Immunogenicity and safety of seasonal and 2009 pandemic A/H1N1 influenza vaccines for patients with autoimmune diseases: a prospective, monocentre trial on 199 patients

A. Kostianovsky[1], P. Charles[1], J.-F. Alves[1], M. Goulet[1], C. Pagnoux[1], V. Le Guern[1], L. Mouthon[1], A. Krivine[2], P. Villiger[1,3], O. Launay[1], L. Guillevin[1]
for the French Vasculitis Study Group (FVSG)

[1]*Department of Internal Medicine, Hôpital Cochin, Assistance Publique-Hôpitaux de Paris, Université Paris-Descartes, Paris;* [2]*Department of Virology, Hôpital Cochin, Assistance Publique-Hôpitaux de Paris, Université Paris-Descartes, Paris, France;* [3]*Department of Rheumatology, University of Bern, Bern, Switzerland.*

**Alex Kostianovsky, MD*
Pierre Charles, MD
Jean-François Alves, MD
Michèle Goulet, MD
Christian Pagnoux, MD, MPH
Véronique Le Guern, MD
Luc Mouthon, MD, PhD
Anne Krivine, MD
Peter Villiger, MD, PhD
Odile Launay, MD, PhD
Loïc Guillevin, MD

**Present address: Internal Medicine Department, CEMIC University Hospital, Buenos Aires, Argentina.*

Please address correspondence and reprint requests to:
Loïc Guillevin, MD,
Department of Internal Medicine,
Hôpital Cochin,
27 rue du Faubourg Saint-Jacques,
75014 Paris, France.
E-mail: loic.guillevin@cch.aphp.fr

Received on March 18, 2012; accepted in revised form on April 13, 2012.

Clin Exp Rheumatol 2012; 30 (Suppl. 70): S83-S89.

Key words: flu, vaccination, vasculitis, lupus, systemic sclerosis, Sjögren's syndrome, immunosuppressants, anti-CD20.

Funding: This study, registered at ClinTrial.gov no. NCT01065285, was supported by grants from INSERM, the Programme Hospitalier de Recherche Clinique (PHRC) and the FVSG, and sponsored by the Assistance Publique-Hôpitaux de Paris.

Competing interests: none declared.

ABSTRACT

Objectives. *The 2009 pandemic A/H1N1 influenza outbreak represented a theoretical risk for patients with autoimmune diseases (AID), especially those immunosuppressed. This study was undertaken to evaluate immunogenicity and tolerance of seasonal (SFV) and A/H1N1 flu vaccines (HFV) in AID patients.*

Methods. *This prospective, open, monocentre, vaccine phase-III study on 199 patients with AID (systemic necrotising vasculitides, progressive systemic sclerosis, systemic lupus erythematosus, Sjögren's syndrome and others), treated or not with immunosuppressants, was conducted from September 2009 to June 2010, to evaluate SFV and HFV efficacy and safety. Subjects received SFV (1 dose, Mutagrip®) and/or non-adjuvant HFV (Panenza®, 2 doses at a 3-week interval). The primary judgment criterion was the seroprotection rate. Secondary outcome measures were seroconversion rates, vaccine tolerance, and numbers of flu syndromes, and AID flares and relapses throughout the 6 month observation period.*

Results. *After SFV inoculation, 1% of the patients became febrile, 18% developed local reactions, 80% were seroprotected and 38% seroconverted. After HFV immunisation, 4% of the patients developed a fever, 23% had local reactions, 65% were seroprotected and 83% seroconverted. Twelve patients developed 15 flu syndromes (3 patients developed 2 syndromes each); 2 of these episodes were temporally consistent with vaccination; 1 patient died of septic shock unrelated to vaccination. Nineteen mild AID flares occurred during follow-up, only 6 being temporally consistent with HFV and SFV.*

Conclusions. *Our findings demonstrated the safety and efficacy of SFV and HFV in AID patients.*

Introduction

The pandemic outbreak of A/H1N1 influenza, in June 2009, in Mexico, the US, and then other countries (1, 2), represented a theoretical risk for patients with autoimmune diseases (AID), especially those taking immunosuppressants. Those patients were encouraged to be vaccinated, independently of their treatment and degree of immunosuppression. In addition to efficacy, the role of vaccination in the occurrence of AID flares has not been definitively elucidated. The results of several reports suggested a link between immunisation (especially virus-based vaccines) and AID flares (3, 4). Notably, vaccination and hyposensitisation have been considered factors triggering Churg-Strauss syndrome (CSS) (5) and anti-neutrophil cytoplasm antibody (ANCA)-associated vasculitides in general (6). However, the benefits of vaccination are now recognised and immunisation in the context of AID, like systemic lupus erythematosus (SLE), is recommended (7). Independently of safety issues, vaccine immunogenicity and impact on flares remain to be evaluated in AID patients treated or not with immunosuppressants.

