Original Article

Immune response to influenza A(H1N1)v in HIV-infected patients

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Abstract

Introduction: HIV infection is considered a risk factor for severe outcomes of influenza A(H1N1)v infection. However, data on immune response against influenza A(H1N1)v virus in HIV-infected patients are lacking.

Methodology: Data from seven HIV-positive and 14 HIV-negative patients infected with A(H1N1)v and from 23 HIV-positive and six HIV-negative asymptomatic controls were analyzed to evaluate the clinical picture, A(H1N1)v viral shedding, and the immune response against the virus.

Results: Patients displayed mainly upper respiratory tract diseases (57.1%), while pneumonia was diagnosed only in HIV-negative patients (23.8% of subjects, of which 4.8% required intensive care unit admission). At day seven, 29% of HIV-infected patients were still positive for A(H1N1)v by RT-PCR on nasopharyngeal swabs. Interestingly, a persistence of CXCL10 secretion at high level and lower IL-6 levels was observed in HIV-positive subjects. The geometric mean haemagglutination inhibition titer (HI-GMT) and anti-influenza IgM levels were lower in HIV-positive individuals while anti-influenza IgG levels remained similar in the two groups.

Conclusions: The immune impairment due to HIV infection could affect A(H1N1)v clearance and could lead to a lower antibody response and a persistent secretion of CXCL10 at high levels. However, the lower IL-6 secretion and treatment with highly active antiretroviral therapy (HAART) could result in a milder clinical picture.

Key words: HIV; influenza A(H1N1)v 2009; cytokines; chemokines; antibody response

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Introduction

On 11 June 2009, the World Health Organization (WHO) gave a pandemic alert in response to the global spread of a novel influenza virus. Since the first human case of infection, the novel pandemic influenza A (H1N1) virus, then named influenza A (H1N1)v virus, rapidly spread in US and Mexico, leading to global transmission through Europe [1]. The new virus was the result of a triple reassortment that combined gene segments from the North America and Eurasian swine H1N1 lineage; its historical origin can be located in 1918, when a virus of avian origin infected humans, causing the Spanish flu outbreak [2]. The distinctive combination of gene segments gives (H1N1)v antigenic features that make it dissimilar from seasonal influenza A [3].

The function of host immune response to eliminating (H1N1)v or the role of host immune response in contributing to respiratory illness of (H1N1)v infections is not clear. Studies on SARS CoV and influenza A (H5N1) have previously identified specific host immune signatures [4]. These studies also included (H1N1)v and showed that in the early phase of the infection, most of the mild outcomes are characterized by the expression of systemic levels of chemokines, such as CXCL10, that are associated with the innate antiviral response [5].

Although (H1N1)v generally leads to mild infection symptoms, thousands of deaths have been

reported from the first confirmed case [1], mostly associated with patients with underlying risk factors that are known to contribute to a severe outcome, such as metabolic dysfunctions, pregnancy, and immunodeficiency [6]. Indeed, reports from Africa have shown that a high proportion of fatal cases of (H1N1)v occurred in HIV-infected people with advanced disease and none under highly active antiretroviral therapy (HAART) [7]. Hence, HIV infection is a possible risk factor for severe (H1N1)v outcome because it is associated with deficiencies in humoral and cell-mediated immunity that is reflected in an impaired development and maintenance of serological memory [8]. Moreover, as a result of HIV infection, T cell depletion might aggravate the clinical outcome, resulting in an extended length of influenza infection [9].

Even if influenza is a common cause of respiratory illness among the HIV-positive population, limited data are available on the clinical course of influenza in this group of people; in this context, the 2009 (H1N1)v pandemic provided a unique opportunity for investigation. To determine if HIV-infected patients are able to clear the (H1N1)v infection, a cohort of HIV-positive patients infected with the 2009 pandemic influenza A virus was monitored; the study focused on the impact of infection on the innate and humoral immune responses. The hemagglutination inhibition assay (HI) was used to evaluate patients' ability to generate antibodies against the (H1N1)v, and characterization of the innate immune signatures was assessed by measuring cytokine and chemokine secretions associated with the development of the adaptive immunity in relation to HIV infection. The results of this study identified a specific hallmark characterizing the host immune response of HIV-infected patients against (H1N1)v.

Methodology

Patient selection

During the 2009-2010 season, a prospective observational study was performed at the Agostino Gemelli Hospital, a reference University Hospital in Rome, Italy. All adults and adolescents seeking medical care for influenza-like illnesses (ILI) were enrolled. The main aim of the study was to describe clinical features of influenza A (H1N1)v virus infection (detailed study design and clinical results are published elsewhere) [10]. At the time of first medical evaluation (day 0), nasopharyngeal swabs and serum samples were obtained from each subject and sent for virological analyses. For the subgroups of HIV-positive and HIV-negative patients testing positive for influenza A(H1N1)v virus by real time reverse transcriptase polymerase chain reaction (RT-PCR), nasopharyngeal swabs and serum samples were collected at seven and 14 days after the first sample was collected. Nasopharyngeal swabs and serum samples were also collected from a control group of asymptomatic HIV-positive and HIV-negative patients who did not have a referred episode of ILI in the previous month.

