CD4/CD8 Ratio Predicts Yellow Fever Vaccine-Induced Antibody Titers in Virologically Suppressed HIV-Infected Patients

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Background: Yellow fever vaccine (YFV) induces weaker immune responses in HIV-infected individuals. However, little is known about YFV responses among antiretroviral-treated patients and potential immunological predictors of YFV response in this population.

Methods: We enrolled 34 antiretroviral therapy (ART)-treated HIV-infected and 58 HIV-uninfected adults who received a single YFV dose to evaluate antibody levels and predictors of immunity, focusing on $CD4^+$ T-cell count, $CD4^+/CD8^+$ ratio, and Human Pegivirus (GBV-C) viremia. Participants with other immunosuppressive conditions were excluded.

Results: Median time since YFV was nonsignificantly shorter in HIV-infected participants than in HIV-uninfected participants (42 and 69 months, respectively, $P = 0.16$). Mean neutralizing antibody (NAb) titers was lower in HIV-infected participants than HIV-uninfected participants (3.3 vs. 3.6 $\log_{10}$mIU/mL, $P = 0.044$), a difference that remained significant after adjustment for age, sex, and time since vaccination ($P = 0.024$). In HIV-infected participants, lower NAb titers were associated with longer time since YFV (rho: −0.38, $P = 0.027$) and lower $CD4^+/CD8^+$ ratio (rho: 0.42, $P = 0.014$), but not $CD4^+$ T-cell count ($P = 0.52$). None of these factors were associated with NAb titers in HIV-uninfected participant. GBV-C viremia was not associated with difference in NAb titers overall or among HIV-infected participants.

Conclusions: ART-treated HIV-infected individuals seem to have impaired and/or less durable responses to YFV than HIV-uninfected individuals, which were associated with lower $CD4^+/CD8^+$ ratio, but not with $CD4^+$ T-cell count. These results supports the notion that low $CD4^+/CD8^+$ ratio, a marker linked to persistent immune activation, is a better indicator of functional immune disturbance than $CD4^+$ T-cell count in patients with successful ART.

Key Words: yellow fever vaccine, yellow fever neutralizing antibodies, HIV, $CD4^+/CD8^+$ ratio, immune activation

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INTRODUCTION

Despite the significant improvements in clinical outcomes for persons living with HIV who receive effective antiretroviral therapy (ART), these patients still experience an increased risk of death and higher prevalence of comorbidities compared with HIV-uninfected persons.[1–3] Studies have consistently demonstrated that immune activation and inflammation, which decrease but fail to return to normal levels on ART, are strong predictors of residual morbidity and mortality.[4–6]

Vaccine responses are among the clinical outcomes that may be impaired for persons living with HIV. Studies performed in different settings have demonstrated that HIV-infected patients present lower responses to several vaccines,[7–9] possibly predicted by lower $CD4^+$ T-cell count,[10,11] detectable HIV viral load,[12–14] and lack of ART use.[14,15] However, studies on vaccine responses restricted to ART-treated HIV-infected patients are lacking,[15] and it remains unknown whether ART can completely reverse the detrimental effect of HIV on vaccine responsiveness.

As with other vaccines, yellow fever vaccine (YFV) has impaired efficacy among HIV-infected persons. Reduced duration of seropositivity and reduced rates of seroconversion have been documented and were associated with both detectable HIV viral load at vaccination[16–21] and lower

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$CD4^+$ T-cell counts.[20] YFV has unique features that make it particularly suitable to assess immune responses among HIV-infected patients. In healthy individuals, YFV efficacy is very high with long duration of protective antibody levels.[22,23] Most patients in areas of low endemicity are vaccinated with a single vaccine dose, and exposure to wild yellow fever (YF) is very uncommon, such that measured antibodies most likely result from the previous vaccination.

