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ORIGINAL ARTICLE

Seroepidemiology of novel influenza A (H1N1) infection among HIV-infected patients in the era of highly active antiretroviral therapy

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Background: The seroprevalence and seroincidence of novel influenza A infection among HIV-infected patients, who were believed to have more severe outcomes than healthy individuals, are rarely investigated in the era of highly active antiretroviral therapy (HAART). Our aim was to determine the seroprevalence and seroincidence of novel influenza A infection among HIV-infected patients in Taiwan.
Methods: Between September and November 2009, before the implementation of a nationwide vaccination for novel influenza A in Taiwan, 931 HIV-infected patients and 566 persons seeking voluntary counseling and testing (VCT) for HIV infection at our university hospital were enrolled in this study. Antibody responses to novel influenza A were determined using a hemagglutination-inhibition (HI) assay.
Results: HIV-infected patients had a significantly lower seroprevalence of novel influenza A infection than VCT clients (14.7% vs. 33.9%, $p < 0.001$). The seroincidence of novel influenza A infection among HIV-infected patients was 9.4% (95% confidence interval [CI]: 7.6—11.4). On the multivariate analysis, heterosexual (odds ratio [OR]: 1.89; 95% CI: 1.105—3.227) and baseline HI titer (OR: 1.02; 95% CI: 1.001—1.038) were significantly associated with seroconversion to novel influenza A virus.

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Conclusion: HIV-infected patients demonstrated a lower seroprevalence of novel influenza A infection than HIV-uninfected patients in Taiwan in the HAART era. Among HIV-infected patients, seroconversion to novel influenza A virus, which was infrequent during the 2009 influenza epidemic, was associated with heterosexual behavior and baseline HI titer.

Introduction

In April 2009, the World Health Organization (WHO) reported that a swine-origin influenza A (H1N1) virus (novel influenza A virus) was spreading via human-to-human transmission and had raised serious public concerns around the world.[1] Between April 2009 and March 2010, an estimated 43—88 million cases were infected in the United States during this H1N1 pandemic.[2] By the end of May 2010, 18,114 deaths from causes related to H1N1 infection had been reported worldwide; pneumonia was reported as a contributing cause of death in 7.9% of all deaths reported in the 122 Cities Mortality Reporting System.[2]

Although it remains controversial whether novel influenza A is more virulent than seasonal influenza, several risk factors associated with adverse outcome following novel influenza A infection have been identified.[3—5] In addition, among individuals who were hospitalized for influenza and related complications, HIV-infected patients who had a lower CD4 count were reported to develop more severe complications to novel influenza A infection, and these patients often developed secondary bacterial infections including pneumonia.[6] Nevertheless, two recent publications reported that the clinical presentation and clinical outcomes of HIV-infected patients who were also infected with novel influenza A were similar to those of the general population.[7,8]

After the identification of the first case of novel influenza A infection in Taiwan in late May 2009, the novel influenza A epidemic started in June 2009.[9] From June 2009 through April 2010, a total of 13,931 specimens, mainly from patients with symptoms of influenza-like illness who sought healthcare at the hospitals or clinics around Taiwan, were screened for novel influenza A, and 3274 (23.5%) of these specimens were confirmed as novel influenza A infection.[9] A nationwide free-of-charge vaccination program for novel influenza A was implemented in November of 2009 for individuals at high risk of developing influenza A-related complications and, in December of 2009 this program was implemented in healthy adults. As of April 2010, the Taiwan Centers for Disease Control estimated that 24.5% (5,667,703/23,133,074) of the people living in Taiwan had received the novel influenza A vaccine.[10]

In this study, our aim is to compare the seroprevalence of novel influenza A virus infection between HIV-infected patients and persons seeking voluntary counseling and testing (VCT) for HIV and to examine the seroincidence of novel influenza A virus infection among HIV-infected patients who received HIV care, including highly active antiretroviral therapy (HAART), in Taiwan, where the novel influenza A epidemic started in June 2009, peaked in late August and November, and subsided in December 2009.[9]

Methods

Study setting and population

In Taiwan, HIV-related care, including HAART and the laboratory tests that monitor virological and immunologic status before or after the initiation of HAART, was introduced in Taiwan in 1997 and is provided free-of-charge to patients at designated hospitals. As a public health response to the HIV epidemic, a program that provides anonymous voluntary counseling and testing (VCT) for HIV has been in use since 1990.

