# Humoral Response to the Influenza A H1N1/09 Monovalent AS03-Adjuvanted Vaccine in Immunocompromised Patients

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***Background.*** Few data are available regarding the immunogenicity and safety of the pandemic influenza vaccine in immunocompromised patients. We evaluated the humoral response to the influenza A H1N1/09 vaccine in solid-organ transplant (SOT) recipients, in patients with human immunodeficiency virus (HIV) infection, and in healthy individuals.

***Methods.*** Patients scheduled to receive the pandemic influenza vaccine were invited to participate. All participants received the influenza A H1N1/09 AS03-adjuvanted vaccine containing 3.75 μg of hemagglutinin. SOT recipients and HIV-infected patients received 2 doses at 3-week intervals, whereas control subjects received 1 dose. Blood samples were taken at day 0, day 21, and day 49 after vaccination. Antibody responses were measured with the hemagglutination inhibition assay (HIA) and a microneutralization assay.

***Results.*** Twenty-nine SOT recipients, 30 HIV-infected patients, and 30 healthy individuals were included in the study. Seroconversion measured by HIA was observed in 15 (52%) of 29 SOT recipients both at day 21 and day 49; in 23 (77%) of 30 at day 21 and 26 (87%) of 30 at day 49 in HIV-infected patients, and in 20 (67%) of 30 at day 21 and in 23 (77%) of 30 at day 49 in control subjects ($P = .12$ at day 21 and $P = .009$ at day 49, between groups). Geometric means of antibody titers were not significantly different between groups at day 21 or at day 49.

***Conclusions.*** Influenza A H1N1/09 vaccine elicited a similar antibody response in HIV-infected individuals and in control subjects, whereas SOT recipients had an overall lower response. A second dose of the vaccine only moderately improved vaccine immunogenicity in HIV-infected patients.

## BACKGROUND

In March 2009, clustered cases of respiratory infection in Mexico led to the discovery of a new subtype of influenza virus A H1N1 [1–3]. The virus spread rapidly worldwide, and it was estimated that ~59 million people in the United States had been infected with the virus as of February 2010. Although overall mortality associated with influenza A H1N1/09 infection was considered to be low [3], several groups of patients at risk for complications were identified: pregnant women [4], young people with chronic diseases [5] and immunocompromised patients [6, 7].

Vaccination against influenza A H1N1/09 was strongly recommended to patients at risk for complications. In particular, guidelines endorsed by the World Health Organization [8] recommended vaccination against influenza virus in human immunodeficiency virus (HIV)–infected individuals and in solid-organ transplant (SOT) recipients because of the potential risk of complications in these patients.

Several different preparations of the influenza A H1N1/09 vaccine were available for large-scale vaccination. In the United States, 2 doses of nonadjuvanted vaccine containing 15 μg of hemagglutinin were recommended for immunocompromised patients, whereas in Europe a vaccine adjuvanted with a squalen oil-in-

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water emulsion (either MF59 or AS03) and a lower dose of antigen was preferably used. Although some studies have compared the immunogenicity of these different vaccines in healthy children and adults [9–14], few data have been published regarding their use in immunocompromised patients [15]. In particular, there was no previous data on the use of the AS03 adjuvant in HIV-infected individuals and SOT recipients, and therefore the safety and efficacy of the vaccine with use of this adjuvant was unknown. Also, there was uncertainty regarding the need for a second dose of the vaccine in patients with impaired immunity.

On November 2009, the Swiss Federal Office of Public Health (FOPH) provided the influenza A H1N1/09 AS03-adjuvanted vaccine free of charge to all patients at risk for influenza, including HIV-infected individuals, SOT recipients, and health care workers. We prospectively assessed the safety and immunogenicity of this vaccine in these populations.

