

# Double blind randomized comparison of a subunit and a virosomal influenza vaccine in HIV positive Patients

John Evison<sup>a</sup>, Kathrin Mühlemann<sup>b</sup>, Hansjakob Furrer<sup>a\*</sup>

Department of Infectious Diseases, University Hospital, Berne, Switzerland <sup>a</sup> and Institut für Infektionskrankheiten University Bern, Switzerland <sup>b</sup>

John Evison, MD  
Klinik und Poliklinik für Infektiologie  
Inselspital, CH- 3010 Bern  
john-marc.evison@insel.ch

## Abstract

**Background:** Virosomal influenza vaccines have been shown to induce better response rates in non-immunocompromised patients. No studies have been performed in HIV positive patients, which have directly compared a virosomal influenza vaccine to other formulations.

**Methods:** Double-blind block-randomized trial with a subunit (Influvac® 2005/2006) and virosomal vaccine (Influvac plus® 2005/2006), manufactured by Solvay Pharma AG (Switzerland). Each vaccine contained 15µg of hemagglutinin (HA) of a A/California/20/99(H3N2)-like, of a A/New Caledonia/20/99 (H1N1)-like and of a B/Shanghai/361/2002-like strain. Sera were collected at baseline and follow up for determination of protective anti-HA titres (1:≥40) and seroconversion rates (1:≥4). Side effects were documented with questionnaires.

**Results:** Sixty-three patients received the subunit and 68 the virosomal vaccine. Baseline characteristics (age, sex, risk for HIV acquisition and CDC stage) did not differ significantly. The groups were similar with regard to median absolute (453 vs 398/µL) and relative (23 vs 24%) CD4 cell counts, rates of HAART (76 vs 78%) and HIV viral loads <50copies/ml.

Both vaccines induced significant anti-HA antibody titres rises in all strains, but these did not differ between vaccines. The A/California strain was associated with the highest titres rises, followed by A/New Caledonia and B/Shanghai. Protective and seroconversion rates behaved similar.

There were no significant differences in side effects for both vaccines. As there were no statistical significant differences between both vaccines, data were pooled to identify factors for trend in achieving protective anti-HA antibody titres against all vaccine strains. In the multivariate analysis CD4 cell count ≥200/µL (OR 3.3, 95% CI 1.1-9.8, p=0.02) and log viral load (OR 0.8, 95% CI 0.8-0.9, p=0.008) predicted the trend for achieving protective anti-HA titres against all vaccine strains.

**Conclusions:** The virosomal vaccine was not associated with higher rates of seroconversion or protective anti-HA antibodies titres in comparison to a subunit vaccine. CD4 cell counts and HIV viral load determine the immune response to influenza vaccine.

## Background

Influenza is an annual recurring event associated with a substantial excess mortality among risk groups, e.g. immunocompromised patients, including HIV positive patients.

The efficacy of influenza vaccination depends on the antigenic shift and drift of the virus and the ability of the immune system to adequately respond to the antigens. Unfortunately, immunocompromised persons not only have a higher risk of morbidity and mortality of influenza infection, but they can also show lower response rates to vaccination. Due to their virus-like structure virosomal vaccines are thought to be closer to the natural infectious process and might be associated with higher immunogenicity. Trials comparing virosomal influenza vaccines to older formulations have so far been scarce and were conducted mainly in elderly persons. Their results have been conflicting. A trial comparing a virosomal influenza vaccine to another formulation in immunocompromised patients has not been conducted so far.

## Methods

**Ethics:** Approved by local ethics board  
**Design:** Prospective block-randomized, double blinded controlled trial  
**Patients:** Adult (≥16 years) HIV positive outpatients, regardless of CD4 cell count or concomitant diseases. Exclusion criteria: acute febrile conditions and known allergy to egg proteins.  
**Vaccines:** Subunit (Influvac®2005/2006) and virosomal (Influvac®plus 2005/2006) influenza vaccine (Solvay Pharma AG (Bern, Switzerland) Antigens 15µg each of A/California/20/99(H3N2)-like, 15µg of A/New Caledonia/20/99 (H1N1)-like and 15µg of HA B/Shanghai/361/2002-like hemagglutinin (HA). Deep intramuscular deltoid injection.  
**Immunogenicity:** Serum anti-hemagglutinin titres (anti-HA) at baseline and 6 weeks after vaccination, (samples were stored at -20 °C until analysis). Anti-HA titres determined according to Palmer et al. at the World Health Organization collaborating centre for virus reference and research (Université Claude Bernard, Lyon, France).  
**Antigens used:** H2N2 A Panama strain for A California H1N1 A New Caledonia for A Caledonia B Hong Kong 330/2001 for B Shanghai strain  
**Protection:** anti-HA titres ≥ 1:40  
**Reactogenicity:** Seroconversion: ≥ fourfold increase of anti-HA titres  
**Safety:** Immediate side effects recorded by study staff. Side effects during the first 7 days documented in a patient administered diary.