Between September and November 2009, residual blood samples were collected from HIV-infected patients who visited the hospital for the determination of CD4 and plasma HIV-RNA load (PVL) and from HIV-negative persons who sought VCT services. The study period spanned 3 months, which is the interval the vast majority of HIV-infected patients who seek regular HIV care go before needing to refill their antiretroviral therapy. We included blood samples from all of the patients who returned to the hospital to monitor their CD4 count and plasma HIV-RNA load, which was performed every 3—6 months according to the DHHS guidelines.[11] In this study, we estimated that the blood samples included in this study were from 60% of all of the HIV-infected patients who sought HIV care during the 3-month study period.

VCT clients were chosen as a comparator group for this seroprevalence study because more than 70% of HIV-infected patients and VCT clients are homosexual males and they might share similar socioeconomic backgrounds in the metropolitan area around Taipei. Patients were excluded from the present study if they had received a novel influenza A vaccine at the time of enrollment. A standardized case collection form was used to retrospectively record data on demographics, clinical characteristics, and laboratory results of the HIV-infected patients. This study was approved by the institutional review board of the hospital and all patients gave written informed consent.

Laboratory investigations

The antibody response of the serum samples to a novel influenza A strain, A/California/07/2009, was determined using a hemagglutination-inhibition (HI) assay, according to the standard protocols of the World Health Organization Collaborating Centre for Reference and Research on Influenza, Melbourne, Australia.[12] The receptor-destroying enzyme (RDE [III], Deka Seiken Co. Ltd, Tokyo, Japan) was used to treat serum samples that were titrated in a 2-fold dilution from 1:10 to 1:1280 in order to examine its

ability to disturb hemagglutination by novel influenza A. For each serum sample, duplicate experiments were performed. Titers were expressed as the reciprocal of the highest dilution of serum that prevented hemagglutination. A Serum HI-antibody titer of $\geq$ 40 was defined as seroreactive to novel influenza A. A $\geq$ 4-fold increase in titers, or conversion from seronegative to HI titers $>$ 40 between two sequential serological samples from the same individual was defined as evidence of novel influenza A seroconversion.

For those subjects who had elevated HI-antibody titers, archived blood samples that were collected during or before the study period were identified and subjected to the HI assay. The HI titer that was determined was designated as the baseline HI titer. The baseline specimens from individuals seeking anonymous HIV screening were identified by their unique code that consisted of the first letter of their identification card, birth year, gender, and the last four digits of their identification card. The interval between the two blood samples was around 3 months. Those patients who did not have paired samples for HI-antibody determination were excluded from the estimation of seroincidence. HI assays were not performed on archived blood samples from patients who did have elevated HI titers due to the assumption that they were seronegative.

Statistical analysis

All statistical analyses were performed using SPSS software version 16.0 (SPSS Inc., Chicago, IL, USA). Associations between categorical variables were analyzed using the χ^2 test or Fisher's exact test, whereas the means or medians of continuous variables were compared using the Student *t* test or Mann-Whitney *U* test. All tests were two-tailed and a *p*-value $<$ 0.05 was considered significant. For the univariate and multivariate analysis, the individuals were divided into three groups based on their year of birth. The year 1957 was chosen because according to previous reports,[13,14] individuals born before 1957 might have been exposed to a strain of H1N1 similar to 1918 strain, which could induce cross-reactivity to the novel influenza A virus. The year 1975 was chosen simply to compare results with a US study,[14] in which the authors reported that individuals born before 1975 might have been exposed to the swine influenza outbreak that occurred in New Jersey in 1976 and had cross-reactivity to novel H1N1. The predictors for antibody response and seroconversion to novel influenza A were analyzed using linear regression and logistic regression models. Based on the findings of previous studies, we assumed that the seroconversion rate of the study population would be 10% and the prevalence would be 30%.[15,16] Therefore, we estimated the sample size, *n*, needed to make *P* (estimated rate-true rate $>$ d) $<$ 0.05 using the formula $n \geq 1.96^2NP(1-P)/(N-1)d^2 + 1.96^2P(1-P) \approx 1.96^2P(1-P)/d^2$, where *P* is the true rate and *N* is the total number of patients in the study population. We assumed that *N* would be large. Thus, we obtained an estimated sample size of 385 cases for seroconversion analysis and 897 cases for seroprevalence analysis would be required to reach 80% statistical power with 3% error.