In this context, we started, in September 2009, a prospective trial designed to evaluate the immunogenicity and safety of seasonal (SFV)- and pandemic A/H1N1-influenza vaccines (HFV) in AID patients.

Patients and methods

Patients

Patients with the following AID were

included after giving their written informed consent: systemic necrotising vasculitides (SNV), systemic sclerosis (SScl), Sjögren's syndrome (SS) with detectable anti-SSA/SSB autoantibodies and satisfying the international criteria (8), SLE and others. All patients were >18 years old, lived in the Paris area, and were followed at our Referral Centre. We did not include patients with allergies to any of the vaccine components or a history of hypersensitivity to anti-flu vaccine, fever or acute infection during the week preceding inoculation, history of active neuropathy or Guillain-Barré syndrome, human immunodeficiency virus infection and/or primary immunodeficiency.

Seventy-four patients with SNV (10 polyarteritis nodosa, 4 microscopic polyangiitis, 28 granulomatosis with polyangiitis (Wegener's) (GPA), 20 CSS (eosinophilic granulomatosis with polyangiitis), 11 Behçet's disease, 1 cryoglobulinaemia), 32 with SScl, 29 with SLE, 23 with SS, and 28 patients with various other AID (polymyositis, dermatomyositis, ankylosing spondylitis, rheumatoid arthritis, sarcoidosis, psoriatic arthritis, idiopathic retroperitoneal fibrosis, polychondritis, autoimmune haemolytic anaemia, primary antiphospholipid syndrome) were included.

Study goals

The primary goal was to evaluate SFV and HFV humoral immunogenicity in the study population. The percentages of patients with haemagglutination-inhibition antibody (HIA) titers ≥1:40 (haemagglutination-inhibition test), determined in serum samples obtained 3 weeks after vaccination against SFV and 3 weeks after the second HFV shot, defined the seroprotection rate. Secondary goals were: 1) comparison of individual seroconversion rates after vaccination (HIA titer <1:10 before and ≥1:40 after vaccination and/or a 4-fold HIA-titer increase after immunisation compared to prevaccination value) for different subgroups; 2) evaluation of the impact of corticosteroids, immunosuppressants or biotherapy on SFV and HFV immunogenicity, assessed as the serum HIA titer; 3) local and systemic tolerance to SFV and HFV (numbers and intensities of local and systemic adverse reactions during the 5 days postinoculation), incidence and severity of flu syndromes, defined as fever >37.8°C, cough and/or sore throat; and 4) the numbers of AID relapses occurring during the observation period.

Methods

Patient selection began in September 2009, with inoculations during the French national flu-vaccination campaign: SFV began in October 2009, and the first HFV dose was given between November and December 2009, 3 weeks after SFV.

Study design

The study design is outlined in Figure 1. Patients fulfilling inclusion criteria were enrolled and gave their written informed consent on day 0. After a comprehensive interview and physical examination, blood was drawn and SFV was administered. Immediate tolerance (local and systemic) was assessed by a physician. In addition, patients were instructed to note any reaction to the immunisation for the next 4 days, filling out a detailed diary form on which they had to grade the severity of the possible reactions, *i.e.*, fever, chills, headache or other general symptoms, and local pain, redness or inflammation. This questionnaire was returned to the physician at the next protocol-scheduled visit. This procedure was followed for every immunisation. Three weeks later, a second consultation took place: after the in-depth interview (during which patients were specifically asked about the presence of flu symptoms, local or systemic adverse reactions to SFV injection, activity of their underlying AID, treatment changes and any other

Action	SFV	HFV dose 1	HFV dose 2	-	-
Post vaccinal Self-administered questionnaire given	-	+	+	+	-
In depht interview & physical exam	+	+	+	+	+
Blood sample drawn	R + S	R + S	R	R + S	R + S