For all patients, demographic and clinical data were collected using a pre-defined form. The evolution of symptoms was then recorded until recovery by reviewing medical charts (for hospitalized patients) and by outpatient visits or telephone medical interviews (for discharged patients).

Viral diagnosis

Nasopharyngeal swabs were immediately sent for microbiological analysis. A 400-µL aliquot of the specimen was used for automated RNA extraction with an EZ1 viral kit (QIAGEN, Hilden, Germany) and then subjected to real time RTPCR. Primers and probes for the H1 gene (swH1) and M gene (InfA) used in this work were recommended by the WHO [11] and were synthesized by Applied Biosystems (Forest City, USA). RT-PCR was performed in a 25 µL reaction volume that contained 5 µl of the RNA dilution, 12.5 µl 2x AgPath-ID One-Step RT-PCR buffer, 1 µL enzyme mix, 0.5 µL assay mix in a fluorometric PCR instrument (ABI 7300). Thermal cycling conditions were 30 minutes at 50°C followed by 10 minutes at 95°C and a subsequent 45 cycle amplification (95°C for 15 seconds, 55°C for 30 seconds; fluorescence was collected at 55°C).

Cytokine and chemokine quantification

Serum samples obtained at each time point were stored at -80°C until analyses were performed. To evaluate the immune response profile during (H1N1)v infection, samples obtained at each time point (day 0, 7, and 14) from HIV-positive or HIV-negative (H1N1)v-infected patients and from their corresponding controls (HIV-positive or HIV-negative (H1N1)v-uninfected patients) were tested to determine levels of cytokines and chemokines (CXCL10, CCL-2, CXCL9, CXCL8, IL-17, IFN-γ, IL-10, IL-6, IL-4, IL-2,IL-12p70, TNF α, IL-1β). Serum chemokine and cytokine levels were evaluated by flow cytometry using BD Cytometric Bead Array (CBA) human inflammatory cytokines and Th1/Th2/Th17 human cytokines kit.

Hemagglutination inhibition assay (HI)

To evaluate antibody response against (H1N1)v, hemagglutination inhibition (HI) assay was performed on sera collected at day 0 and 14. Sera were treated with receptor-destroying enzyme (RDE-Sigma) of *V. cholera* by diluting one part serum with four parts of the enzyme and were incubated overnight in a 37°C water bath. The enzyme was inactivated by a 30-minute incubation at 56°C followed by the addition of three volumes of sodium citrate 2.5 % for a final dilution of 1/10. HI assays were performed in V-bottom 96-well microtiter plates with 0.5% chicken erythrocytes, as previously described, [12] using inactivated pandemic influenza A/California/07/2009 antigens.

ELISA

To further investigate the expression of class-specific IgM and IgG antibodies during (H1N1)v infection, a modified enzyme-linked immunosorbent assay (ELISA) protocol was performed as described previously [13]. Briefly, BPL-California/07/09 H1N1 virus (adjusted to 20 hemagglutination units/well in PBS) was used as a coating antigen. Plates were blocked with PBS containing 1% BSA and incubated for 1 hour at 37°C. Serial dilutions of each human serum sample in PBS were added to the plates and incubated for two hours at room temperature. Bound antibodies were detected with goat anti-human IgG or IgM, and conjugated with horseradish peroxides (AbCam). Plates were stained with o-Phenylenediaminedihydrochloride (OPD, Sigma Aldrich, Saint Louis, USA) in 0.05 M citrate-phosphate buffer pH 5 (Sigma) as a substrate, and the absorbance was measured (wavelength, 492 nm) after stopping the reaction with 3 M H_2SO_4 solution.

Statistical analysis

Data analysis was performed using SPSS version 15.0. Categorical variables were compared between groups using the Chi-square test or Fisher's exact test, as appropriate; for continuous variables, comparisons were based on the non-parametric Mann-Whitney *U* test. GMT was compared by means of Student's *t* test on the log10-transformed titers. A two-sided p value of less than 0.05 was considered statistically significant.

Table 1: Patient cohort description

	Total patients (N=21)	Etiology			
		Hiv-positive (n=7)	vs	Hiv-negative (n=14)	p[b]
Male sex	10 (47.6)	3 (42.9)		7 (50)	1.000
Age (years)[a]	44 (38-49)	40 (31-49)		44 (41-53)	0.263
Smokers	2 (9.5)	1 (14.3)		1 (7.1)	1.000
Co-morbidities					
Cardiac Disease	3 (14.3)	0 (0)		3 (21.4)	0.521
Diabetes	2 (9.5)	0 (0)		2 (14.3)	0.533
Hypertension	3 (14.3)	0 (0)		3 (21.4)	0.521
Obesity	1 (4.8)	1 (14.3)		0 (0)	0.333
Epidemiological contact with confirmed or suspected Influenza A(H1N1)v case	2 (9.5)	2 (28.6)		0 (0)	0.100
Receipt of seasonal trivalent influenza vaccine	3 (14.3)	1 (14.3)		2 (14.3)	1.000
Diagnosis:					
Upper respiratory tract infection	12 (57.1)	5 (71.4)		7 (50)	0.642
Bronchitis	4 (19.0)	2 (28.6)		2 (14.3)	574
Pneumonia	5 (23.8)	0 (0)		5 (35.7)	0.123
Complications:					
Hospitalization	7 (33.3)	1 (14.3)		6 (42.9)	0.337
Lenght of hospitalization (days)[a]	7 (4-14)	7 (7-7)		9 (3-14)	1.000
Intensive Care Unit admission	1 (4.8)	0 (0)		1 (7.1)	1.000
Oxygen supplementation requirement	1 (4.8)	0 (0)		1 (7.1)	1.000
Therapy:					
Oseltamivir	15 (71.4)	7 (100)		8 (57.1)	0.061
Antibiotics	13 (61.9)	3 (42.9)		10 (71.4)	0.346