Recent findings suggest that persistent excessive immune activation may be associated with reduced cellular and humoral responses to YFV.[24] A low $CD4^+/CD8^+$ ratio is a simple, clinically available biomarker of immune activation, also correlated with higher risk of mortality among ART-treated HIV-infected patients.[25] In addition, chronic coinfection with GBV-C, also known as human pegivirus, has been previously associated with reduced markers of innate and adaptive immune activation among HIV-infected patients.[26–28]

In this study, we compared YFV efficacy, as assessed by YFV-specific neutralizing antibody (NAb) titers, between treated HIV-infected patients and HIV-uninfected controls who received a single YFV dose. We examined whether time since vaccination, $CD4^+$ T-cell count, $CD4^+/CD8^+$ ratio, and GBV-C viremia were associated with YFV NAb titers in this cohort.

METHODS

Study Population

Potential participants aged 18 years and above vaccinated with a single dose of the YFV derived from the 17D strain (Bio-Manguinhos; Fiocruz, Rio de Janeiro, Brazil) were selected from vaccine registers and from direct survey in outpatient clinics in a tertiary hospital in Sao Paulo, Brazil. All participants were enrolled between November 2010 and April 2014, and had a registered YFV in a vaccination document. Any time point after vaccination was accepted for inclusion.

HIV-negative persons underwent a rapid HIV test at enrollment. All HIV-infected patients were under ART and had undetectable HIV viral load at enrollment and in at least 2 inspections before enrollment.

For both groups, participants with immunosuppressive conditions other than HIV infection were excluded. These included diabetes, chronic liver, or kidney diseases, any type of non-skin cancer (except resolved Kaposi Sarcoma for HIV-infected participants), and use of oral or parenteral immunosuppressive therapy in the 3 months before enrollment.

Statistical Analysis

Continuous variables were assessed using a *t* test or the nonparametrical Wilcoxon rank-sum test when applicable. Categorical variables were evaluated with Pearson χ^2 or Fisher exact test. Correlation of log-transformed YFV NAb titers with continuous predictors was performed using Spearman rank correlation test. A linear regression model using robust variance estimation was used to assess the association between HIV status and NAb titers adjusted for age, sex, and time since vaccination. We also used a linear regression model adjusted for age and sex to assess the association between GBV-C viremia and $CD4^+/CD8^+$ ratio. For all comparisons, 2-tailed *P* values less than 0.05 were considered statistically significant. All analyses were performed in Stata Version 13.1 (StataCorp; StataCorp LP, College Station, TX).

Ethical Aspects

The study was approved by The Ethics Committee at University of Sao Paulo Medical School. All participants signed an informed consent form. HIV tests were performed with pretest and posttest counseling. Confidentiality regarding participant's identifiable information was assured by data storage in locked cabinets and/or secure server, and only deidentified records were available during data management.

Laboratory Methods

The HIV plasma viral load was determined by reverse-transcriptase (RT)-PCR using an Amplicor HIV-1 Monitor Test (Roche Diagnostic Systems, Branchburg, NJ), which has a lower detection limit of 200 copies of HIV per cubic millimeter. $CD4^+$ and $CD8^+$ T lymphocyte counts were determined by flow cytometry (FACSCalibur, BD Biosciences, CA) using Multitest reagent (BD Biosciences, Franklin Lakes, NJ).

NAb titers against YF virus were defined by the plaque reduction neutralization test (PRNT) performed at Virologic Technology Laboratory of Bio-Manguinhos (LATEV; FIOCRUZ, Brazil). For the analysis of the protective immune response after vaccination, the PRNT is considered the gold standard,[29] producing results that are correlated with protection.[30,31] PRNT was conducted in serial 2-fold dilutions starting at 1:5, in 20 μL aliquots of heat-inactivated (at 56°C for 30 minutes) serum, in 96-well tissue culture plates as previously described.[32] A positive serum control for YF NAb (a standard serum prepared in house), properly calibrated by the First International Reference Preparation (NIBSC code: YF),[33] was included in each run of the PRNT. For neutralization step, each well received a YF viral suspension at concentration approximately 30 plaque forming units per well. After incubation at room temperature for 1 hour, a suspension of Vero cells was added, and the plates were incubated again for 3 hours at 37°C in 5% CO_2. The medium was then discarded and the cells overlaid with 100 μL per well of medium containing carboxymethylcellulose. After incubation for 6 days at 37°C in 5% CO_2, the cell monolayers were fixed with 10% formalin, stained with 0.04% crystal violet, and plaques were counted. PRNT titer was defined as the reciprocal of the last serum dilution that reduced the plaque numbers in 50% relative to the virus control. Linear regression was used to determine NAb titers. Titers expressed in mIU/mL were calculated relative to the antibody content in the international reference serum (143 IU/mL), which was used to determine the nominal value of the positive serum control (1115 mIU/mL). Therefore, it was possible to transform NAb titer represented by dilution into mIU/mL. In this study, results are presented in Log_{10} mIU/mL.