## METHODS

### Patients

Patients observed at the outpatient clinics of the Transplantation Centre and the Infectious Diseases Service from the University Hospital of Lausanne (CHUV) (Lausanne, Switzerland) were approached by nursing and medical staff to recommend the administration of the influenza A H1N1/09 vaccine. If the patients expressed interest in receiving the vaccine, then the study was presented by the investigators to the patients. Control subjects were enrolled from among members the staff of the hospital who were undergoing regular vaccination by announcing the study during staff meetings. Inclusion criteria for the SOT recipients group were age $\geqslant$18 years, $\geqslant$3 months after kidney and/or liver transplantation, creatinine clearance level $>$30 mL/min, and no episode of acute rejection over the previous month. Inclusion criteria for the HIV group were age $\geqslant$18 years and a CD4+ T cell count $>$200 cells/mL. Inclusion criteria for the control group were age $\geqslant$18 years and absence of an immunosuppressive condition. Exclusion criteria for all groups were pregnancy, allergy to egg, and previous serious adverse event related to receipt of influenza vaccine. Patients and control subjects who received the seasonal influenza vaccine before or simultaneously with the pandemic influenza vaccine were allowed to participate in the study. All subjects provided written informed consent. The study was approved by the local institutional review board.

The study intervention consisted in obtaining blood samples before vaccination (day 0), at day 21 after vaccination (ie, before the second dose of vaccine in the HIV-infected and SOT recipients groups), and at day 49 after the first vaccination. Subjects were contacted by telephone to determine whether they had experienced adverse events related to vaccination 24 h, 48 h, and 7 days after receipt of each vaccination. All adverse events were graded according to the following classification: mild, defined as no interference with normal activities; moderate, defined as some interference with normal activities; and severe, defined as preventing subjects from engaging in normal daily activities. After vaccination, patients were observed during a 6-month period for the development of influenza infection, and they were instructed to return to the outpatient clinics in case of symptoms compatible with influenza infection (respiratory illness, temperature $>$38°C, or myalgia). A real-time polymerase chain reaction for influenza A H1N1/09 from a nasopharyngeal swab sample was performed in all cases of clinical suspicion of influenza.

### Vaccine

The vaccine used in this study was the influenza A H1N1/09 AS03-adjuvanted vaccine (Pandemrix; GlaxoSmithKline). The vaccine was composed from a split inactivated A/California/07/2009 (H1N1)–derived strain of influenza virus containing 3.75 μg of hemagglutinin antigen. The AS03 adjuvant was composed of squalene (10.69 mg), DL-α-tocopherol (11.86 mg), and polysorbate 80 (4.86 mg). The vaccine was provided free of charge by the FOPH by 16 November 2009. SOT recipients and HIV-infected patients received 2 doses at a 3-week interval, whereas control subjects received 1 dose.

### Clinical Definitions

We used standard clinical definitions to evaluate the response to the vaccine. Seroconversion rate was defined as the rate of patients with $\geqslant$4-fold increase in antibody titers against influenza A H1N1/09 after vaccination. Seroconversion factor was defined as the level of increase in antibody titers before and after vaccination. Seroprotection rate was defined as the percentage of patients with an antibody titer after vaccination of $\geqslant$32 measured by hemagglutination inhibition assay (HIA) or $\geqslant$40 measured by microneutralization. The European Medicines Agency (EMEA) recommendations to evaluate the efficacy of the influenza vaccine require a seroconversion rate of $\geqslant$40%, a seroconversion factor of $\geqslant$2.5, and a seroprotection rate of $\geqslant$70%. The vaccination end points were primarily evaluated using the HIA, because only this assay has been correlated with vaccine protection.

### Laboratory Methods

Antibody responses were detected by means of microneutralization and HIA, according to standard methods [16], at the Centre for Infections, Health Protection Agency (London, UK) using egg-grown NIBRG-121 virus, a reverse-genetic virus containing hemagglutinin and neuraminidase from the influenza A/California/7/2009 strain (seed virus kindly provided by *National Institute for Biological Standards and Control*). Serum samples obtained from subjects were tested with the use of 1:2 serial dilutions. For HIAs, serum samples were treated with receptor-destroying enzyme (RDEII; Denka Seiken) according