Notes: values are expressed as n (%) except for [a] median (interquartile range); [b]comparisons are based on Chi square test or Student's *t* test.
Abbreviations: COPD, chronic obstructive pulmonary disease; C-PAP, continuous positive airway pressure.
None of the subjects received Influenza A (H1N1) 2009 monovalent Vaccine.

Results

Description of patient cohort and (H1N1)v infection

Complete clinical data, nasopharyngeal swabs, and serum samples at each time point (day 0, 7, and 14) were available for a total of 21 (H1N1)v-infected (7 HIV-positive and 14 HIV-negative) patients. All subjects were enrolled during the second wave of the (H1N1)v pandemic in the fall between October and December of 2009. The main demographical and clinical characteristics are shown in Table 1. Subjects were mainly young adults with classic ILI symptoms. All patients (HIV positive and HIV negative) were mainly affected by upper respiratory tract diseases (57.1%), while pneumonia was diagnosed only in HIV-negative individuals (23.8% of subjects, of which 4.8% required intensive care unit admission and four of five were treated with oseltamivir). Hospitalization occurred in 33.3% of patients; the median length of hospitalization was seven days (interquartile range (IQR) 4-14). HIV-positive patients had a median value for CD4 and CD8 cells count of 637 cell/mm3 (IQR 373-757) and 775 cell/mm3 (IQR 637-1115), respectively; six of seven (86%) were enrolled in combined antiretroviral therapy, and all seven (100%) had a HIV RNA < 50 copies/mL. No statistically significant differences in the main demographical and clinical characteristics were observed between HIV-infected and uninfected patients (Table 1).

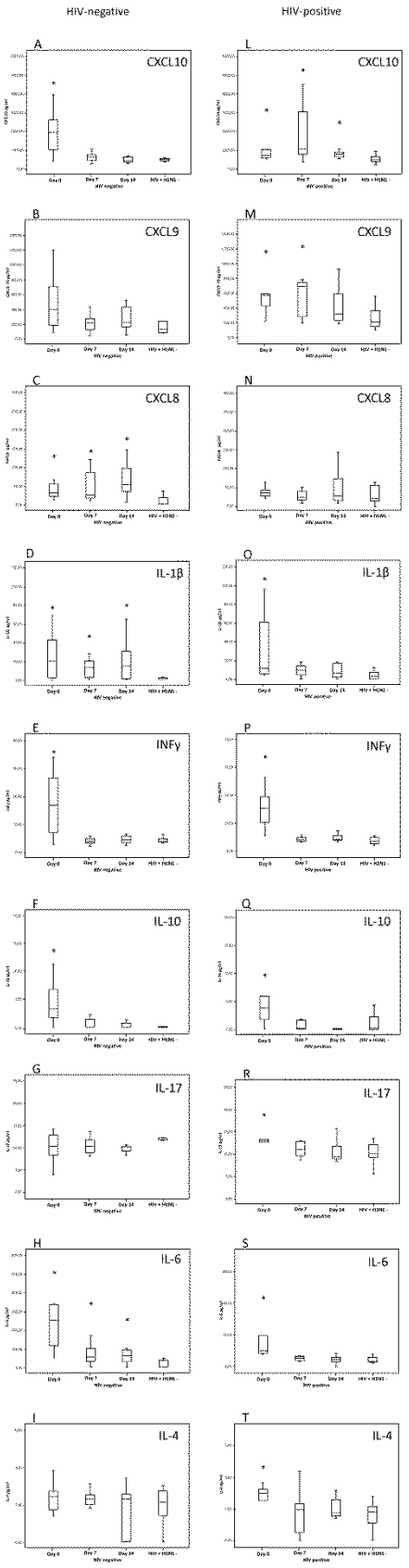
Seven days following the first diagnosis, as assessed by RT-PCR, all HIV-uninfected patients were negative for (H1N1)v, while 29% (n = 2) of HIV-infected patients were still positive for (H1N1)v (p = 0.100). At day 14, all patients (HIV-positive and negative) tested negative for (H1N1)v and were free of any disease symptoms.

To compare the basal immune profile in the absence of (H1N1)v infection, a control group of 29 asymptomatic (H1N1)v-uninfected patients (23 HIV positive and six HIV negative) with available serum samples was also included in the study.

