a] Department of Maternal and Pediatric Sciences, Università degli Studi di Milano, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Via Commenda 9, 20122 Milano, Italy
[b] Department of Epidemiology, Istituto di Ricerche Farmacologiche Mario Negri, Milan, Italy

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ABSTRACT

In order to evaluate the immunogenicity, safety, and tolerability of monovalent 2009 pandemic influenza A/H1N1 MF59-adjuvanted vaccine in patients with β-thalassemia major, 31 subjects (19 males; mean age 17.8 ± 8.7 years) with β-thalassemia major and 28 age- and gender-matched healthy controls were enrolled. Four weeks after vaccination, seroconversion rates were about 80% and seroprotection rates 100% in both groups. Three months after vaccination, most of the subjects remained seroconverted and the seroprotection rates were 93.5% among the patients and 100% among the controls. Safety and tolerability were also very good, with no differences between the groups.



## 1. Introduction

The list of patients for whom influenza vaccination is recommended by all health authorities includes those with chronic blood disorders such as β-thalassemia major [1] because chronic anemia with tissue hypoxia, multiple blood transfusions, increased iron deposits, and iron chelation therapy can all reduce host defences and increase susceptibility to bacterial and viral infections [2]. Furthermore, most patients with β-thalassemia major are splenectomised or have functional spleen deficits, both of which can cause a further increase in the risk of influenza-related complications as they are associated with a reduced specific antibody response, an impaired complement-dependent opsonisation pathway, and decreased phagocytosis [3,4].

The findings of studies of the immune response to vaccines of patients with functional or anatomical asplenia indicate that their vaccine-induced immunogenicity is similar to that of healthy subjects, but the fact that vaccine failures have been reported in properly immunised asplenic subjects suggests that protection can sometimes be less than expected [5]. There are few data regarding the immunogenicity of influenza vaccines in asplenic patients and those that are available seem to indicate that, at last in the case of seasonal A/H1N1 antigen, the seroprotection rates evoked by the inactivated vaccine are lower than in healthy subjects [6].

The recent influenza pandemic caused a new A/H1N1 virus led to a higher incidence of hospitalisations and death than the annual rates associated with seasonal influenza viruses [7]. A number of specific vaccines against this virus can generate adequate antibody responses in healthy subjects [8], but there are no published data concerning their efficacy in spenectomised and non-splenectomised patients with chronic anemia. The aim of this study was to evaluate the immunogenicity, safety, and tolerability of the 2009 monovalent pandemic influenza A/H1N1 MF59-adjuvanted vaccine in patients with β-thalassemia major.

## 2. Material and methods

### 2.1. Study population

Adolescents and young adults with β-thalassemia major regularly attending the outpatient clinic of the Department of Maternal and Pediatric Sciences of the University of Milan (Italy) were considered eligible for the study. As the pandemic A/H1N1 influenza vaccine was available in Italy only about four weeks after the first documented episode of pandemic influenza, those who had suf-

* Corresponding author. Tel.: +39 02 55032498; fax: +39 02 50320206.
*E-mail address:* Nicola.principi@unimi.it (N. Principi).

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fered from an influenza-like illness in the four weeks before the beginning of the study were excluded in order to avoid the risk of enrolling patients who had already had the disease. A similar number of healthy age- and gender-matched subjects were enrolled as controls.

The study protocol was approved by the Ethics Committee of the Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan, Italy. Written informed consent to participate in the study was obtained from all of the subjects and from the parents or legal guardians of those aged less than 18 years.

### 2.2. Study procedures

All of the subjects received a single dose of the monovalent pandemic influenza A/H1N1 MF59-adjuvanted vaccine produced by Novartis (Focetria, Siena, Italy). Each dose contained 7.5 μg of H1 hemoagglutinin, 9.75 mg of the squalene MF59, 1.175 mg of polysorbate 80 and 1.175 mg of sorbitan trioleate in buffer, and was administered by means of an injection into the deltoid muscle of the non-dominant arm.

Serum samples were collected for antibody assay immediately before the vaccine was administered, and four weeks ($28 \pm 2$ days) and 3 months ($90 \pm 2$ days) later.

The subjects were observed for 30 min after the injection, and they or their parents recorded the occurrence of solicited and unsolicited local symptoms (erythema, swelling/induration, and pain) or systemic symptoms (an axillary temperature of $\geq$38 °C, irritability, sleepiness, changes in eating habits, vomiting, diarrhea, malaise, muscle ache) for the next 14 days. The symptoms were considered mild if they did not interfere with normal everyday activities, and severe if they prevented them and required medical attention. Adverse reactions were defined as any reaction that persisted for longer than seven days after the vaccination, and serious adverse reactions as any reaction that required medical attention or hospitalisation during the study period.