## Results

### Study population

One hundred and thirty-one HIV positive patients were enrolled between 17<sup>th</sup> October and 16<sup>th</sup> December 2005. The characteristics of the study population are shown in Table 1. The study groups were well balanced for all parameters except for comorbidities, patients in the virosomal group presenting a higher Charlson Index.

**Table 1. Baseline characteristics of 131 HIV positive patients**

Vaccine	Virosome		Subunit		P
	N	%	N	%	
<b>Total</b>	68	51.9	63	48.1	
<b>Age, years (mean, SD)</b>	42.8	9.7	42.9	9.7	0.9
<b>Male</b>	48	70.5	48	76.1	0.6
<b>HIV Transmission</b>					0.3
MSM	28	41.1	27	42.9	
Heterosexual	26	38.3	30	47.6	
Intravenous drug abuse	9	13.3	5	8.0	
Unknown	5	7.3	1	1.5	
<b>Charlson Index (median, range)</b>	2	1-3	1	0-2	0.04
<b>Nicotine consumption</b>	29	43.9	30	47.6	0.6
<b>Patients with first influenza vaccination</b>	9	13.8	4	6.6	0.1
<b>Stage of HIV infection</b>					0.5
Stage A	31	45	31	51	
B	25	37	21	33	
C	12	18	10	16	
<b>CD4 cell count/µL</b>					0.1
≥ 500/	25	37	25	40	
≥ 200-500	35	51	31	49	
< 200	8	12	7	11	
<b>CD4 absolut (mean, SD)</b>	461	236.2	463	238.9	0.9
<b>CD4 % (mean, SD)</b>	25	8.2	23	9.3	0.3
<b>CD4/CD8 ratio (median, IQR)</b>	0.49	0.35-0.76	0.51	0.28-0.77	0.5
<b>HIV-1 RNA &lt;50 copies/ml</b>	43	63.2	43	68.2	0.3
<b>HAART</b>	53	77.9	48	76.1	0.8
<b>Interval baseline to follow up, days (mean, SD)</b>	55.9	46.8	47.1	31.3	0.2
<b>Interval to last influenza vaccination, days (median, IQR)</b>	389	373-426	395	368-730	0.5

### Immunogenicity and Reactogenicity

Baseline and follow up geometric mean anti-HA titres (GMT) were comparable for both vaccines (Table 2). The GMTs showed a gradient with highest titres for the A/California, followed by the A/New Caledonia and the B/Shanghai strain. Immunogenicity (anti-HA titres 1:≥40) were comparable for both vaccines at baseline and follow up, but showed a similar gradient for the individual vaccine strains (Table 3). Fifty to 75% of patients had protective anti-HA antibody titres at follow up. Of those patients without protective anti-HA antibody titres at baseline between 40 to almost 75% achieved titres ≥1:40. Reactogenicity, defined as an ≥fourfold increase of anti-HA from baseline to follow up was comparable between both vaccines (Table 5).

**Table 2. Geometric mean anti-HA titres (95% CI) at baseline and follow up**

	A/California (H3N2)		A/New Caledonia (H1N1)		B/Shanghai	
	Baseline	Follow up	Baseline	Follow up	Baseline	Follow up
<b>Virosome</b>	25.1 (18.3-34.6)	113.7 (83.0-155.9)	33.2 (23.7-46.4)	75.5 (57.9-98.6)	20.7 (16.6-25.7)	36.2 (28.2-46.6)
<b>Subunit</b>	28.9 (18.3-34.6)	126.4 (91.5-174.6)	27.1 (18.7-39.4)	72.0 (57.9-98.6)	28.2 (16.6-25.7)	57.8 (42.2-79.3)
	<b>P=0.7</b>	<b>P=0.5</b>	<b>P=0.8</b>	<b>P=0.3</b>	<b>P=0.4</b>	<b>P=0.7</b>