Comparison of immune markers expression between (H1N1)v-infected and uninfected patients

The levels of the immune markers, cytokines, and chemokines were evaluated in serum samples by flow cytometry analysis using the CBA assay as a standard method.

Figure 1. Immune marker expressions in HIV-negative and HIV-positive (H1N1)v-infected patients



In HIV-negative patients, an increase of the chemokines CXCL10 (p = 0.003), CXCL8 (p = 0.005) and the cytokines IFNγ (p = 0.01), IL-10 (p = 0.002), IL-6 (p = 0.002), and IL-1β (p = 0.01) was observed during the acute phase of (H1N1)v infection (day 0) when compared to healthy controls (HIV-negative, (H1N1)v-uninfected). Moreover, whereas CXCL10 (p = 0.2), IFNγ (p = 0.3), and IL-10 (p = 0.1) returned to a basal level at day 7, CXCL8 (day 7, p - = 0.01; day 14, p - 0.004), IL-6 (day 7, p = 0.01; day 14, p = 0.02) and IL-1β (day 7, p = 0.003; day 14, p = 0.02) remained high later on (day 14), when compared to healthy controls (Figure 1A to I) (Table Supplementary 1).

In HIV-positive (H1N1)v-infected patients, significantly higher levels of CXCL10 (p = 0.02), CXCL9 (p = 0.01), IFNγ (p = 0.0004), IL-10 (p = 0.01), IL-6 (p = 0.003), IL-1β (p = 0.03), IL-17 (p = 0.01), and IL-4 (p = 0.04) were observed compared to their corresponding controls (HIV-positive and (H1N1)v-uninfected) at day 0. While most of these cytokines returned to a basal level seven days following the first diagnosis, CXCL10 (p = 0.02) and (CXCL9 p = 0.03) still remained at higher levels in (H1N1)v-infected patients compared to their corresponding controls. In the late phase of infection (day 14) CXCL9 (p = 0.2) returned to a basal level, whereas CXCL10 (p = 0.02) remained at higher levels in the (H1N1)v-infected patients (Figure 1 L to T) (Table Supplementary 1).

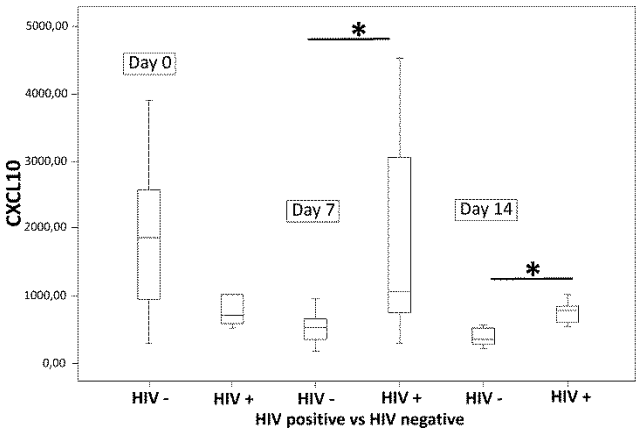
Comparison of immune markers expression between HIV-positive and HIV-negative (H1N1)v-infected patients

A comparative analysis was performed to evaluate the differential secretion of cytokine and chemokine levels in HIV-positive and HIV-negative (H1N1)v-infected individuals.

During the acute phase (day 0) of infection, the serum cytokines levels appeared to be elevated in both HIV-positive and HIV-negative cohorts compared to their corresponding (H1N1)v negative controls (Figure 1 L to T and A to I), though significant differences in the expression levels of immune mediators among these two groups of (H1N1)v-infected patients were not observed (Table Supplementary 1).

Conversely, 7 and 14 days following the first diagnosis, significantly higher levels of CXCL10 were observed in the HIV-positive group compared to the HIV-negative cohort (day 7, p = 0.04; day 14, p = 0.03) (Figure 2). Higher levels of CXCL10 observed in HIV-positive patients infected with (H1N1)v seems

Figure 2. Level of CXCL10 in HIV-positive and HIV-negative patients



not to be ascribable to oseltamivir treatment, as demonstrated by the analysis comparingCXCL10 levels in oseltamivir treated HIV-negative against HIV-positive patients (Figure Supplementary 1). Such analysis confirmed that increased CXCL10 expression by HIV-positive (H1N1)v-infected patients was not due to oseltamivir treatment, since HIV-positive patients maintained high levels of CXCL10 expression, while the CLCL10 levels in HIV-negative/(H1N1)v patients treated with oseltamivir decreased after 7 days of treatment. IL-6 expression was further analyzed comparing HIV-negative and HIV-positive patients treated with oseltamivir, confirming that antiviral therapy does not statistically influence immune mediators levels in HIV-negative patients treated with oseltamivir. Oseltamivir treatment lead to a strong decrease of the immune mediators in the HIV-negative group 7 days after administration. Conversely, in the HIV-positive patients, the level of CXCL10 remained higher. Although there was a decrease in statistical significance at day 7 (p = 0.18) when only the oseltamivir treated patients were compared, the HIV-positive cohort had a delay in the normalization of CXCL10 levels compared to the HIV-negative cohort as the higher levels at day 14 show (p < 0.05) (Figure Supplementary 1).