### 2.3. Laboratory assays

Serum antibody levels were determined by means of a hemagglutination inhibition (HI) assay using a standard method [9]. The serum samples were tested in duplicate at an initial dilution of 1:10, and those that were negative for the antibody were assigned an arbitrary titre of 1:5. The HI antibody titres were expressed as the reciprocal of the highest dilution of serum which completely inhibited hemagglutination.

The parameters used as expressions of a humoral immune response were the seroconversion rate (defined as the percentage of subjects experiencing at least a 4-fold increase in HI antibody titre from a seropositive pre-vaccination titre or an increase from <10 to $\geq$40 in those who were seronegative), geometric mean titres (GMTs), the difference between the mean pre- and post-vaccination titre, and the seroprotection rate (defined as the percentage of subjects reaching an HI titre of $\geq$40).

### 2.4. Statistical analysis

The continuous variables are given as mean values ± standard deviation (SD), and the categorical variables as numbers and percentages. The continuous data were analysed using a two-sided Student's test if they were normally distributed (on the basis of the Shapiro–Wilk statistic) or a two-sided Wilcoxon rank-sum test if they were not. Categorical data were analysed using contingency table analysis and the chi-squared or Fisher's test, as appropriate. All of the analyses were two-tailed, and $p$ values of 0.05 or less were considered significant.

**Table 1**
Immunogenicity endpoints against the 2009 pandemic A/H1N1 influenza strain in patients with β-thalassemia major and healthy controls (a), and in splenectomised and non-splenectomised patients with β-thalassemia major (b).

| Pandemic A/H1N1 strain | Before dose | | 4 weeks after dose | | 3 months after dose | |
|---|---|---|---|---|---|---|
| | Thalassemic patients ($n$ = 31) | Healthy controls ($n$ = 28) | Thalassemic patients ($n$ = 31) | Healthy controls ($n$ = 28) | Thalassemic patients ($n$ = 31) | Healthy controls ($n$ = 28) |
| (a) | | | | | | |
| Seroconversion, no. (%) | NA | NA | 27 (87.1) | 22 (78.6) | 24 (77.4) | 22 (78.6) |
| GMT (fold increase) | 137.27 (NA)[a] | 139.60 (NA)[b] | 1156 (8.42) | 747.69 (5.35) | 715.80 (5.21) | 555.38 (3.97) |
| Seroprotection, no. (%) | 11 (35.5)[a] | 10 (35.7)[b] | 31 (100.0) | 28 (100.0) | 29 (93.5) | 28 (100.0) |
| | Before dose | | 4 weeks after dose | | 3 months after dose | |
| Pandemic A/H1N1 strain | Non-splenectomised thalassemic patients ($n$ = 16) | Splenectomised thalassemic patients ($n$ = 15) | Non-splenectomised thalassemic patients ($n$ = 16) | Splenectomised thalassemic patients ($n$ = 15) | Non-splenectomised thalassemic patients ($n$ = 16) | Splenectomised thalassemic patients ($n$ = 15) |
| (b) | | | | | | |
| Seroconversion, no. (%) | NA | NA | 15 (93.7) | 12 (80.0) | 13 (81.2) | 11 (73.3) |
| GMT (fold increase) | 143.16 (NA)[c] | 131.73 (NA)[d] | 1349 (9.4) | 973.74 (7.39) | 916.18 (6.39) | 570.18 (4.32) |
| Seroprotection, no. (%) | 6 (37.5)[c] | 5 (33.3)[d] | 16 (100.0) | 15 (100.0) | 15 (93.7) | 14 (93.3) |

NA: not applicable; GMT: geometric mean titres.
[a] $p < 0.05$ vs. thalassemic patients 4 weeks and 3 months after dose.
[b] $p < 0.05$ vs. healthy controls 4 weeks and 3 months after dose; no significant differences between patients with β-thalassemia major and healthy controls.
[c] $p < 0.05$ vs. non-splenectomised thalassemic patients 4 weeks and 3 months after dose.
[d] $p < 0.05$ vs. splenectomised thalassemic patients 4 weeks and 3 months after dose; no significant difference between splenectomised and non-splenectomised patients with β-thalassemia major.