Results

Clinical characteristics and seroprevalence of novel influenza A

Between September and November 2009, a total of 1497 patients, including 931 HIV-infected patients and 566 HIV-uninfected VCT clients, were tested for antibody responses to novel influenza A infection (Fig. 1). The interval between the collection of blood samples was the same in both groups (September 2009 to November 2009). Compared with HIV-uninfected persons, HIV-infected patients were older (median age: 37 vs. 27 years, $p < 0.001$) and were predominately male (94.2% vs. 90.5%, $p = 0.007$) (Table 1). Among HIV-infected patients, 49.2% were born between 1957–1975 and 12.7% were born before 1957, while among HIV-uninfected individuals, 85.2% were born after 1975. For HIV-infected patients, the median CD4 cell count and the percentage of patients with plasma HIV-RNA load $<$ 1.60 $\log_{10}$ copies/mL were 446 cells/μL (range: 3–3,078 cells/μL) and 56%, respectively (Table 1). When HI assays were performed, almost 75% of HIV-infected patients (74.9%; 697/931) were receiving HAART with a median duration of 47.2 months (range: 0.03–161.7 months). During the study period, none of the study patients were hospitalized due to novel influenza A infection.

Compared with the HIV-uninfected subjects, HIV-infected patients had a statistically and significantly lower overall seroprevalence of novel influenza A (14.7% vs. 33.9%, $p < 0.001$) (Table 1). The seroprevalence of novel influenza A in each of the two groups of patients are plotted according to age and month in Figs. 2A and 2B, respectively. The seroprevalence of novel influenza A was significantly higher in HIV-uninfected individuals than HIV-infected patients across all age groups (Fig. 2A). A significant lower seroprevalence of influenza virus infection was observed among HIV-1-infected individuals than HIV-1 uninfected individuals in October and November (9.6% [35/365] vs. 78/191 [40.8%] in October, $p < 0.001$; 22.9% [72/314] vs. 76/163 [46.6%] in November, $p < 0.001$) (Fig. 2B). On the multivariate logistic analysis, receipt of HAART (odds ratio [OR]: 2.10; 95% confidence interval [CI]: 1.110–3.955; $p = 0.02$), heterosexual behavior (OR: 2.14; 95% CI: 1.335–3.428; $p = 0.002$), and baseline HI titer (OR: 1.03; 95% CI: 1.007–1.045; $p = 0.008$) were statistically and significantly associated with an elevated antibody level to novel influenza A (Supplementary Table 1). Age was not statistically or significantly associated with an elevated antibody level to novel influenza A, although a significantly higher proportion of those who received HAART were indeed older than those who had not received HAART ($p < 0.001$) (data not shown).

Incidence of novel influenza A infection among HIV-infected patients and contributing factors

Paired serum samples were available from 113 of 137 seroreactive HIV-infected patients (82.5%) that were then used to determine the seroincidence of novel influenza A (median interval between collection of the two paired samples: 6.05 months; range: 2.59–10.61 months), of