Assessment of differential basal expression of immune mediators in HIV-positive and HIV-negative (H1N1)v-uninfected control subjects

To determine whether the differential basal levels of cytokine expression observed were the results of HIV infection, serum samples from HIV-negative and HIV-positive asymptomatic (H1N1)v-uninfected patients were analyzed. Most of the cytokines tested

were found at similar levels in the two groups, though in HIV-positive patients, higher levels of IL-6 ($p = 0.02$), IL-1β ($p = 0.04$), and CXCL8 ($p = 0.02$) were detected compared to the HIV-negative individuals (Figure 3) (Table Supplementary 2). To determine whether this observation could have been influenced by oseltamivir treatment, oseltamivir-treated HIV-negative/(H1N1)v patients were compared to the HIV-positive/(H1N1)v cohort (Figure Supplementary 2). This analysis showed that the higher IL-6 basal level was not influenced by oseltamivir treatment.

The low influence of oseltamivir treatment on this analysis was further confirmed by the analysis of HIV-negative/(H1N1)v treated or untreated patients with the antiviral-treated patients (Figure 2). As shown by bar graphs, none of the cytokines analyzed were significantly different between the treated and untreated groups. Only a difference on day 0 was seen for CXCL10 and IL-6 in HIV-negative/(H1N1)v patients who were then treated with oseltamivir because they developed strong symptoms and the antiviral therapy was then supplied.

Finally, in order to exclude that these observations could have been influenced by comorbidities in the HIV-negative/(H1N1)v cohort, an analysis was performed, excluding from this group the patients with other diseases specified in Table 1. Such analysis confirmed that, even excluding comorbidities, the difference between CXCL10 and IL-6 expression in HIV-positive and HIV-negative (H1N1)v-infected patients remains statistically significant (Figure 3).

Comparison of antibody response between HIV-infected and uninfected patients

To evaluate antibody response against (H1N1)v, an HI assay was performed on sera obtained from HIV-positive and HIV-negative (H1N1)v-infected individuals at day 0 and day 14. In HIV-negative patients, the geometric mean titer (GMT) significantly increased over time from 22.08 at day 0 (95% confidence intervals (CI) 10.60-45.99) to 204.9 at day 14 (95% confidence intervals (CI) 112.5-373; $p = 0.006$) and in the same time frame also in HIV-positive patients from 14.96 at day 0 (95% confidence intervals (CI) 7.183-30.74) to 107.7 at day 14 (95% confidence intervals (CI) 47.63-243.4; $p = 0.02$).

While an increase in IgM secretion was observed in both HIV-positive and HIV-negative patients, at day 14, a significantly higher level of IgM was observed in HIV-negative patients ($p = 0.004$) (Figure 4A). A similar pattern was observed for IgG, though HIV-negative subjects showed a significantly higher level of IgG at day 0 compared to their HIV-positive counterparts ($p = 0.045$) (Figure 4B).

Discussion

The pandemic spread of the novel influenza virus has raised concerns on the possible increased risk of severe infection in immune-suppressed populations. As it has already been shown, the 2009 (H1N1)v infection seems to be associated with mild clinical outcomes. Nevertheless, experiments conducted in animal models indicated that (H1N1)v infection may result in more severe lung lesions in humans compared to mice, ferrets, and non-human primates infected with the seasonal human H1N1 virus [14], suggesting that (H1N1)v can cause distinctive clinical effects, especially in patients with a compromised immune system [15]. HIV infection, which is associated with anomalous humoral and T-cell–mediated immune responses, could result in increased susceptibility to viral respiratory infections [16]. However, recent studies have reported that the susceptibility of HIV-infected patients without advanced disease to 2009 H1N1v influenza may not be increased [17].

Figure 3. Cytokine levels in HIV-negative and HIV-positive (H1N1)v-uninfected patients

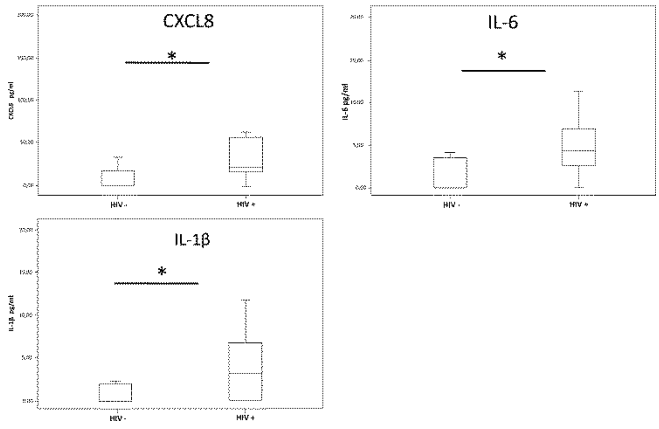
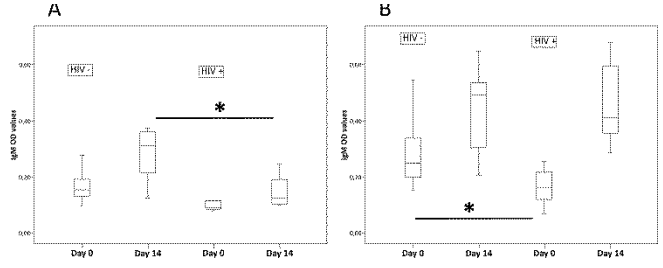


Figure 4. IgG and IgM response between HIV-infected and HIV-uninfected patients



Here, we investigated the clinical outcomes following (H1N1)v infection in HIV-positive and HIV-negative patients and attempted to identify the immune signatures associated with the two groups. We found that (H1N1)v infection presents different clinical outcomes in HIV-positive compared to HIV-negative patients, and that this relates to a different immune response in these two groups.