**Table 2**
Summary of solicited local and systemic reactions in the 14 days following vaccination with the 2009 pandemic A/H1N1 MF-59 adjuvanted influenza vaccine in children with β-thalassemia major and healthy controls.

| Adverse events | Thalassemic patients ($n = 31$) | Healthy controls ($n = 28$) |
|---|---|---|
| Local reactions, no. (%) | | |
| Erythema | 1 (3.2) | 2 (7.1) |
| Swelling/induration | 1 (3.2) | 3 (10.7) |
| Pain | 6 (19.3) | 7 (25.0) |
| At least one local event | 7 (22.6) | 7 (25.0) |
| Systemic reactions, no. (%) | | |
| Fever ≥38 °C | 4 (12.9) | 7 (25.0) |
| Rhinitis | 1 (3.2) | 5 (17.9) |
| Malaise | 1 (3.2) | 5 (17.9) |
| Spleepiness | 1 (3.2) | 3 (10.7) |
| Changed eating habits | 1 (3.2) | 4 (14.3) |
| Vomiting | 1 (3.2) | 1 (3.6) |
| Diarrhea | 1 (3.2) | 2 (7.1) |
| At least one systemic event | 8 (25.8) | 10 (35.7) |
| At least one local or systemic event | 11 (35.5) | 12 (42.9) |
| Required drugs for local or systemic events | 4 (12.9) | 8 (28.6) |
| Serious adverse events | 0 (0.0) | 0 (0.0) |

Percentages in parentheses. No significant differences between the patients with β-thalassemia major and healthy controls.

## 3. Results

Of the 47 eligible patients with β-thalassemia major followed up, 31 (65.9%) were enrolled in the study (19 males; mean age 17.8 ± 8.7 years), including 15 (48.4%) who had undergone splenectomy at least three years before entering the study. All of the patients had received transfusions of filtered red blood cell concentrates at regular intervals (a mean of 15 transfusions/year), and seasonal influenza vaccine in the two years preceding this study. The control group consisted of 28 healthy subjects with similar characteristics in terms of gender (20 males), age (17.6 ± 7.0 years) and previous influenza vaccinations.

Table 1 shows the immune responses of the two groups after the administration of the 2009 pandemic influenza A/H1N1 MF59-adjuvanted vaccine. About 35% of the subjects in all the groups had baseline specific antibody titres of 40 or more upon HI assay and a measurable GMT. Four weeks after the administration of the vaccine, thalassemic children and healthy controls had seroconversion rates of about 80% and seroprotection rates of 100%. GMTs and the increase in antibody levels were higher in the group of β-thalassemia patients but not significantly so. Three months after the vaccination, most of the subjects remained seroconverted without any statistically significant difference between the groups, and 93.5% of the thalassemic patients and 100% of the controls were still seroprotected. GMTs and their increase from baseline remained higher in the β-thalassemic patients, but there was still no statistically significant difference in comparison with the controls.

There was no significant difference in seroconversion rates, GMTs or seroprotection rates between the splenectomised and non-splenectomised β-thalassemic patients.

Table 2 summarises the incidence of solicited and unsolicited local and systemic reactions in thalassemic patients and healthy controls during the 14 days following vaccination. Local reactions occurred in 25% of the subjects in both groups, whereas systemic reactions were more frequent among the controls, but this difference was not statistically significant. No serious adverse event occurred in any of the enrolled subjects.

## 4. Discussion

Our findings indicate that the antibody responses to the administration of a single dose of the monovalent 2009 pandemic influenza A/H1N1 MF59-adjuvanted vaccine are similar in patients with β-thalassemia major and healthy subjects, and enough to suggest long-lasting protection from pandemic influenza infection as more than 90% of the thalassemic patients had seroprotective antibody titres three months after the administration of the vaccine. Moreover, the immunogenicity of the pandemic MF59-adjuvanted vaccine was the same in the splenectomised and non-splenectomised patients. This can be explained by the fact that the adolescents and young adults who had undergone splenectomy had done so at least three years before their enrolment in the study. It is well known that the negative impact of splenectomy is greater in younger patients and, in most cases, no longer relevant three years after surgery [5]. The safety and tolerability of the vaccine were also very good, with no difference between the groups or in comparison with the results of other studies of MF59-adjuvanted vaccines [10].

Clinical experience with avian and human influenza vaccines in subjects who are presumed to have no pre-existing antibody (as in the case of the 2009 pandemia) suggests that two doses are required to induce a protective HI antibody titre, but we found that a single dose was sufficient. This is clinically relevant because it is well known that compliance to a double-dose regimen is usually poor and that a number of subjects remain unprotected even if they have received the first vaccine dose [11]. However, it is not clear whether the increase in the speed and magnitude of the antibody response in our subjects with β-thalassemia major was due to the addition of MF59 to the A/H1N1 antigen or to other factors, or a combination of both. High seroprotection rates have also been found in healthy subjects administered pandemic vaccines without adjuvants [12,13], which suggests that the pandemic H1 hemagglutinin is a strong antigen that can adequately stimulate the immune system even in hosts, as in our study, whose defences are a little less efficient than those of healthy subjects.