GBV-C Viral RNA was extracted from 140uL serum samples using QIAamp Viral RNA Mini Kit (QIAGEN Inc, Valencia, CA), according to the manufacturer's instructions. A 5uL aliquot of the RNA extracted was used to perform the qRT-PCR with the kit SuperScript III Platinum One-Step Quantitative RT-PCR System with ROX (Life Technologies, Carlsbad, CA) with primers, and a TaqMan probe that amplified and quantified a fragment of 72 pb of the 5' nontranslated region (5′UTR). The reaction was made with 0.5 μL of SuperScript III RT/Platinum Taq Mix, 12.5 μL of 2X reaction mix with ROX, 0.75 μL of 10 μM forward primer RTG1 (5′GTGGTGGATGGGTGATGACA3′) (Sigma), 1.25 μL of 10 μM reverse primer RTG2 (5′GACCCACCTATA-GTGGCTACCA3′) (Sigma), and 0.4 μL of 25 μM TaqMan probe [(6′FAM)CCGGGATTTACGACCTACC(TAMRA-6-FAM)] (Life Technologies), and the reaction final volume of 25 μL was completed with DEPC-treated water. The synthesis of cDNA was performed during the first 15 minutes of reaction at 50°C. After 2 minutes maintained at 95°C, the amplification and the quantification were performed during 40 cycles with the following times and temperatures: 95°C, 15 seconds; 60°C, 30 seconds. The reading of FAM fluorescence was made during the annealing period at 60°C.

RESULTS

Table 1 shows the main demographic and clinic characteristics of the 34 HIV-infected participants and 58 HIV-uninfected participants. Median age was 46 years old for HIV-infected participants and 38 years old for controls. The HIV-infected group had a higher percentage of males (79% vs. 29%). Among the 34 HIV-infected participants, 30 (88%) reported receiving the YFV after the diagnosis of HIV infection. Median time since YFV was nonsignificantly shorter in HIV-infected participants (41 months) than in HIV-uninfected participants (69 months; $P = 0.16$). Few participants in either group recalled developing any adverse events after YFV.

TABLE 1. Characteristics of Participants*

	HIV-Infected, ART-Suppressed, N = 34	HIV-Negative Controls, N = 58	*P*
Age, yrs	46 (41–49)	38 (29–48)	0.088
Male gender, no. (%)	27 (79)	17 (29)	<0.001
Time since YFV, mo	41 (25–119)	69 (35–131)	0.156
AE after YFV, no. (%)	3 (9)	12 (21)	0.158
$CD4^+$ T count,† cells/mm^3	790 (603–956)	1120 (945–1308)	<0.0001
$CD4^+/CD8^+$ ratio†	0.7 (0.5–1.2)	1.8 (1.3–2.5)	<0.0001
GBV-C viremia,‡ no. (%)	8 (42)	15 (38)	0.735
Undetectable HIV viral load,§ no. (%)	34 (100)	—	—

*Continuous variables are presented as median and interquartile ranges.
†Only available on 49 controls.
‡Only available for 40 controls and 19 HIV-infected participants.
§With plasma HIV RNA level <200 copies per milliliter.
AE, adverse events; ART-suppressed, antiretroviral therapy-suppressed.