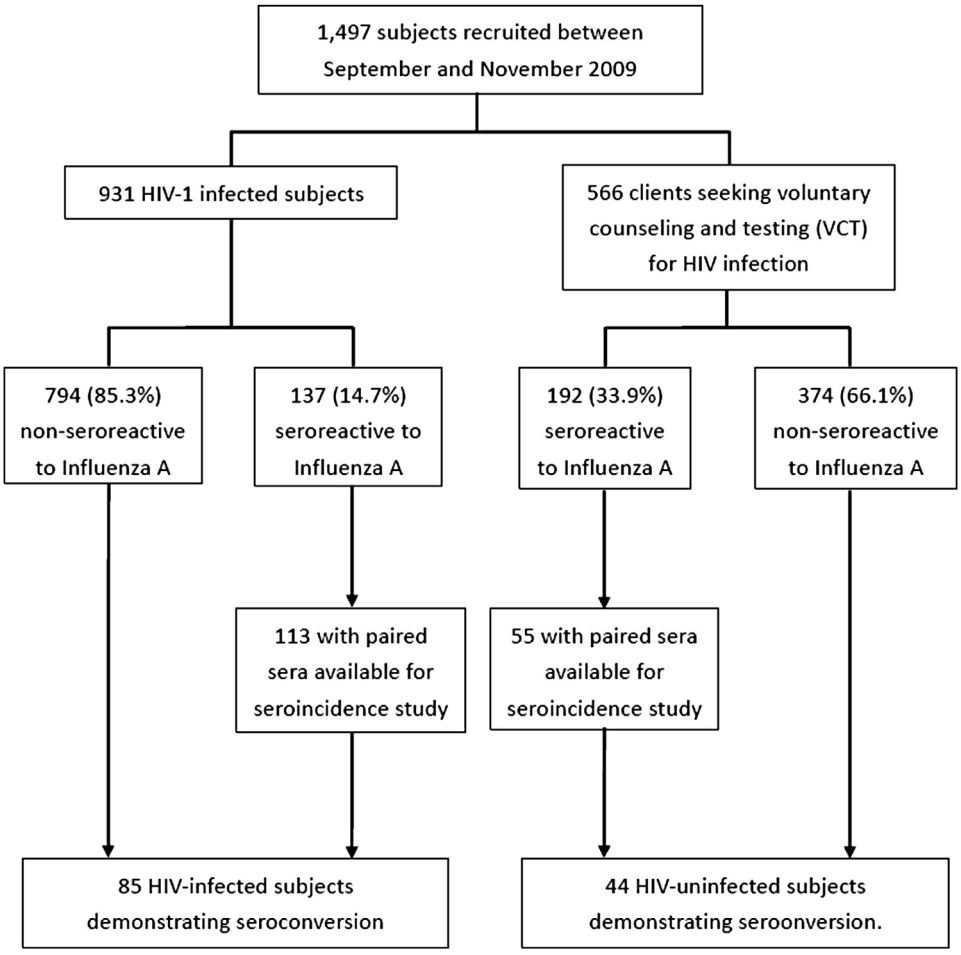

Figure 1. Study flow of seroprevalence and seroincidence of novel influenza A infection in HIV-infected and HIV-uninfected individuals.

Table 1 Clinical characteristics of HIV-infected individuals and HIV-uninfected patients who sought VCT for HIV infection

Characteristics		HIV-infected patients	HIV-uninfected VCT clients	*p*
Total screened		931	566	
Male gender, *n* (%)		877 (94.2)	512 (90.5)	0.007
Age (y), median (range)		37 (1—87)	27 (16—60)	<0.001
Birth year	Before 1957, *n* (%)	118(12.7)	8(1.4)	<0.001
	Between 1957—1975, *n* (%)	458 (49.2)	76 (13.4)	<0.001
	After 1975, *n* (%)	355 (38.1)	482 (85.2)	<0.001
Risky behavior for HIV infection, *n* (%)	MSM	654 (70.2)	389 (68.7)	0.54
	Heterosexual	151 (16.2)	175 (30.9)	<0.001
	IDU	41 (4.4)	0 (0)	<0.001
	Others or unknown	85 (9.1)	2 (0.4)	<0.001
CD4 cells/μL, median (range)		446 (3—3078)	NA	—
CD4 ≥ 200 cells/μL, *n*/*N* (%)		811/907 (89.4)	NA	—
Plasma HIV-1 RNA load ≥1.60 $\log_{10}$ copies/mL, *n*/*N* (%)		507/906 (56.0)	NA	—
Receipt of HAART, *n* (%)		697 (74.9)	NA	—
Duration of HAART (mo), median (range)		47.2 (0.03—161.7)	NA	—
HI titer ≥ 1:40, *n* (%)		137 (14.7)	192 (33.9)	<0.001

Abbreviations: HAART, highly active antiretroviral therapy; HBsAg, hepatitis B virus surface antigen; HCV, hepatitis C virus; HI, hemagglutination-inhibition assay; IDU, injecting drug users; MSM, men who have sex with men; NA, not applicable; VCT, voluntary counseling and testing.