**Table 1. Baseline Characteristics of Solid-Organ Transplant (SOT) Recipients, Human Immunodeficiency Virus (HIV)–Infected Patients, and Control Subjects**

| Variable | SOT recipients (n = 29) | HIV-infected patients (*n* = 30) | Control subjects (*n* = 30) | *P* |
|---|---|---|---|---|
| Age, mean years (± SD) | 47.7 ± 12.8 | 48.0 ± 9.8 | 41.8 ± 8.91 | .04 |
| Sex, M/F | 15/14 | 23/7 | 13/17 | .02 |
| Previous seasonal influenza vaccine (2009), no. (%) of subjects | 21 (72) | 24 (80) | 21 (70) | .85 |
| Time from transplant, median months (range) | 38 (5–231) | | | |
| Type of transplant, no. (%) of subjects | | | | |
| Kidney | 25 (86) | | | |
| Liver | 4 (14) | | | |
| Induction therapy, no. (%) of subjects | | | | |
| Thymoglobulin | 12 (41) | | | |
| Basiliximab | 15 (52) | | | |
| None | 3 (10) | | | |
| Maintenance immunosuppression, no. (%) of subjects | | | | |
| Prednisone | 16 (55) | | | |
| Tacrolimus | 22 (76) | | | |
| Cyclosporin | 5 (17) | | | |
| Mycophenolate | 21 (72) | | | |
| Azathioprine | 4 (14) | | | |
| mTOR inhibitor | 2 (7) | | | |
| Time from HIV infection diagnosis, years; median (range) | | 13 (1–29) | | |
| Nadir CD4+ T cell count, median cells/mL (range) | | 181 (2–464) | | |
| Current CD4+ T cell count, median cells/mL (range) | | 587 (312–1368) | | |
| Current undetectable viral load, no. (%) of patients | | 29 (97) | | |
| Patients receiving antiretroviral therapy, no. (%) of subjects | | | | |
| Type of antiretroviral therapy | | 30 (100) | | |
| NRTI + NNRTI | | 13 (43) | | |
| NRTI + PI | | 9 (30) | | |
| NRTI + NNRTI + PI | | 3 (10) | | |
| Other | | 5 (17) | | |

**NOTE.** mTOR, mammal target of rapamycine; NNRTI, nonnucleoside reverse-transcriptase inhibitor; NRTI: nucleoside reverse-transcriptase inhibitor; PI: protease inhibitor.

to the manufacturer's instructions and tested at an initial dilution of 1:8, and those samples that had results that were negative for the hemagglutinin antibody were assigned a titer of 1:4. Serum specimens were analyzed to determine absolute end point titers, and the final dilution was 1:32,768. For microneutralization assays, serum samples were tested at an initial dilution of 1:10, and those samples that had negative results were assigned a titer of 1:5. The final dilution was 1:320, and samples for which the end point titers were greater were assigned a value of 1:640. Specimens were tested in duplicate, and the geometric mean titers were used in the analyses.

### Statistical Analysis

Geometric mean titers of antibodies between groups were compared using the analysis of variance test. Seroconversion and seroprotection rates after vaccination were compared using the $\chi^2$ test. Seroconversion rates measured by HIA or microneutralization assays were compared using a κ correlation. A logistic regression model was used to describe the independent variables associated with vaccine response measured by the HIA. Variables of interest for SOT recipients included age, sex, time from transplantation, creatinine level, type of organ transplanted, induction therapy, and maintenance therapy. Statistical analysis was performed by PASW Statistics 18 software (SPSS).

## RESULTS

### Patient Population

A total of 89 subjects were enrolled in the study between November and December 2009; 29 SOT recipients (25 kidney transplant recipients and 4 liver transplant recipients), 30 HIV-infected individuals, and 30 healthy control subjects. Baseline

**Table 2. Adverse Events within the Week after Administration of Influenza Vaccine**

| | SOT recipients ($n = 29$) | | HIV-infected patients ($n = 30$) | | Control subjects ($n = 30$) | $P$ |
|---|---|---|---|---|---|---|
| Variable | After first dose | After second dose | After first dose | After second dose | After first dose | |
| Total adverse events, no. (%) of subjects | 28 (97%) | 20 (69%) | 28 (93%) | 22 (73%) | 27 (90%) | 0.38 |
| Local adverse events, no. (%) of subjects | 25 (86%) | 17 (59%) | 25 (93%) | 18 (60%) | 27 (90%) | 0.16 |
| Redness, total no. of subjects (mild/moderate/severe) | 2 (0/2/0) | 1 (1/0/0) | 5 (5/0/0) | 4 (3/1/0) | 3 (2/1/0) | |
| Induration, total no. of subjects (mild/moderate/severe) | 6 (5/1/0) | 5 (4/1/0) | 12 (10/2/0) | 9 (9/0/0) | 16 (10/5/1) | |
| Tenderness, total no. of subjects (mild/moderate/severe) | 25 (18/7/0) | 17 (13/4/0) | 25 (16/9/0) | 16 (12/4/0) | 27 (16/11/0) | |
| Systemic adverse events, no. (%) of subjects | 19 (65%) | 10 (34%) | 16 (53%) | 11 (37%) | 16 (53%) | 0.31 |
| Fever, no. of subjects | 2 | 2 | 2 | 0 | 3 | |
| Nausea, total no. of subjects (mild/moderate/severe) | 4 (2/2/0) | 3 (3/0/0) | 2 (2/0/0) | 2 (2/0/0) | 3 (3/0/0) | |
| Fatigue, total no. of subjects (mild/moderate/severe) | 18 (10/8/0) | 10 (8/2/0) | 15 (6/8/1) | 8 (6/1/1) | 16 (7/7/2) | |
| Other, total no. of subjects (Mild/Moderate/Severe) | 3 (3/0/0) | 5 (5/0/0) | 7 (7/0/0) | 5 (4/1/0) | 9 (9/0/0) | |