All HIV-infected participants were under stable ART, and a high median $CD4^+$ T-cell count (790 cells per cubic millimeter, interquartile range: 603–956) was observed in this group. However, $CD4^+$ T-cell count and $CD4^+/CD8^+$ ratio were lower among HIV-infected participants compared with HIV-uninfected controls ($P < 0.0001$ for both comparisons). More participants in the HIV-infected group had detectable GBV-C viremia, but this difference did not reach statistical significance (42% vs. 38%, $P = 0.74$).

Mean YF-specific NAb titers were significantly lower in HIV-infected participants (3.3 $\log_{10}$mUI/ml, 95% CI: 3.1 to 3.5) than HIV-uninfected participants (3.6 $\log_{10}$mIU/ml, 95% CI: 3.4 to 3.7; $P = 0.044$, Fig. 1). This difference remained significant after adjustment for covariates in a multivariate model; mean YF-specific NAb titers among HIV-infected participants was 0.44 times the titers predicted for HIV-uninfected subjects adjusted for age, sex, and time since vaccination (95% CI: 0.22 to 0.90, $P = 0.024$). In HIV-infected participants, lower NAb titers were not correlated with $CD4^+$ T-cell count (Spearman Rho: −0.11, $P = 0.52$). However, in this group of patients, lower NAb titers were correlated with longer time since YFV (Spearman Rho: −0.38, $P = 0.027$, Fig. 2) and lower $CD4^+/CD8^+$ ratio (Spearman Rho: 0.42, $P = 0.014$, Fig. 3). None of these factors were correlated with NAb titers in HIV-uninfected participants.

GBV-C viremia was not associated with difference in NAb titers overall ($P = 0.98$) or among HIV-infected participants ($P = 0.56$). Furthermore, among HIV-infected participants, GBV-C viremia was not associated with $CD4^+/CD8^+$ ratio in a multivariate model adjusted for age and sex (predicted change in $CD4^+/CD8^+$ ratio = 0.10, 95% CI: −0.60 to 0.81, $P = 0.76$).

DISCUSSION

In this study, YF-specific NAb titers were significantly lower among ART-treated HIV-infected patients compared

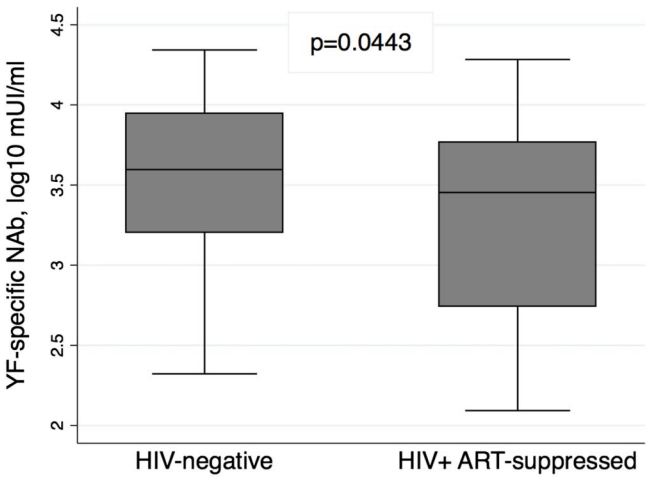

FIGURE 1. NAb titers to YF in HIV-infected participants and HIV-uninfected participants. YF virus-specific NAb titers were $\log_{10}$-transformed. ART, antiretroviral therapy with plasma HIV RNA level <200 copies per milliliter.