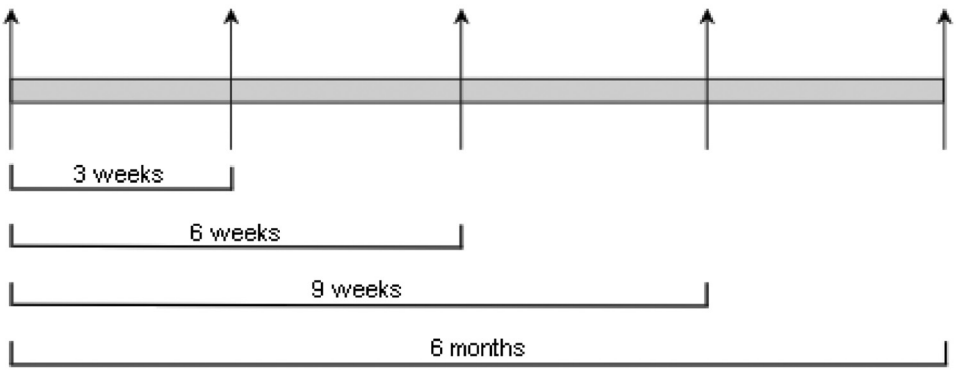

Fig. 1. Study design. +: present; –; absent; R: routine; S: serum library.

remarkable medical event) and assuring the absence of any abnormality in their physical examination, blood was drawn and, once available, the first HFV dose was administered. Because HFV was not available for all patients 3 weeks after SFV administration, an additional visit was scheduled 3 weeks later for the first HFV shot. Follow-up visits were repeated until the final visit, 6 months after the study began (4.5 months after the second HFV shot).

To obtain more detailed follow-up information, in addition to the 3 postvaccination self-monitoring questionnaires, patients were asked to post monthly self-administered questionnaires reporting the presence of fever, cough and/or sore throat, and emergency medical visits, prescriptions of antiviral drugs or antibiotics, subjective activity level of the underlying AID and any immunosuppressive treatment changes. All questionnaire responses were entered into the database by a member of our group (AK). When the H1N1 pandemic started in Europe, we decided to vaccinate all patients who had not been previously vaccinated against it as soon as possible, omitting the SFV, which was no longer needed at that time.

Vaccines

SFV was Mutagrip® (Sanofi-Pasteur MSD), a trivalent, inactivated-influenza single-dose vaccine. The vaccine licensed for the 2009–2010 season contained A/Brisbane/59/2007/3/2006 (H1N1), A/Brisbane/10/2007 (H3N2) and B/Brisbane/60/2008 strains, and was formulated to contain 15 µg of each strain's haemagglutinin antigen. Vaccines were prepackaged in 0.5-mL syringes and injected intramuscularly into the deltoid muscle.

HFV was Panenza® (Sanofi-Pasteur MSD), a monovalent, inactivated, split-virion, A/H1N1 vaccine. The vaccine seed virus was prepared from the reassortant virus NYMC X-179A (New York Medical College, New York City, NY) generated from the A/California/7/2009 strain, as recommended by World Health Organisation (WHO). HFV was formulated to contain 15 µg of haemagglutinin antigen in a 0.5-mL dose that was injected intramuscularly into the deltoid muscle. In accordance with French Ministry of Health guidelines, 2 doses (given at a 3-week interval) of non-adjuvant vaccines were administered.

Serological/immunological assays were performed in a centralised laboratory (Sanofi-Pasteur Global Clinical Immunology Laboratory, Swiftwater, PA). The HIA titer against the vaccine strain was measured in all samples by a validated haemagglutination-inhibition (HI) assay, as described by the WHO Collaborating Centre for Influenza (Centres for Disease Control, Atlanta, GA) (9). Serum samples were subjected to an enzymatic treatment and heated to destroy non-specific inhibitors. The HI assay was run in microtiter test plates, with turkey erythrocytes and the A/California/7/2009 (H1N1v) strain serving as the antigen. HI assays were run in duplicate for each treated serum sample, with serial 2-fold dilutions starting at 1:10. The sample titer retained was the highest dilution that completely inhibited haemagglutination. Negative samples were assigned a titer of 1:5. The HIA geometric mean titers (GMT) at each time were used for the analyses.

Statistical analyses

Parameter values for descriptive analyses are expressed as means ± standard deviation or median (range) for continuous variables, according to their distribution, and n (%) for qualitative variables. The following factors expected to be associated with the immune response to HFV were analysed: age, sex, AID type, corticosteroid use and SFV immunisation. Generalised linear models taking into account repeated measures were used to identify the factors independently associated with that response. Two models were built: one considering $\log_{10}$/GMT titers and the other seroconversion; normality of residuals was verified for the former. For all analyses, significance was defined as $p \leq 0.05$.