**NOTE.** A patient may present >1 adverse event. SOT, solid-organ transplant.

characteristics of the patients are shown in Table 1. Control subjects were younger than the other groups, and there were more male subjects included in the HIV-infected group. Overall, 66 (74%) of 89 subjects had previously received the seasonal influenza vaccine (72% in the SOT group, 80% in the HIV-infected group, and 70% in the control group) and 7 (7.9%) of 89 received both vaccines (seasonal and pandemic influenza) simultaneously.

Ninety percent of SOT recipients had received induction therapy at the time of transplantation with either thymoglobulin or basiliximab, and most of the patients received a maintenance combination therapy with tacrolimus and mycophenolate, with or without steroids. All HIV-infected individuals were receiving antiretroviral therapy at the time of vaccination, and 97% of them had undetectable viral load.

### Safety of the Influenza A H1N1/09 Vaccine

A majority of patients who received the vaccine experienced an adverse event (90% in the control group, 93% in the HIV-infected group, and 97% in the SOT group), although most of the reactions were considered to be mild and resolved without sequellae (Table 2). Patients who simultaneously received both seasonal and pandemic influenza vaccines did not develop more adverse events than did patients who received only the pandemic influenza vaccine (data not shown). Only 4 patients experienced an adverse event that was considered to be severe: 1 patient in the HIV-infected group experienced severe fatigue after each dose of vaccine, 2 patients in the control group also experienced severe fatigue, and 1 patient in the control group experienced an induration at the site of injection. Interestingly, 1 of the control subjects who experienced fatigue also had fever, and antibody levels before vaccination were elevated (1:512) in this patient; therefore, it is possible that this patient may have been infected coincidently by influenza.

### Immunogenicity of the Influenza A H1N1/09 Vaccine

The results regarding the immunogenicity of the vaccine are summarized in Table 3. A higher percentage of SOT recipients had detectable antibody levels at baseline (24% of SOT recipients, compared with 13% of HIV-infected individuals and 3% of control subjects, as measured by HIA; $P = .06$). Figure 1 shows the date of enrollment in the study according to patient group and the presence of antibody before vaccination.

Overall, HIV-infected patients and control subjects had a higher rate of seroconversion after vaccination than did SOT recipients. The administration of a second dose of the vaccine did not improve the rate of seroconversion among SOT recipients as measured by HIA. Most patients reached a seroprotected status after 1 dose of the vaccine, ranging from 72% to 96% in SOT recipients and from 80% to 100% in HIV-infected individuals (measured by HIA and by the microneutralization assay, respectively). Geometric means of antibody titers were comparable between groups, although there was a trend towards higher titers in HIV-infected patients (especially as measured by microneutralization assay). There were no significant differences in geometric means antibody titers between day 21 and day 49 for any group. Reverse cumulative distribution of antibody titers according to patient group and the assay used is shown in Figure 2.

We compared the seroconversion rate at day 49 for all subjects according to the test used. Twelve patients seroconverted by HIA but not by microneutralization assay, and 4 patients seroconverted by microneutralization assay but not by HIA. The overall agreement between tests was 82%, with a κ-value of .51 ($P < .001$), indicating a moderate agreement.