In this study, the clinical picture following (H1N1)v-infection in HIV-positive patients appeared milder overall compared to what was observed in HIV-negative subjects. In fact, cases of severe complications with onset of pneumonia that led to hospitalization and that, in one case, required oxygen supplementation, were detected only in HIV-negative patients. This is in apparent contrast with previous findings, where no remarkable differences in clinical outcomes were observed in HIV-positive patients compared to HIV-negative subjects [18,19]. In a study by Peters *et al.*, 911 subjects were analyzed in three separate cohorts, which included HIV+ patients with CD4 counts below 200, with half taking ART. The results of the study showed that receiving ART approximates significance in terms of being admitted to an ICU and/or death. The majority of the patients receiving ART therapy were HIV-infected patients with a CD4 cell count <200 cells/μL, while in the present study, the median value for CD4 counts was more than 600; this could explain the differences in the findings [20].

However, other studies have shown that influenza infection in HIV-positive subjects usually has a milder outcome, probably as a result of the immune impairment due to CD4-deficiency [21]. Indeed, it has been shown that complications following influenza infection result from an inflammatory burst triggered by a cytokine and chemokine storm induced by viral replication in the lung parenchyma, which directly involves CD4 T cells [22].

To determine whether the different clinical outcomes observed among HIV-positive and HIV-negative patients were dependent upon a diverse immune response, we dissected the immune response elicited following (H1N1)v infection using the human beads cytokines array assay (CBA, BD). With this method we were able to detect and quantify different chemokines, cytokines, and immune mediators through flow cytometry analysis in the serum of (H1N1)v infected patients and controls.

Interestingly, HIV-positive patients did not show remarkable differences in the cytokine and chemokine patterns in response to H1N1 infection compared to HIV-negative patients, although the chemokine CXCL10 appeared elevated along the time of the infection in the HIV-positive group, showing a delay in the long-term normalization of its level compared to what was reported in HIV-negative patients. CXCL10 or IP-10 is an IFN-γ-inducible protein that plays an important role in the host response to a diversity of viral infections [23]; it is expressed in a variety of cells in response to IFN-γ stimulation [24]. High levels of CXCL10 have been associated with a poor control of viral infection by the immune system [25,4].Moreover, increased levels of CXCL10 on plasma have been already detected by Kamat *et al.* in HIV patients on HAART [26]. Hence, the immune dysfunction during HIV infection mainly reflected in the CD4 T cell impairment could lead to a persistent secretion of CXCL10 at high levels, as observed in HIV-positive patients.

Another interesting observation comes from the analysis of IL-6 that was found to be lower in HIV-positive compared to HIV-negative patients. The higher secretion of IL-6, which is considered an activator of the acute phase responses [27] and also a marker of critical illness during influenza infection [28,5], may also explain the more severe clinical outcomes reported in HIV-negative patients, supporting the hypothesis that clinical complications following influenza infection may result from an inflammatory burst. It was shown that higher levels of IL-6 in pre-HAART HIV patients correlate with higher morbidity and mortality rates [29]. These data suggest that IL-6 can effectively be considered as a valuable marker of severe infection.

Protection and viral clearance during influenza infection relies on the humoral arm of the immune response. To determine the H1N1v serum antibody levels, the GMT level in HIV-positive and negative patients at day 0 and day 14 of the H1N1 infection were analyzed. While HIV-positive patients were able to mount an effective antibody response, they showed a significantly lower GMT at day 14 compared to HIV-negative patients. Interestingly, the difference in GMT observed was dependent on the impairment of HIV-positive subjects to mount a solid IgM response, since IgG levels were similar among the two groups. While the immunological basis of the difference between IgG and IgM response remains unknown, it is of interest to note that IgMs are known to be highly neutralizing and may contribute to viral clearance. The reduced viral clearance observed in the HIV-positive group may result from the impaired ability to mount an IgM response.

However, our data consider HIV-infected patients to be in a no-advance immune suppression, as the CD4+ and CD8+ cell counts show (CD4+ > 200 cells/mm3 and CD8+ < 1000 cells/mm3), and under HAART, which reduces the incidence of opportunistic infection; this suggests that influenza severity could be different in a situation of advanced HIV disease or in the presence of comorbidities. Furthermore, it should be taken into account that HAART is not accessible to HIV-infected people in many parts of the world, especially in developing countries, where pulmonary complications of HIV infection are major causes of morbidity and mortality [9].