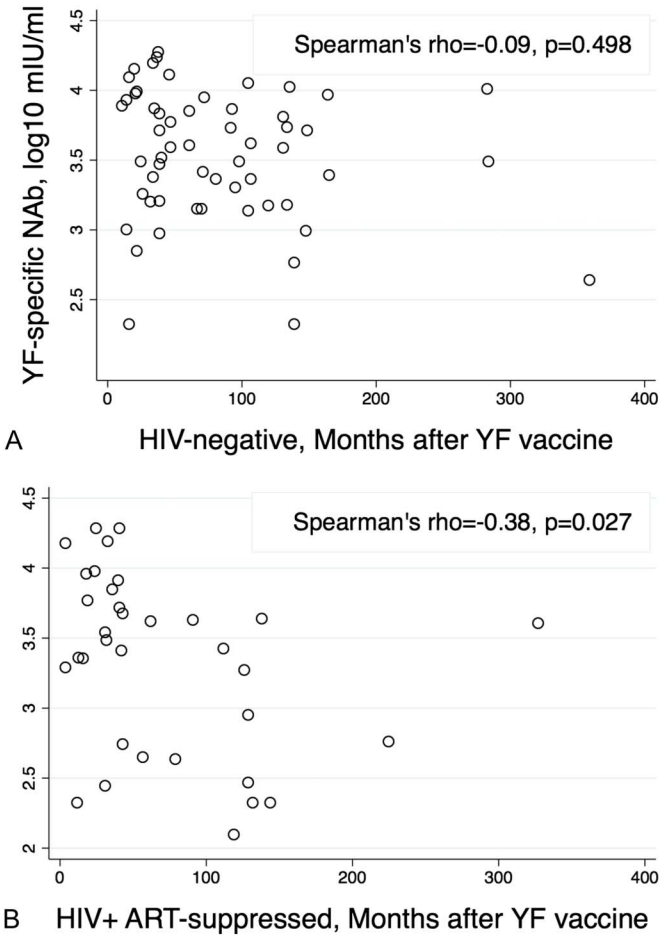

FIGURE 2. Correlation between time since vaccination and YF NAb titers in HIV-uninfected participants (A) and HIV-infected participants (B).

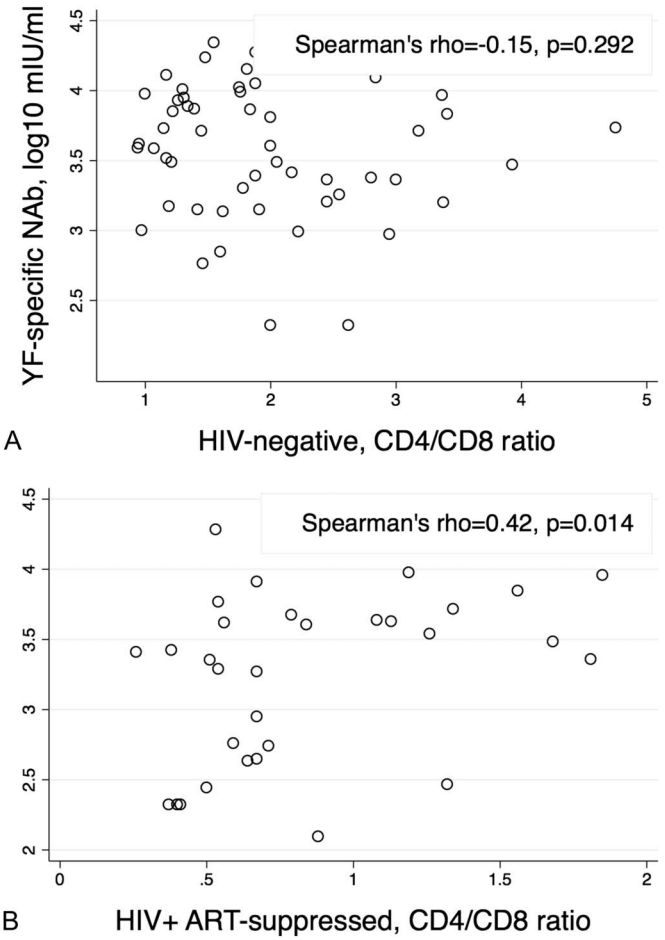

FIGURE 3. Correlation between $CD4^+/CD8^+$ ratio and YF NAb titers in HIV-uninfected participants (A) and HIV-infected participants (B).

with HIV-uninfected controls, despite similar time since YFV. Our study also found that among HIV-infected participants, but not among controls, lower NAb titers were correlated with the time elapsed since vaccination and with lower $CD4^+/CD8^+$ ratio. Interestingly, $CD4^+$ T-cell count was not significantly correlated with YFV NAb titers in our cohort ART-treated HIV-infected patients, and GBV-C viremia had no statistical association with titers of YFV NAb overall or among HIV-infected participants.