### Variables associated with Vaccine Response

Because only 4 patients in the HIV-infected group and 7 patients in the control group did not respond to the vaccine, we analyzed

**Table 3. Geometric Mean Titers Measured by Hemagglutinin Inhibition Assay and by Microneutralization Assay before and after Influenza Vaccination**

| | Hemagglutinin inhibition assay | | | | Microneutralization assay | | | |
|---|---|---|---|---|---|---|---|---|
| Variable | SOT recipient ($n$ = 29) | HIV-infected patients ($n$ = 30) | Control subjects ($n$ = 30) | $P$ | SOT recipients ($n$ = 29) | HIV-infected patients ($n$ = 30) | Control subjects ($n$ = 30) | $P$ |
| Seroconversion rate, % of subjects | | | | | | | | |
| Day 21 | 52 | 77 | 67 | .12 | 52 | 83 | 97 | <.001 |
| Day 49 | 52 | 87 | 77 | .009 | 65 | 90 | 87 | .03 |
| Geometric mean titer (95% confidence interval) | | | | | | | | |
| Day 0 | 8.6 (5.1–14.6) | 5.9 (4.2–8.1) | 5.9 (3.9–8.9) | .35 | 64.8 (41.8–100.5) | 28.6 (19.6–41.9) | 11.8 (7.0–19.8) | <.001 |
| Day 21 | 54.8 (27.3–110.0) | 97.0 (46.3–203.2) | 75.2 (32.5–173.7) | .64 | 274.6 (182.9–412.3) | 360.8 (255.6–509.2) | 366.1 (228.2–587.3) | .31 |
| Day 49 | 56.8 (28.6–112.9) | 116.7 (68.2–199.7) | 79.7 (39.1–162.5) | .61 | 322.6 (232.2–448.0) | 500.7 (409.3–612.4) | 323.7 (197.8–529.6) | .06 |
| Seroconversion factor (95% confidence interval) | | | | | | | | |
| Day 21 | 6.4 (3.1–13.1) | 16.5 (8.0–33.9) | 16.2 (7.3–35.8) | .12 | 4.2 (2.3–7.9) | 13.0 (8.3–20.6) | 31.1 (18.6–51.9) | <.001 |
| Day 49 | 6.6 (3.2–13.7) | 19.9 (11.3–34.9) | 15.3 (7.7–30.2) | .09 | 5.0 (2.7–9.3) | 18.1 (11.6–28.2) | 27.5 (16.0–47.1) | <.001 |
| Seroprotection rate, % of subjects | | | | | | | | |
| Day 0 | 24 | 13 | 3 | .06 | 72 | 40 | 17 | <.001 |
| Day 21 | 72 | 80 | 70 | .65 | 96 | 100 | 93 | .36 |
| Day 49 | 72 | 93 | 80 | .10 | 100 | 100 | 93 | .13 |

**NOTE.** SOT, solid-organ transplant.

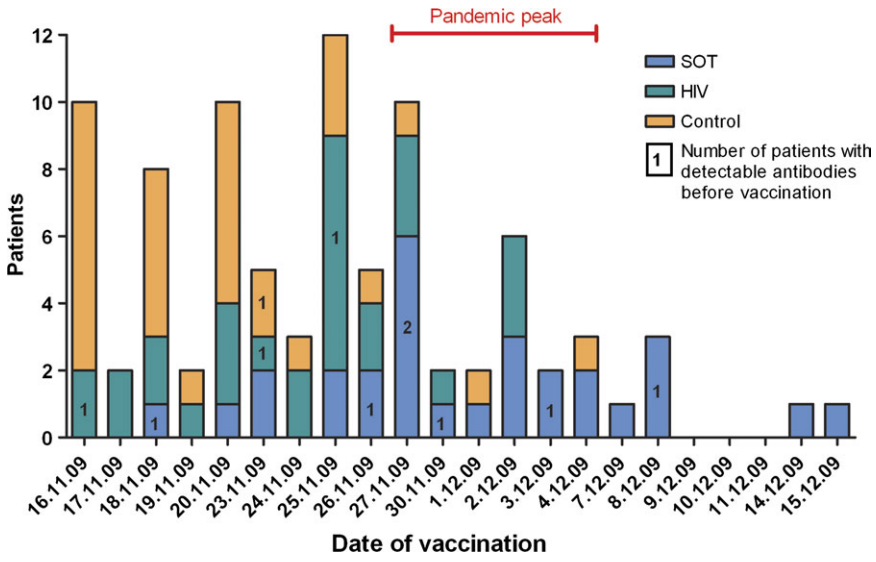


**Figure 1.** Date of vaccination according to patient group. The red line shows the week when the influenza A H1N1/09 pandemic reached its peak in Switzerland (Swiss Federal Office of Public Health) Grippe pandémique (H1N1) 2009 en Suisse, semaines 17 (2009) à 8 (2010). Bull OFSP 2010; no 20: 535-543. Numbers inside the columns denote the number of patients with detectable antibodies before vaccination. SOT, solid-organ transplant.