In summary, HIV-infected patients under HAART show a distinctive feature in the immune response against 2009 pandemic influenza, characterized by a persistent secretion of high levels of CXCL10 and lower antibody responses. It may be hypothesized that the immune impairment due to the HIV infection in patients under HAART could lead to a higher CXCL10 secretion at long term, and to a delay in normalization of its levels during recovery. Moreover, the poorer response of specific antibodies thus potentially affects virus clearance. Nonetheless, the concomitant lower IL-6 secretion, which has been described to be associated with critical illness during influenza infection [27,30], could explain the milder symptom manifestation observed in HIV-positive patients.

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Supplementary Items

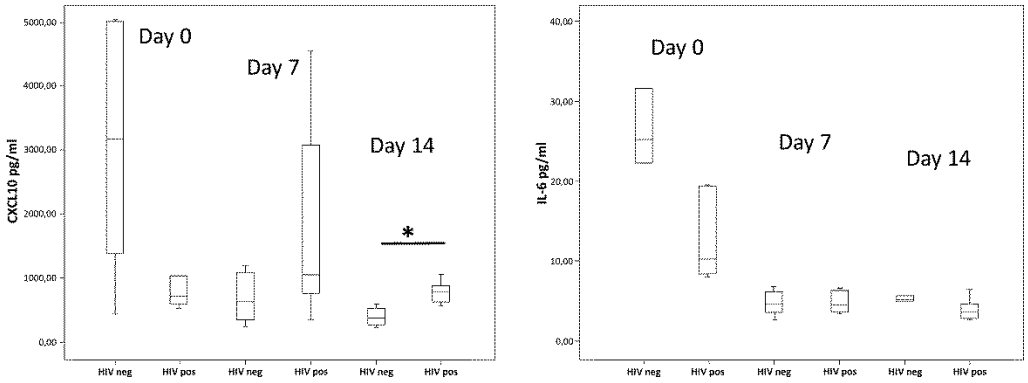
Table Supplementary 1: Median and interquartile range for chemokines and cytokines in HIV-infected and HIV-uninfected (H1N1)v-positive patients

		HIV-infected				HIV-uninfected	
		Median	**Range**			**Median**	**Range**
CXCL10	Day 0	712,35	2.386	**CXCL10**	Day 0	1860,5	4.796
	Day 7	1050,8	4.201		Day 7	533	2.451
	Day 14	780,7	491		Day 14	365	2.168
CCL2	Day 0	809	839	**CCL2**	Day 0	486	2.565
	Day 7	764	1.352		Day 7	477	836
	Day 14	375	582		Day 14	479	692
CXCL9	Day 0	567,25	1.952	**CXCL9**	Day 0	403	1.107
	Day 7	693,1	1.234		Day 7	231	2.023
	Day 14	418	739		Day 14	235	2.688
IL-17	Day 0	12,9	11	**IL-17**	Day 0	10	10
	Day 7	11	4		Day 7	10	29
	Day 14	9	7		Day 14	10	7
INFɣ	Day 0	15,4	20	**INFɣ**	Day 0	16,85	352
	Day 7	4	5		Day 7	4	15
	Day 14	4	4		Day 14	4	17
IL-10	Day 0	3,7	24	**IL-10**	Day 0	4,8	54
	Day 7	2	24		Day 7	4	7
	Day 14	0	2		Day 14	3	15
IL-4	Day 0	2,9	4	**IL-4**	Day 0	2	4
	Day 7	2	4		Day 7	2	13
	Day 14	2	3		Day 14	2	3
IL-2	Day 0	4	6	**IL-2**	Day 0	4	5
	Day 7	4	1		Day 7	4	9
	Day 14	4	1		Day 14	3	2
IL-12p70	Day 0	3	37	**IL-12p70**	Day 0	2	48
	Day 7	3	32		Day 7	2	40
	Day 14	3	77		Day 14	2	198
TNFα	Day 0	0	1	**TNFα**	Day 0	0	4
	Day 7	0	2		Day 7	0	2
	Day 14	1	3		Day 14	0	9
IL-6	Day 0	9,9	23	**IL-6**	Day 0	25,2	171
	Day 7	5	15		Day 7	4,95	51
	Day 14	4	33		Day 14	5,8	104
IL1-β	Day 0	11,08	92	**IL1-β**	Day 0	20,2	114
	Day 7	9	17		Day 7	11,8	61
	Day 14	6	48		Day 14	11,6	73
CXCL8	Day 0	36	41	**CXCL8**	Day 0	29,6	556
	Day 7	31	41		Day 7	23,45	223
	Day 14	31	134		Day 14	42,6	141

Table Supplementary 2: Median and interquartile range for chemokines and cytokines for asymptomatic control patients