Previous studies including both ART-treated and untreated patients with a wider range of $CD4^+$ T-cell counts suggested that higher $CD4^+$ T-cell counts were associated with higher levels of YFV antibody response.[18,20] In our cohort, HIV-infected participants had a remarkably high median $CD4^+$ T-cell count, consistent with the clinical scenario observed in the present in our setting, where most HIV-infected patients have immediate access to medical care and ART. In this selected sample, YFV response was correlated with $CD4^+/CD8^+$ ratio, but not with $CD4^+$ T-cell count. It is possible that our findings are not applicable to other low-income or middle-income countries, where HIV-infected patients present with a broader range of $CD4^+$ T-cell count. However, with the recent changes in the recommendations for early antiretroviral initiation, we believe that the range of $CD4^+$ T-cell count shown in our study will become increasingly representative of HIV-infected patients overall.

Our results are consistent with an extensive literature that suggests treated HIV-infected patients with normal $CD4^+$ T-cell counts and undetectable HIV viral load still experience increased morbidity, mortality, and lower responses to vaccines compared with HIV-uninfected subjects.[1,15,34] In addition, in previous publications, this residual dysfunction was at least partially attributed to a state of persistent immune activation, which improves but fails to normalize after ART initiation and viral suppression.[5,35,36] Accordingly, impaired responses to vaccines were also correlated with increased immune activation in this population[15,37,38] and in other chronic inflammatory conditions.[39] An emerging literature suggests that $CD4^+/CD8^+$ ratio is a better surrogate for functional immune defects than the $CD4^+$ T-cell count in treated HIV infection and may be a strong correlate of chronic immune activation predicting detrimental outcomes in this setting.[25,40,41]

Besides $CD4^+/CD8^+$ ratio, other correlates of immune activation may also be associated with impaired vaccine responses. In a recent study of healthy volunteers, Muyanja et al analyzed characteristics of the immune microenvironment before YFV and demonstrated that activation of $CD8^+$ T cells and B cells, and also activated monocytes before vaccination was correlated with reduced NAb titers after vaccination. Interestingly, African volunteers in this study exhibited less durable T-cell and B-cell YFV responses, which were boosted after a second YFV dose.[24] In other studies, immune activation was also associated with impaired responses to serogroup C *Neisseria meningitidis* vaccine[42] and influenza vaccine[37] among HIV-infected patients. Unfortunately, we were unable to investigate the effect of other biomarkers of immune activation on YFV responses in our study. However, in most clinical settings, $CD4^+/CD8^+$ ratio will be the only readily available, easily understood biomarker of immune activation, and may be a useful tool for the prediction of residual morbidity and mortality in treated HIV-infected patients.

We have found an unexpectedly high rate of GBV-C viremia among both HIV-infected participants and HIV-uninfected participants, which was not associated with levels of YFV NAb overall or among HIV-infected patients and did not predict $CD4^+/CD8^+$ ratio in the latter group. Chronic infection with GBV-C was consistently associated with improved survival in studies that included HIV-infected patients treated before widespread use of highly active antiretroviral therapy (HAART) or patients in early periods of HAART use.[43–46] Few studies in the late-HAART period have not been consistent in demonstrating a beneficial effect of GBV-C coinfection,[47,48] and studies addressing this association among patients with early ART initiation are lacking. Since the hypothesized beneficial effect of GBV-C is to reduce HIV replication and excessive immune activation,[27,28,49] it is possible that effective and early ART might extinguish any advantage previously conferred by GBV-C coinfection. However, we cannot rule out that our study was underpowered to detect a true difference on YFV NAb titers related to GBV-C viremia.