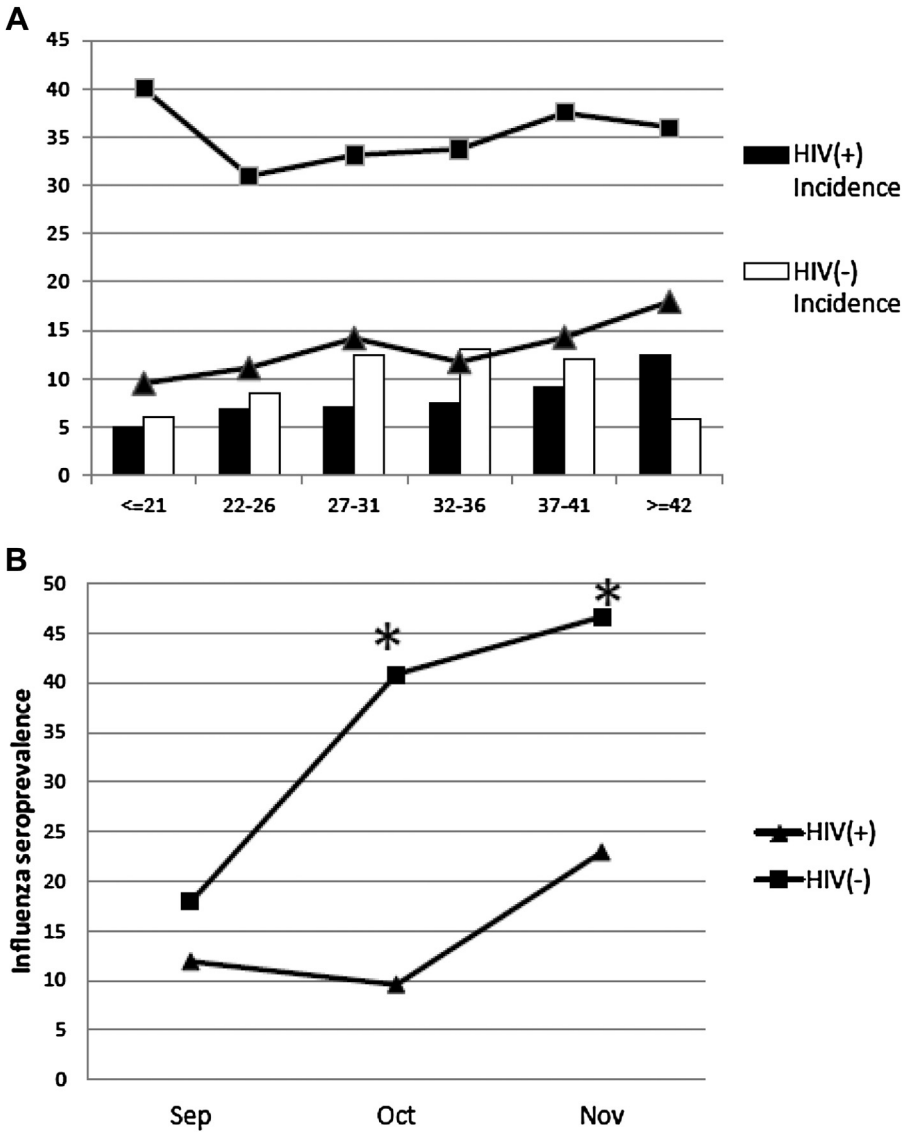

Figure 2. (A) Seroprevalence and seroincidence of novel influenza A infection among HIV-infected and HIV-uninfected individuals, according to age (years). Serum samples that were collected from 931 HIV-infected patients (filled triangle) and 566 HIV-uninfected persons (filled square) were tested using a hemagglutination-inhibition (HI) assay. The percentage of subjects with antibody titers of $\geq$40 (defined as having an antibody response to novel influenza A) was plotted according to the age of the subject. The percentage of HIV-infected subjects (filled bar) and HIV-uninfected persons (open bar) with seroconversion (defined as having at least a 4-fold increase in an antibody response to novel influenza A or conversion from seronegative to HI titers $>$40 between 2 sequential serological samples from the same individual) was plotted as bars according to the age of the subjects. (B) The dynamic of seroprevalence between the HIV-infected and HIV-uninfected patients stratified by month. The percentage of subjects with antibody titers of $\geq$40 (defined as having an antibody response to novel influenza A) was plotted according to the time of sample recruitment. The percentage of HIV-infected subjects (filled triangle) and HIV-uninfected persons (filled square) with seroreactivity was plotted.

which 85 showed seroconversion, resulting in an estimated incidence of 9.4% (85/907; 95% CI: 7.6—11.4)(Fig. 1). Among VCT clients, 55 of 192 seroreactive subjects (28.6%) had paired serum samples available for analysis, of which 44 showed seroconversion, resulting in an estimated incidence of 10.3% (44/429) (95% CI: 7.6—13.4). There was no statistical or significant difference in terms of the incidence between the two groups of subjects ($p = 0.60$). The range of the baseline HI titers was the same in both groups (negative to 1:40). An increase in incidence from 5.0% of HIV-infected patients aged $\leq$ 21 years to 12.4% of patients aged $\geq$ 42 years was observed (Fig. 2A), although the trend of this increase was not statistically significant ($p = 0.32$). All of the HIV-infected patients maintained regular clinic appointments every 1—3 months during the study period and no hospitalizations were required due to H1N1 or respiratory tract infections, as verified by a review of their medical records.

Using multivariate analysis, we found that only heterosexual behavior (OR: 1.89; 95% CI: 1.105—3.227; $p = 0.02$)