	HIV-infected			HIV-uninfected	
	Median	**Range**		**Median**	**Range**
CXCL10	480	860	**CXCL10**	457	235
CCL2	371	806	**CCL2**	341	393
CXCL9	214	1.145	**CXCL9**	144	872
IL-17	10	28	**IL-17**	12	4
INFɣ	4	15	**INFɣ**	4	3
IL-10	0	11	**IL-10**	3	2
IL-4	2	6	**IL-4**	2	3
IL-2	3	6	**IL-2**	3	1
IL-12p70	3	632	**IL-12p70**	0	8
TNFα	0	38	**TNFα**	0	0
IL-6	4	343	**IL-6**	0	4
IL1-β	3	114	**IL1-β**	0	2
CXCL8	21	952	**CXCL8**	0	34

Figure Supplementary 1. Cytokine levels for CXCL10 (A) and IL6 (B) in oseltamivir-treated HIV-negative and HIV-positive (H1N1)v-infected patients at day 0, 7, and 14 following diagnosis



*Significant difference $p < 0.05$

Figure Supplementary 2. Cytokine levels in oseltamivir-treated (-) and oseltamivir-untreated (-) HIV-negative (H1N1)v-infected patients at day 0, 7, and 14 following diagnosis

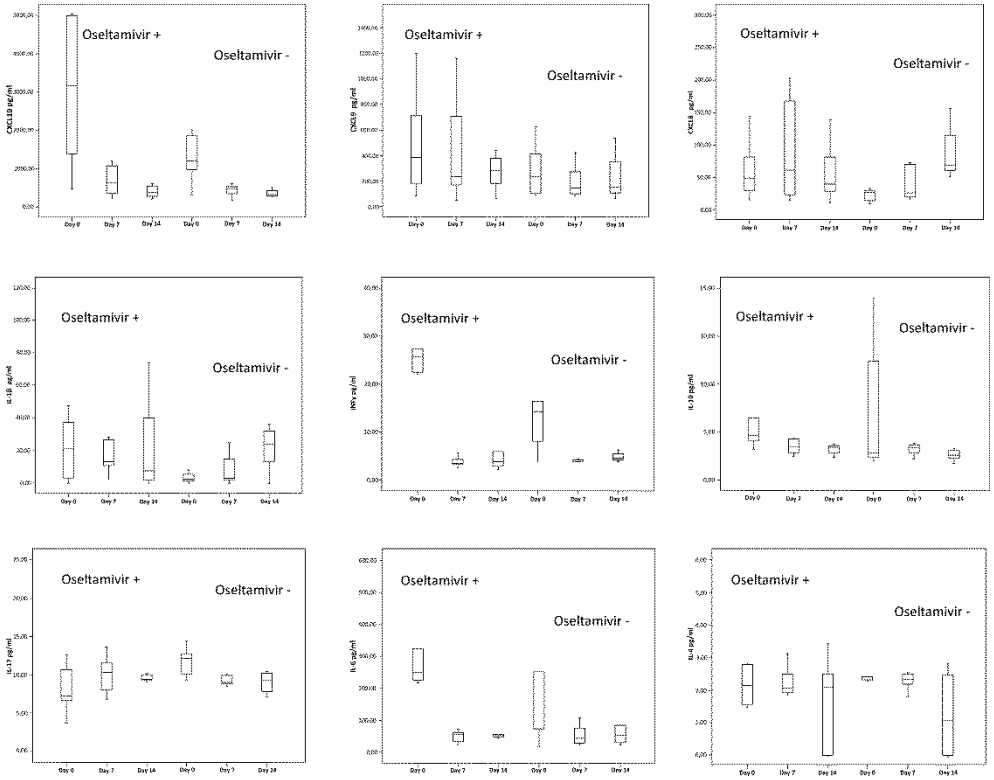
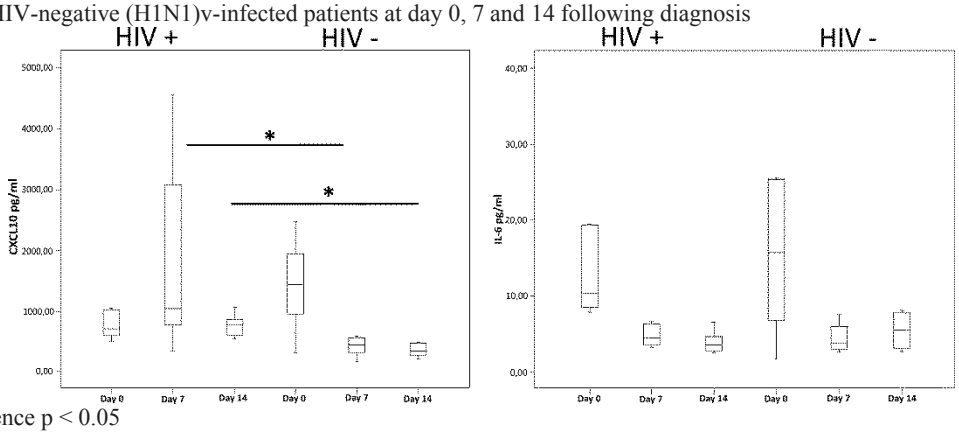


Figure Supplementary 3. Cytokine levels excluding HIV-negative patients with underlying comorbidities, for CXCL10 (A) and IL6 (C) in HIV-positive and HIV-negative (H1N1)v-infected patients at day 0, 7 and 14 following diagnosis



*Significant difference $p < 0.05$