YFV has been used for several decades as the main strategy for prevention of wild YF.[50] In healthy individuals, YFV efficacy is elevated, with up to 99% seroconverting rates,[22] and the protective response is long lasting, with detectable antibodies up to 35 years after vaccination.[23] Based on this enduring effect, World Health Organization (WHO) has recently modified its position, suggesting that a single YFV dose is sufficient to confer lifelong protective immunity, and a booster dose is no longer required.[51] However, as also suggested in the WHO position paper,[51] our findings indicate that this recommendation may not apply to HIV-infected individuals even in the current era of widespread use of ART. Earlier studies have shown that the duration of YFV response is reduced among HIV-infected persons. We confirmed that this trend persists in our cohort of ART-treated HIV-infected patients, for whom time elapsed after vaccination was inversely correlated with NAb titers. Although median NAb titers among HIV-infected patients in our study were above the previously established seropositivity cutoff point of 2.9 mIU/ml,[32] the decreasing trend in titers over time suggests that this population may benefit from a booster YFV if exposure to wild YF is likely to occur, as has been suggested previously.[52] The ideal interval between first vaccination and a booster dose for HIV-infected subjects is at the present unknown. At 5 years or more, 7 of 13 (54%, 95% CI: 25% to 81%) HIV-infected participants in our study had protective antibodies. At 10 years, 4 of 8 (50%, 95% CI: 16% to 84%) HIV-infected participants had protective antibodies. Although not conclusive, this roughly suggests that revaccination may be necessary less than 5 years after first vaccine dose.

Early ART initiation has already been shown to improve immune activation[35] and hepatitis B virus vaccine response.[15] We believe that early ART initiation could improve the efficacy of other immunization regimens among HIV-infected individuals, and additional studies addressing how early or late ART initiation would influence vaccine responses could further support the recommendations of immediate ART initiation. It is possible that early ART could even revert the detrimental effect of HIV infection on response to vaccines, but this hypothesis requires additional investigation. However, residual immune activation has been demonstrated despite early ART initiation,[35] and efficacious, clinically useful interventions to normalize immune activation are still unavailable. At the present, a booster vaccination seems to be the best available recommendation for HIV-infected individuals who are exposed to wild YF.

Our study has several limitations. We did not retrieve the information on ART and HIV suppression status in the HIV-infected group at the time the YFV was received. HIV-infected groups and HIV-uninfected groups were significantly unbalanced by sex, with higher proportion of HIV-infected men ($P < 0.001$). This reflects the prevalence of HIV in our setting, affecting disproportionately men who have sex with men.[53] Sex may impact vaccine responses in general,[54,55] and YFV immunogenicity has been shown to be higher among males than females in studies conducted among HIV-uninfected participants.[56,57] Nevertheless, one study in HIV-infected individuals found that females had higher NAb responses than males.[18] Thus, the effect of sex imbalance in our study is unclear but should not undermine the conclusions of the study because sex was controlled for the in the multivariate analyses. We also had a nonsignificant imbalance of age, with median age of 46 years old in the HIV-infected group and 38 years old in the HIV-uninfected group. Although some studies have suggested that older adults may have impaired response to YFV, the effect of age seems to be more relevant beyond 60 years old, and it is unlikely that the age difference seen in our study had an important impact in results. Furthermore, the difference in NAb titers between groups persisted in the model adjusted for age. Our study also had a limited sample size, which was appropriate for the analysis of NAb titers but was underpowered to analyze the effect of HIV status on seropositivity to YF. Finally, we assessed chronic GBV-C infection with a single measurement of GBV-C viremia and could not discriminate recent unresolved from chronic infections.

In conclusion, the decreased YFV efficacy among HIV-infected patients demonstrated in previous studies seems to persist among HIV-infected individuals on stable ART, who

seem to have impaired NAb responses to YFV compared with HIV-uninfected individuals with similar time after YFV. Moreover, lower NAb titers were correlated with $CD4^+/CD8^+$ ratio and time since YFV only among HIV-infected participants. This strengthens the hypotheses that persistent immune activation is a predictor of functional immune defects in ART-treated, HIV-infected patients, and that strategies to reduce excessive residual inflammation in this population are urgently needed. In addition, it suggests YF-exposed, HIV-infected patients should receive more than a single lifetime YFV dose. Possible strategies for preventing YF in this population include performing periodical measures of NAb titers or delivering a booster dose of YFV. The ideal time point for the booster vaccination remains unknown in this population.

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