the variables associated with vaccine response only in SOT recipients. In the univariate analysis, vaccine responders were younger, had a longer follow-up period after transplantation, and were not receiving a triple-drug regimen (Table 4). In the multivariate analysis, only age remained a significant factor for vaccine response (odds ratio [OR], 0.90; 95% confidence interval [CI], 0.83–0.98 for each increase of 1 year; $P = .021$).

### Outcomes at 6 Months

No patient developed clinically or microbiologically documented influenza A H1N1/09 infection during the follow-up period. A kidney transplant recipient received a diagnosis of breast cancer after receiving the second dose of the vaccine, and a liver transplant recipient developed a transitory ischemic attack between receiving the first and the second dose of the vaccine. No episode of acute rejection was diagnosed in SOT recipients during the 6-month follow-up period after vaccination. No HIV-infected individual developed an opportunistic infection after vaccination.

## DISCUSSION

In this prospective study, we assessed the immunogenicity and safety of the influenza A H1N1/09 A03-adjuvanted vaccine in immunocompromised patients, and we compared the results to those for a control group of healthy volunteers. We observed a similar response in antibody levels in HIV-infected individuals, compared with the response in control subjects, whereas the response in SOT recipients was lower, overall, than was the response in the other 2 groups. Of note, all groups reached the recommended target for vaccine approval according to the EMEA after receipt of the first dose of vaccine. Improvement in the vaccine response was only moderate after administration of the second dose of the vaccine in the HIV group.

A number of studies have evaluated the immunogenicity of the influenza A H1N1/09 vaccine in different populations, mostly in immunocompetent adults and children [9–14]. Most studies have found an appropriate response to the vaccine using both adjuvanted and nonadjuvanted vaccines, although a higher response was generally seen with adjuvanted vaccines. For example, seroconversion rates after 1 dose of 15 μg of nonadjuvanted vaccine was 63%–72% in a cohort of 176 adults, whereas the response to 7.5 μg of MF59-adjuvanted vaccine was 77%–96% [9]. A randomized trial involving 937 children from 6 months to 13 years of age [12] showed a better immunogenicity associated with receipt of 2 doses of 1.875 μg of the AS03-adjuvanted vaccine than with receipt of 2 doses of 7.5 μg of the nonadjuvanted vaccine (98% vs 80% in children $<3$ years of age and in 99% vs 95% in those $>3$ years of age). In this trial, systemic reactions were more commonly seen in association with receipt of the adjuvanted vaccine, although serious adverse events were rare [12]. In our study, the control group had a lower response rate to the vaccine than was expected (67% seroconversion rate at day 21, measured by HIA). It is not clear why we observed this slightly lower-than-expected response, although it may be attributable to the relatively modest sample size of our study.

Experience with the influenza A H1N1/09 vaccine in immunocompromised patients is limited, because the immunogenicity of the vaccine has only been evaluated in HIV-infected