Results

Patients and global immune responses

One hundred and ninety-nine patients, 134 women and 65 men, mean age 53.4±15.1 (range 19–90) years, participated in the study: 173 received SFV and, among the 197 given HFV, 174 received both doses.

Immune responses to SFV and HFV achieved seroprotection (80% and 64.5%, respectively) and seroconversion (39.6% and 71.7% of those seroprotected, respectively), as detailed in Table I. Because SFV was administered to 59 patients by their general practitioners, they were not tested for HIA on day 0. The successive available GMT for SFV and HFV are reported for the indicated groups in Table 1 and individually in Figure 2A and B. After SFV inoculation, anti-H1N1 antibody GMT rose, albeit not to protective levels: from 8.22 (n=103) on SFV day 0 to 13.4 (n=197 Table I) 3 weeks later, on HFV day 0.

Immune responses according to AID

Among the 62 SNV patients immunised with SFV, 79% were seroprotected and 49% of them seroconverted (Table I). Seventy-nine SNV patients were inoculated with HFV: 64.5% achieved seroprotection and 78.4% of them seroconverted. Figure 3A and C, respectively, report the successive individual GMT HIA titers after SFV and HFV injections according to AID. Among the 33 SScl patients immunised against SFV, 87.8% were seroprotected and 41.4% of them seroconverted. Thirty-four SScl patients were vaccinated with HFV: 70.6% became seroprotected and 54.2% of them seroconverted. Among 28 SLE patients who received SFV, 71.4% were seroprotected and 55% of them seroconverted. Thirty-two SLE patients were inoculated with HFV, 65.6% were seroprotected and 85.7% of them seroconverted.

Immune responses according to treatment

The immune responses according to therapy are reported in Table I. Figure 3C and D, respectively, report the successive individual GMT HIA titers after SFV and HFV inoculations according to therapy. At the time of SFV vaccination, 75 patients were non-immunosuppressed, *i.e.* not receiving cytotoxic agents and/or taking <10 mg of corticosteroids/day. Among them, 86.7% were seroprotected and 47.7% of them seroconverted. Among the 86 non-im-

Table I. Seroprotection and seroconversion rates and GMT after SFV and HFV administration to AID patients, according to disease and its therapy.

Parameter	All AID patients	AID type			Ongoing treatment		
		SNV	SScl	SLE	Non-IS	IS	Biotherapy
Anti-SFV							
n.	173	62	33	28	75	94	15
Seroprotected, n (%)	139 (80.3)	49 (79.0)	29 (87.9)	20 (71.4)	65 (86.7)	75 (79.8)	9 (60)
Seroconverted, n (%)	55 (39.6)	24 (49)	12 (41.4)	11 (55)	31 (47.7)	30 (40)	1 (11.1)
Pre-SFV titer, n (%)	32 (18.5)	14 (22.6)	6 (18.2)	3 (10.7)	13 (17.3)	19 (20.2)	1 (6.7)
GMT day 0	22.1	20.1	24.3	18.4	22.11	23.7	13.48
GMT SFV week 3	88.7	82.9	88.3	89.1	106.9	92.1	31.4
GMT SFV week 9	77.3	75.7	75.9	79.8	85	83.7	32.2
Anti-HFV							
n	197	79	34	32	86	108	16
Seroprotected, n (%)	127 (64.5)	51 (64.6)	24 (70.6)	21 (65.6)	59 (68.6)	68 (63)	5 (31.3)
Seroconverted, n (%)	91 (71.7)	40 (78.4)	13 (54.2)	18 (85.7)	42 (71.9)	47 (69.1)	5 (100)
Pre-HFV titer, n (%)	32 (16.2)	12 (15.2)	7 (20.6)	5 (15.6)	16 (18.6)	18 (16.7)	0
GMT day 0 (SFV week 3)	13.4	12.9	19.7	12.1	15	13.6	7.4
GMT HFV week 3	40.7	41.8	45.5	49	48.2	40.2	12.5
GMT HFV week 6	50.3	52.3	60.3	62.4	62.5	47.4	14.6
GMT month 6	27.8	30.4	32.8	24.9	30.5	27.1	10

Non-IS: non-immunosuppressed (no treatment or <10 mg of corticosteroids/day); IS: Immunosuppressed (≥10 mg of corticosteroids/day and/or immunosuppressant(s)); biotherapy: rituximab, adalimumab, etanercept or infliximab; SNV: systemic necrotising vasculitides; SScl: systemic sclerosis; SLE: systemic lupus erythematosus.