It is also possible that there may be a greater degree of pre-existing immunity against the pandemic virus in the population than originally presumed. As previously reported by others [14], we found that about one-third of our patients and controls had antibodies against HI and, although we excluded subjects who had suffered a recent influenza-like illness, it is not possible to exclude asymptomatic infection with the pandemic virus as local viral activity had already begun before the beginning of the study. Finally, it cannot be excluded that a previous vaccination with the traditional seasonal A/H1N1 strain may play a role. Although the 2009 pandemic A/H1N1 virus is antigenically very distant from recently circulating seasonal H1N1 strains, previous vaccinations may have led to the development of a certain degree of immune memory. The pandemic virus is still of the same H1N1 subtype, and there are

reports of cross-protection arising from exposure to antigenically drifted strains of the same influenza subtype [15].

In conclusion, the immune response to monovalent 2009 pandemic influenza A/H1N1 MF-59 adjuvanted vaccine is similar in splenectomised and non-splectomised patients with β-thalassemia major and in healthy subjects. Protection can be assured by a single vaccine dose. Moreover, also the safety and tolerability of the vaccine is similarly good. Although these data need to be confirmed in larger study populations, they could be extrapolated when considering influenza vaccine use in subjects with other hemoglobinopathies (i.e. sickle cell disease) or other types of splenectomised patients. Further studies are required to elucidate the greater immune response to the pandemic MF59-adjuvanted product in comparison with non-adjuvanted or differently adjuvanted influenza vaccines, and to evaluate immune responses in younger patients.

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## References

[1] Centers for Disease Control Prevention (CDC), Recommendations of the Advisory Committee on Immunization Practices (ACIP). 2009 prevention and control of seasonal influenza with vaccines. MMWR Morb Mortal Wkly Rep 2009;58:1–52.
[2] Dwyer J, Wood C, McNamara J, Williams A, Andiman W, Rink L, et al. Abnormalities in the immune system of children with β-thalassemia major. Clin Exp Immunol 1987;68:621–9.
[3] Deodlar HA, Marshall RJ, Barnes JN. Increased risk of sepsis after splenectomy. BMJ 1993;307:1408–9.
[4] Sullivan JL, Ochs HD, Schiffman G, Hammerschlag MR, Miser J, Vichinsky E, et al. Immune response after splenectomy. Lancet 1978;1:178–81.
[5] Castagnola E, Fioredda F. Prevention of life-threatening infections due to encapsulated bacteria in children with hyposplenia or asplenia: a brief review of current recommendations for practical purposes. Eur J Haematol 2003;71:319–26.
[6] Brydac LB, Machala M, Laguna P, Rokicka-Milewska R. Antibody response to influenza vaccination in splenectomized patients in Poland. J Clin Immunol 2004;24:225–36.
[7] Donaldson LJ, Rutter PD, Ellis BM, Greaves FE, Mytton OT, Pebody RG, et al. Mortality from pandemic A/H1N1 2009 influenza in England: public health surveillance study. BMJ 2009;339:b5213.
[8] Kelly H, Barr I. Large trials confirm immunogenicity of H1N1 vaccines. Lancet 2010;375:6–9.
[9] Menegon T, Baldo V, Bonello C, Dalla Costa D, Di Tommaso A, Trivello R, et al. Influenza vaccines: antibody responses to split virus and MF59-adjuvanted subunit virus in an adult population. Eur J Epidemiol 1999;15:573–6.
[10] Clark TW, Pareek M, Hoschler K, Dillon H, Nicholson KG, Groth N, et al. Trial of 2009 influenza A (H1N1) monovalent MF59-adjuvanted vaccine. New Engl J Med 2009;361:2424–35.
[11] Nelson JC, Bittner RC, Bounds L, Zhao S, Baggs J, Donahue JG, et al. Compliance with multiple-dose vaccine schedules among older children, adolescents, and adults: results from a vaccine safety datalink study. Am J Public Health 2009;99(Suppl. 2):S389–97.
[12] Greenberg ME, Lai MH, Hartel GF, Wichems CH, Gittleson C, Bennet J, et al. Response to a monovalent 2009 influenza A (H1N1) vaccine. New Engl J Med 2009;361:2405–13.
[13] Vajo Z, Tamas F, Sinka L, Jankovics I. Safety and immunogenicity of a 2009 pandemic influenza A H1N1 vaccine when administered alone or simultaneously with the seasonal influenza vaccine for the 2009–10 influenza season: a multicentre, randomized controlled trial. Lancet 2010;375:49–55.
[14] Hancock K, Veguilla V, Lu X, Zhong W, Butler EN, Sun H, et al. Cross-reactive antibody responses to the 2009 pandemic H1N1 influenza virus. New Engl J Med 2009;361:1945–52.
[15] Parkman PD, Hopps HE, Rastogi SC, Meyer Jr HM. Summary of clinical trials of influenza virus vaccines in adults. J Infect Dis 1977;136(Suppl.):S722–30.