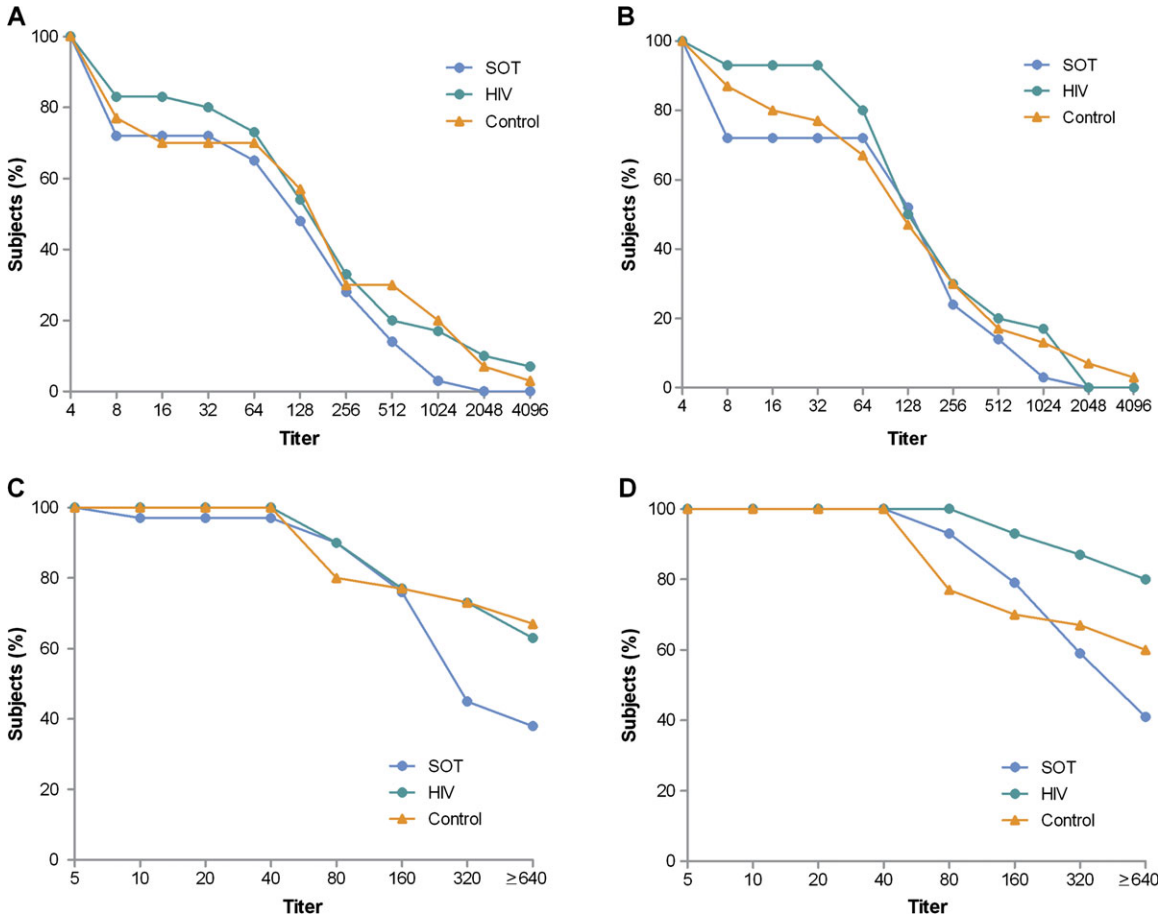


**Figure 2.** Reverse cumulative-distribution curves of antibody titers measured by hemagglutinin inhibition assay in serum samples obtained on day 21 (*A*) and on day 49 (*B*) and measured by microneutralization in serum samples obtained on day 21 (*C*) and on day 49 (*D*), according to the patient population.

individuals [15]. Bickel et al [15] observed a 69% seroconversion rate among 160 HIV-infected individuals who received 1 dose of the AS03-adjuvanted influenza vaccine, which was the same vaccine used in our study. Variables associated with a favorable vaccine response included younger age and higher CD4+ T cell count. Compared with their findings, we observed an 8% higher seroconversion rate in our cohort of treated HIV-infected patients after receipt of 1 dose of the vaccine. Regarding SOT

**Table 4. Variables associated with Vaccine Response in the Group of Solid-Organ Transplant (SOT) Recipients**

| Variable | Responders ($n$ = 15) | Nonresponders ($n$ = 14) | $P$ |
|---|---|---|---|
| Age, mean years (±SD) | 41.9 ± 14.2 | 54.3 ± 7.1 | .006 |
| Sex, M/F | 8/7 | 7/7 | >.99 |
| Time from transplant, median months | 78 | 19 | .07 |
| Creatinine, mean μmol/L (±SD) | 122.4 ± 37.8 | 126.4 ± 47.5 | .39 |
| Type of transplant, no. of patients | | | .037 |
| Kidney | 11 | 14 | |
| Liver | 4 | 0 | |
| Induction therapy, patients | | | .36 |
| Thymoglobulin | 5 | 7 | |
| No thymoglobulin | 10 | 7 | |
| Maintenance immunosuppression, patients | | | |
| Triple therapy[a] | 5 | 10 | .04 |
| Dual or monotherapy | 10 | 4 | |

[a] Calcineurine inhibitor (cyclosporine or tacrolimus) plus prednisone plus antimetabolite (mycophenolate or azathioprine).

recipients, no studies have been published, to our knowledge, that have assessed the immunogenicity of the influenza A H1N1/09 vaccine.