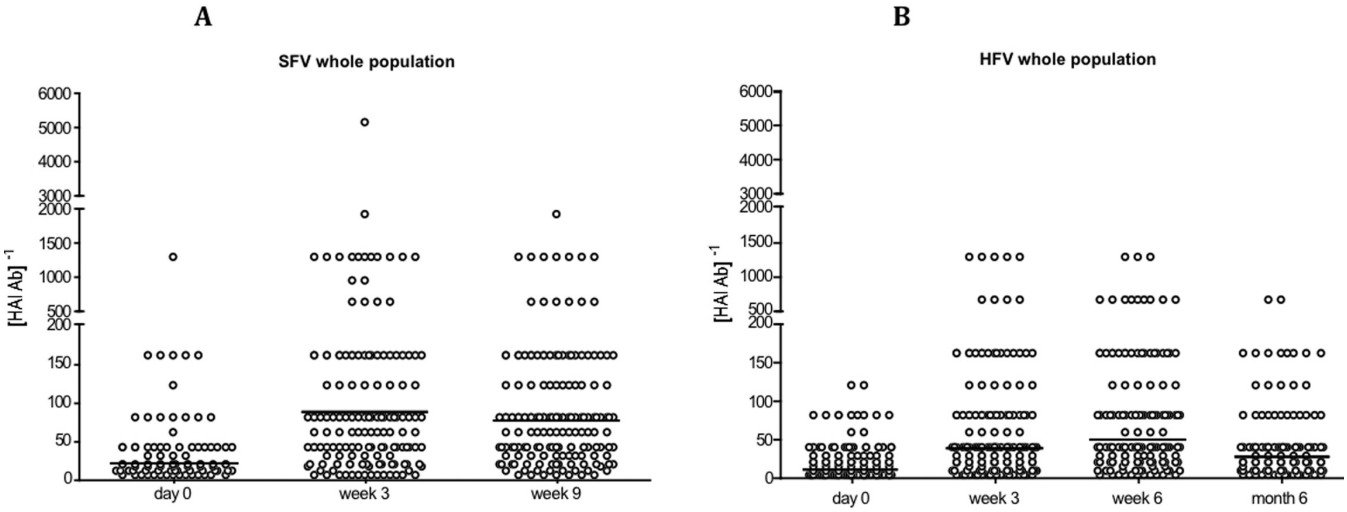

Fig. 2. All AID patients inoculated with SFV (**A**) or HFV (**B**). Each circle represents an individual patient. Horizontal lines represent the group's haemagglutinin-inhibition antibody (HIA) GMT.

munosuppressed patients injected with HFV, 68.6% became seroprotected and 71.1% of them seroconverted.

At the time of SFV administration, 94 patients were being treated with immunosuppressant(s) (azathioprine, pulse cyclophosphamide, mycophenolate mofetil, tacrolimus, cyclosporine and/or methotrexate) and/or ≥10 mg of corticosteroids/day. Among them, 79.8% became seroprotected and 40% of them seroconverted. Among the 108 immunosuppressed patients vaccinated against HFV, 63% were seroprotected and 69.1% of them seroconverted.

At the time of SFV inoculation, 16 patients were receiving a biotherapy: rituximab for 8, etanercept for 4, adalimumab for 3 and infliximab for 2 (1 patient received both etanercept and adalimumab). Among them, 60% achieved seroprotection and 11.1% of them seroconverted. Among the 16 biotherapy patients administered HFV, 31.3% became seroprotected and all 5 of them seroconverted. Although a trend was observed for weaker responses mounted under biotherapies, too few patients were included in this group to allow any conclusion to be drawn.

Factors associated with vaccinal response

Younger age and SFV immunisation were independently associated with higher $\log_{10}$/GMT anti-HFV titers (Table II), but not type of AID, corticosteroid use or sex. Younger age was the only factor significantly associated with seroconversion.