Table 2 Multivariate logistic analysis of the predictors of seroconversion to novel influenza A (H1N1) in HIV-1-infected patients

Covariate	Estimate	Standard error	Wald 95% confidence limits	Wald Chi-Square	*p*
Intercept	−3.0747	0.3317	—	85.9080	<0.0001
Receipt of HAART	0.6203	0.3546	0.928 − 3.726	3.0593	0.0803
Heterosexual	0.6359	0.2733	1.105 − 3.227	5.4140	0.0200
Baseline HI titer	0.0192	0.0093	1.001 − 1.038	4.2403	0.0395

Abbreviations: HAART, highly active antiretroviral therapy; HI, hemagglutination inhibition.
Number of total observations (n) = 825; percentage of concordant pairs = 51%; percentage of discordant pairs = 28.6%; adjusted generalized R^2 = 0.0443; estimated area under the receiver operating characteristic (ROC) curve = 0.612; and Hosmer and Lemeshow goodness-of-fit test, p = 0.3390−0.05 (df = 5).

and baseline HI titer (OR: 1.02; 95% CI: 1.001−1.038; p = 0.04) were statistically and significantly associated with seroconversion to novel influenza A among HIV-1-infected individuals (Table 2).

Discussion

In this study, which was conducted during the 2009 epidemic of novel influenza A infection in Taiwan, we found that a significantly lower seroprevalence of novel influenza A infection in HIV-infected patients than HIV-uninfected subjects. This observation, that the incidence was similar between HIV-infected patients and HIV-uninfected VCT clients (85/907 vs. 44/429, p = 0.60), suggests that our study groups might have had similar risk of exposure to novel influenza A during or prior to the epidemic.

The reasons for the lower seroprevalence of HIV-infected patients may be related to several epidemiologic and clinical factors. Compared with HIV-infected patients, more VCT clients are heterosexual and female. On multivariate analysis, heterosexuality, receipt of HAART, and baseline HI titer were associated with seropositivity. It is possible that heterosexuals are more likely to be exposed to children with influenza, although we did not perform interviews to obtain information on clinical symptoms indicative of influenza, upper respiratory tract infections, or possible exposure via the family or community. The immunodeficiency status of the HIV-infected patients may have also contributed to reduced antibody responses once exposed to novel influenza A infection, and thus the receipt of HAART, which has been shown to normalize the memory B-cell subpopulation that is critical for antibody production,[17] and also have contributed to seropositivity.