**Table 3. Protective anti-HA titres (1:≥40) at baseline and follow up**

	A/California (H3N2)		A/New Caledonia (H1N1)		B/Shanghai	
	Baseline N (%)	Follow up N (%)	Baseline N (%)	Follow up N (%)	Baseline N (%)	Follow up N (%)
<b>Virosome</b>	15/68 (22.0)	54/68 (79.4)	20/68 (29.4)	52/68 (76.4)	10/68 (14.7)	32/68 (47.0)
<b>Subunit</b>	20/63 (31.7)	49/63 (77.7)	17/63 (26.9)	43/63 (68.2)	14/63 (22.2)	34/63 (53.9)
		<b>P=0.6</b>		<b>P=0.8</b>		<b>P=0.4</b>

**Table 4. Rate of protective anti-HA titres (1:≥40) at follow up in patients with non-protective titres at baseline**

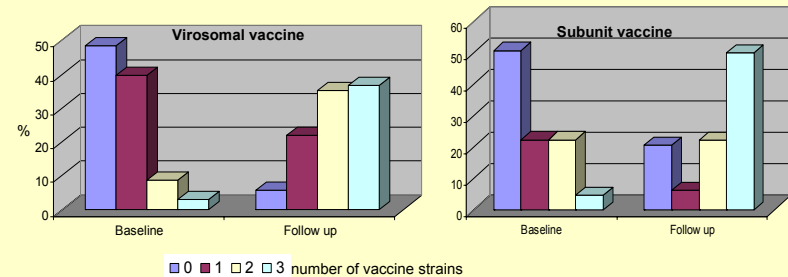
	A/California (H3N2) N (%)	A/New Caledonia (H1N1) N (%)	B/Shanghai N (%)
<b>Virosome</b>	39/53 (73.5)	32/48 (66.6)	23/58 (39.6)
	<b>P=0.6</b>		<b>P=0.8</b>
<b>Subunit</b>	29/43 (67.4)	26/46 (56.5)	20/49 (40.8)
			<b>P=0.8</b>

**Table 5. Seroconversion rate (≥ fourfold increase in anti-HA titre)**

	A/California (H3N2) N (%)	A/New Caledonia (H1N1) N (%)	B/Shanghai N (%)
<b>Virosome</b>	42/68 (61.7)	33/68 (48.5)	16/68 (23.5)
	<b>P=0.7</b>	<b>P=0.7</b>	<b>P=0.8</b>
<b>Subunit</b>	43/63 (68.2)	29/63 (46.0)	17/63 (26.9)

Figure 1 shows the number of vaccine strains to which patients had protective anti-HA titres at baseline and at follow up.

**Figure 1. Number of vaccine strains with protection at baseline and follow up**



### Safety

Both vaccines were well tolerated. There were no severe immediate reactions. Side effects occurring during the first 7 days after vaccination were of minor severity and consisted mostly of tiredness followed by malaise, headache, muscle pains and arthralgias (data not shown).

### Predictors of immunogenicity and reactogenicity

Data of both vaccine groups were combined in order to identify predictors of immunogenicity and reactogenicity using ordered logistic regression. HIV-1 RNA <50 copies/ml and CD4 ≥200/µL predicted better seroprotection and seroconversion, whereas hepatopathy (elevated ALAT or ASAT) was associated with lower seroprotection in the multivariate analysis, which included also age, sex and Charlson index (Table 6).

**Table 6. Multivariate analysis for trend of seroprotection and seroconversion**

	OR	95% CI	p
<b>Protective anti-HA titres (1:≥40)</b>			
CD4 ≥200/µL	4.3	1.6-11.9	0.005
HIV-1 RNA <50c/mL	3.4	1.7-7.0	0.001
Hepatopathy	0.35	0.14-0.88	0.025
<b>Seroconversion rate (≥4fold increase of titer)</b>			
CD4 ≥200 /µL	3.1	1.1-8.7	0.03
HIV-1 RNA <50c/mL	2.0	1.0-3.8	0.048
Hepatopathy	0.5	0.2-1.2	0.1

## Conclusion

- Virosomal and subunit influenza vaccines have similar rates of immunogenicity and reactogenicity in HIV positive patients and are equally well tolerated
- A high rate of patients achieve protection against ≥ 2 vaccine strains
- CD4 cell counts > 200/µl and successful ART (plasma HIV-RNA <50c/mL) predict a better response to influenza vaccination