Adverse reactions, flu syndromes and deaths

Local reactions (local pain, redness or swelling) and systemic symptoms (fever, chills, headache) after SFV and

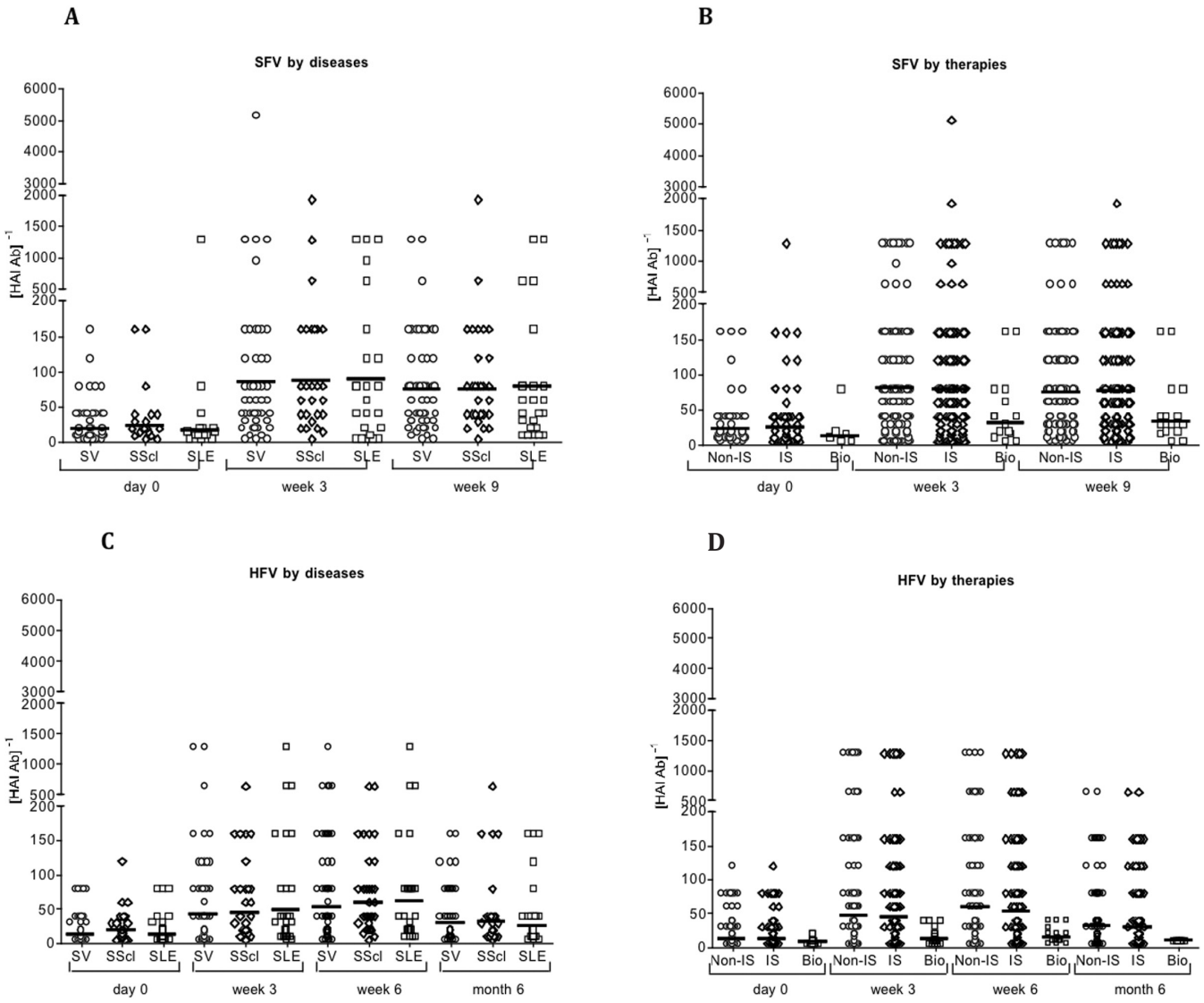

Fig. 3. Sequential anti-SFV (**A** and **B**) and anti-HFV (**C** and **D**) humoral responses according to AID (**A** and **C**) and its treatment (**B** and **D**). Each symbol represents an individual single patient. Horizontal lines represent the corresponding group's GMT. Non-IS: non-immunosuppressed (no treatment or <10 mg of corticosteroids/day); IS: immunosuppressed (≥10 mg of corticosteroids/day and/or immunosuppressant(s)); Bio: biotherapy (rituximab, adalimumab, etanercept or infliximab); SNV: systemic necrotising vasculitides; SScl: systemic sclerosis; SLE: systemic lupus erythematosus.