The incidence of novel influenza A infection was low (9.4%) among HIV-infected patients, which is close to or lower than that observed in a healthy community cohort in Singapore (13%) and the estimated incidence (14.5%) reported in a cross-sectional study, which mainly including patients accessing healthcare in the United Kingdom.[15,16] On the multivariate analysis, baseline HI titer, which was influenced by age (p = 0.003), receipt of HAART (p = 0.01), and homosexual behavior (p = 0.01) (Supplementary Table 2) were associated with seroconversion in HIV-infected individuals. The baseline HI titer most likely reflects the cross-reactive antibody response induced by the 1957 influenza epidemic.[14] The steadily increasing trend of seroincidence in older HIV-infected patients could be boosted by the pre-existing antibody response to the 1957 influenza strain, which suggests that, at least among those > 52 years old, the memory cells that might have been exposed to the influenza virus antigen before HIV infection could still be boosted in those receiving HAART.[17]

There are several limitations to our study, and interpretation of our data should be cautious. First, there are significant differences in terms of baseline characteristics between the two study populations. The HIV-infected patients were older and were more likely to be male. Since a proportion of elderly persons may have cross-reactive antibodies to novel influenza A,[14] our observations may be biased. Second, < 30% of the VCT clients who were seropositive for novel influenza A also provide paired serum samples for analysis. Therefore, without a sufficient sample size of sequential blood specimens from HIV-uninfected subjects available for analysis, the incidence in the HIV-uninfected population could have been underestimated. Third, the incidence of novel influenza A in HIV-infected patients may have been underestimated because their immune functions are compromised by HIV infection. The absence of seroconversion may simply reflect the incompetence of their immune systems to mount an antibody response to novel influenza A.

In conclusion, HIV-infected patients who received regular HIV care at our university hospital had a lower seroprevalence of novel influenza A infection than HIV-uninfected subjects in Taiwan. Among HIV-infected patients, seroconversion for novel influenza A virus that was infrequent in the 2009 influenza epidemic was associated with heterosexual behavior and baseline HI titer.

Conflicts of interest

None of the authors have any conflicts of interest to declare.

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Appendix A

Supplementary Table 1. Multivariate logistic analysis of the predictors of seroreactivity to novel influenza A (H1N1) in HIV-1 infected patients

Covariate	Estimate	Standard error	Wald 95% confidence limits	Wald Chi-Square	*P* value
Intercept	−2.9387	0.3073	—	91.4778	<0.0001
Receipt of HAART	0.7398	0.3241	1.110 − 3.955	5.2117	0.0224
Heterosexual	0.7605	0.2406	1.335 − 3.428	9.9940	0.0016
Baseline HI titer	0.0256	0.0096	1.007 − 1.045	7.0841	0.0078

Abbreviation: HAART, highly active antiretroviral therapy; HI, hemagglutination inhibition.
Note: *Seroreactivity was defined as having hemagglutination inhibition (HI) titer higher than 1:40.
Number of total observations (n) = 825, percentage of concordant pairs =55%, percentage of discordant pairs = 26.7%, adjusted generalized R^2 = 0.0695, estimated area under the Receiver Operating Characteristic (ROC) curve = 0.642, and Hosmer and Lemeshow goodness-of-fit test p = 0.5972 ≈ 0.05 (df = 5).

Supplementary Table 2. Multivariate logistic analysis of the predictors of baseline hemagglutination inhibition (HI) titer to novel influenza A (H1N1) among the HIV-1-infected patients

Covariate	Parameter Estimate	Standard error	t Value	Variance inflation	*p*
Intercept	1.0593	0.1440	7.36	0	<0.0001
Age younger than 30 years	−0.3621	0.1228	−2.95	1.1697	0.0033
Age older than 52 years	0.4498	0.1505	2.99	1.1467	0.0029
Receipt of HAART	0.2900	0.1185	2.45	1.1262	0.0146
Homosexual	−0.2958	0.1167	−2.54	1.1344	0.0114

Abbreviation: HAART, highly active antiretroviral therapy.
Number of total observations (n) = 813; adjusted generalized R^2 = 0.0640.