The AS03-adjuvanted vaccine was, overall, well-tolerated in the 3 groups. Safety was a potential concern for SOT recipients, because experience with adjuvanted influenza vaccines is limited in this population, and some clinicians were reluctant to use adjuvanted influenza vaccines in this population because of the possible risk for acute rejection. In addition, recent data suggest that influenza vaccination can temporarily increase nonspecific cellular alloreactivity in SOT recipients[17]. This has not been observed in our study, nor has it been observed in previous studies that have used MF59-adjuvanted seasonal influenza vaccine in kidney and heart transplant recipients [18, 19]. Several recent studies with nonadjuvanted influenza vaccine also provide evidence against an association between influenza vaccination and immunologic dysregulation in SOT recipients [20, 21].

A significant proportion of patients in our study had test results that were positive for antibodies before vaccination. Although, especially in older patients, this can be the result of a cross-reactivity with nonpandemic influenza H1N1 infection [22], it may also represent the consequence of previously unrecognized infection [23], because the vaccination campaign in Switzerland was performed during the peak of the pandemic influenza season. The group that included more individuals with preexisting antibodies was the SOT recipient group, which included more individuals who were enrolled slightly later in the study (although patients who were seropositive for antibodies before vaccination included individuals who were enrolled throughout the inclusion period). The baseline seroprotection rate was high in the SOT group (76%) when the antibody response was measured by microneutralization assay. In addition, seroconversion rates were also higher when measured by microneutralization assay than when measured by HIA. This may be explained by the fact that the HIA only measures the proportion of antibodies that are directed to the receptor-binding site of viral hemagglutinin, whereas the microneutralization assay detects a broader range of neutralizing antibodies. However, only the HIA has been correlated with vaccine protection [24]. A higher rate of seroprotection against influenza A H1N1 when measured by the microneutralization assay was also seen in a serological survey in England [23].

We evaluated the variables associated with vaccine response in the group of SOT recipients. In the multivariate analysis, a younger age was the only variable significantly associated with vaccine response, which is a consistently recognized factor for a favorable vaccine response. Regarding the influenza A H1N1/09 vaccine, a recent study that also used the AS03-adjuvanted vaccine showed that immunogenicity was better in healthy adults $<60$ years of age than it was in older individuals [25]. In addition, a large trial in China that used the nonadjuvanted vaccine also showed a lower rate of seroprotection among the elderly population (79%) than among younger adults (97%) [11].

Our study has several limitations. First, this was not a randomized trial, and therefore we did not control for the vaccine administration. For example, a significant number of patients simultaneously received the seasonal vaccine, and this may have influenced the antibody response to the influenza A H1N1/09 vaccine. However, this was not seen in a randomized trial that involved 355 patients [26], in which immunogenicity of the pandemic influenza vaccine was similar irrespective of whether the patient had simultaneously received the seasonal vaccine. Second, we enrolled 2 different groups of immunocompromised patients (likely with different net states of immunosuppression); therefore, extrapolation of these results to other immunocompromised populations should be done with caution. Indeed, treated HIV-infected individuals can elicit a response similar to that for healthy control subjects, and we would expect a lower vaccine response rate among HIV-infected individuals with low CD4+ T cell counts [27]. However, the information derived from our study is useful, because it encourages the use of the influenza vaccine in similar patients.

In conclusion, the immunogenicity of the influenza A H1N1/09 AS03-adjuvanted vaccine was appropriate in all 3 groups, although higher responses were observed among treated HIV-infected patients and healthy control subjects. A second dose of the vaccine only slightly improved the immunogenicity, and therefore, a single dose would probably be more cost-effective in large-scale vaccinations. However, additional data from larger studies would be needed to confirm our results. These data on influenza A H1N1/09 have practical implications for strategies surrounding influenza vaccination in specific immunocompromised patients during the next influenza season.

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