HFV inoculations, detailed in Table III, were mild. Among 12 patients developing 15 flu syndromes (3 patients developed 2 syndromes each), 2 were temporally consistent with both vaccinations; none required hospitalisation. During the study, 1 patient died of septic shock unrelated to the flu or vaccination.

AID flares

Throughout the study, 19 AID flares occurred. Six were temporally related to vaccination (within 30 days of inoculation): polyneuritis in a CSS patient (3 days after the 1st HFV dose); arthritis and purpura in a Wegener's disease patient (20 days after the 1st HFV dose); skin rash in an SS patient (10 days after receiving SFV); aphthae in a patient with Behçet's disease (the day of the 1st HFV dose); arthralgias in a patient with ankylosing spondylitis (2 days after the 1st HFV dose); and asymptomatic hypereosinophilia in a CSS patient (3 days after the 1st HFV dose).

Thirteen mild flares observed during the study were temporally unrelated to vaccination: cough and haematuria in a Wegener's disease patient; ulcers in a patient with Behçet's disease; migraine in a giant cell arteritis patient; neurolupus (seizures) in a SLE patient, myositis-arthritis and calcinosis abscess in a SScl patient (2 different flares in the same patient); arthritis in a SLE patient and a rash in another; keratitis in an SS patient, sinusitis in a Wegener's disease patient; arthralgias in a sarcoidosis patient and another with Behçet's disease; and arthralgias in an ankylosing spondylitis patient.

Discussion

Since the first descriptions of hepatitis viruses triggering cryoglobulinaemia (10) or polyarteritis nodosa (11) almost 40 years ago, much attention has been paid to the role of infections as a contributor to the development of autoimmunity. Since most vaccines are based on microbial particles and/or in-

Table II. Model for titer values.

Model items	Estimate	Standard error	*p*-value
3 weeks	1.10366	0.11258	<0.0001
6 weeks	1.28044	0.10988	<0.0001
9 weeks	0.69014	0.11369	<0.0001
Connective tissue disease	–0.06631	0.10708	0.536
Other diseases	–0.20994	0.13647	0.124
Age	–0.08770	0.03424	0.010
Sex	0.01783	0.10462	0.865
Corticosteroid use	0.00834	0.01090	0.444
No SFV	–0.57712	0.12276	<0.0001

Generalised linear model taking into account repeated measures of study parameters that might influence log titers of anti-H1N1 antibodies.

Table III. Adverse reactions, flares and deaths.

Event	Vaccination	
	Related	Unrelated
Reactions postvaccination		
Local		
SFV	32 (18%)	
HFV	45 (23%)	
Systemic		
SFV	2 (1%)	
HFV	8 (4%)	
Flu syndromes	2	13
AID flares	6	13
Deaths	0	1

activated pathogen forms, whether or not to recommend vaccination for AID patients has been a matter of serious debate (12, 13).

At present, the decision to immunise AID patients is accepted in clinical practice, especially those with SLE. For other AID, the benefit/risk ratio of vaccination also favours immunisation (9, 14-16). To date, vaccination has been considered a factor that could induce autoimmunity or favour flares only for CSS (17). This prospective study, as some others (18), was designed to determine SFV and HFV efficacy and tolerance in patients with various AID, and to try to answer to some questions about their seroprotection and seroconversion, according to disease, treatments, tolerance, safety and, especially, AID flares/relapses.

Among all the AID patients who received SFV and HFV, 80% and 65%, respectively, became seroprotected. No difference was observed according to AID type, among those included in the study. Surprisingly, being on immunosuppressive therapy was not associated with a lower seroprotection level than non-immunosuppressed patients. However, patients receiving biotherapies had a trend to lower protection rates after both SFV and HFV, compared to immunosuppressed and non-immunosuppressed patients.

Intriguingly, SFV positively enhanced the anti-HFV antibody titers. Although this "booster effect" might represent a synergic potentiation, probably due to shared antigenic motifs between the 2 vaccines, it was not sufficiently strong to elicit a protective immune response; the latter was achieved only after HFV inoculation. However, the second HFV dose did not significantly increase the seroprotection rate, since maximum protective antibody titers had already been reached at week 6, 3 weeks after the first dose. Therefore, according to our data, the second HFV dose failed to provide any additional protective benefit.

Overall outcomes were good after 6 months of follow-up. The low flu incidence in France after the outbreak of 2009 pandemic A/H1N1 in the Americas might have negatively biased our findings: the occurrence of 15 flu syndromes during follow-up (7.5% of the 199 recruited patients) is lower than would be expected; however, because we do not have an unvaccinated control population for comparison, it is difficult to reach definitive conclusions regarding protection. Nonetheless, it is clear that overall tolerance and safety of SFV and HFV was acceptable, in light of the low numbers of adverse local and systemic reactions and vaccine-related AID flares observed.

In conclusion, our findings demonstrated SFV and HFV safety and efficacy of in AID patients.

References

1. DAWOOD FS, JAIN S, FINELLI L *et al*.: Emergence of a novel swine-origin influenza A (H1N1) virus in humans. *N Engl J Med* 2009; 360: 2605-15.
2. CHOWELL G, BERTOZZI SM, COLCHERO MA *et al*.: Severe respiratory disease concurrent with the circulation of H1N1 influenza. *N Engl J Med* 2009; 361: 674-9.
3. ZAFRIR Y, AGMON-LEVIN N, SHOENFELD Y: Post-influenza vaccination vasculitides: a possible new entity. *J Clin Rheumatol* 2009; 15: 269-70.
4. POU MA, DIAZ-TORNE C, VIDAL S *et al*.: Development of autoimmune diseases after vaccination. *J Clin Rheumatol* 2008; 14: 243-4.
5. VANOLI M, GAMBINI D, SCORZA R: A case of Churg-Strauss vasculitis after hepatitis B vaccination. *Ann Rheum Dis* 1998; 57: 256-7.
6. BIRCK R, KAELSCH I, SCHNUELLE P, FLORES-SUAREZ LF, NOWACK R: ANCA-associated vasculitis following influenza vaccination: causal association or mere coincidence? *J Clin Rheumatol* 2009; 15: 289-91.
7. LU CC, WANG YC, LAI JH, LEE TS, LIN HT, CHANG DM: A/H1N1 influenza vaccination in patients with systemic lupus erythematosus: safety and immunity. *Vaccine* 2011; 29: 444-50.
8. VITALI C, BOMBARDIERI S, JONSSON R *et al*.: Classification criteria for Sjögren's syndrome: a revised version of the European criteria proposed by the American-European Consensus Group. *Ann Rheum Dis* 2002; 61: 554-8.
9. SCHATTNER A: Consequence or coincidence? The occurrance, pathogenesis and significance of autoimmmune manifestations after viral vaccines. *Vaccine* 2005; 23: 3876-86.
10. ELIAKIM M, SLAVIN S, ZLOTNICK A: Chronic liver disease associated with mixed cryoglobulinemia. Review of the literature and presentation of two cases. *Med Chir Dig* 1972; 1: 67-72.
11. GOCKE D, HSU K, MORGAN C, BOMBARDIERI S, LOCKSHIN M, CHRISTIAN C: Association between polyarteritis and Australia antigen. *Lancet* 1970; 2: 1149-53.
12. AGMON-LEVIN N, PAZ Z, ISRAELI E, SHOENFELD Y: Vaccines and autoimmunity. *Nat Rev Rheumatol* 2009; 5: 648-52.
13. CLARKE AJ, GULATI P, ABRAHAM SM: A cross-sectional audit of the uptake of seasonal and H1N1 influenza vaccination among patients with rheumatoid arthritis in a London hospital. *Clin Exp Rheumatol* 2011; 29: 596.
14. STASSEN P, SANDERS J, KALLENBERG C, STEGEMAN C: Influenza vaccination does not result in an increase in relapses in patients with ANCA-associated vasculitis. *Nephrol Dial Transplant* 2008; 23: 654-8.

15. ISRAELI E, AGMON-LEVIN N, BLANK M, SHOENFELD Y: Adjuvants and autoimmunity. *Lupus* 2009; 18: 1217-25.
16. LITINSKY I, BALBIR A, ZISMAN D *et al*.: Vaccination against influenza in patients with systemic sclerosis. *Clin Exp Rheumatol* 2012 Mar 9. [Epub ahead of print]
17. GUILLEVIN L, PAGNOUX C, MOUTHON L: Churg-Strauss syndrome. *Semin Respir Crit Care Med* 2004; 25: 535-45.
18. BOURNIA VK, SFONTOURIS C, KONSTA M, ILIOPOULOS A: H1N1 influenza outcome in rheumatic patients under biological therapy. *Clin Exp Rheumatol* 2012 Feb 28. [Epub ahead of print].