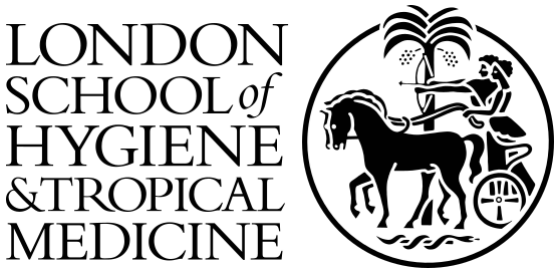

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Identification of Elite and Viremic Controllers From a Large Urban HIV Ambulatory Center in Kampala, Uganda

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Background: Throughout the world, there are antiretroviral therapy–naive HIV+ individuals who maintain elevated peripheral CD4$^+$ T-cell counts, historically referred to as long-term nonprogressors (LTNPs). With recent improvements in viral load (VL) detection methods to levels as low as 20 copies per milliliter, 2 subsets of LTNPs have been defined: elite controllers (ECs), with undetectable VLs for at least 6–12 months, and viremic controllers (VCs), with VLs between 200 and 2000 copies per milliliter. ECs and VCs have been extensively studied in the developed world to determine underlying mechanisms responsible for virologic control. In sub-Saharan Africa, most studies have characterized LTNPs based on immunologic criteria making it difficult to compare findings with the Western cohorts, which use virologic criteria. Here, we describe a cohort of Uganda ECs and VCs attending a large HIV ambulatory center in Kampala, Uganda, based initially on CD4 counts and confirmed by repeated VL measurements.

Methods: A cross-sectional study was conducted among 14,492 HIV-infected, antiretroviral therapy–naive individuals aged 18 years and older under care for at least 5 years with serial peripheral CD4 counts ≥500 cells/μL. Among those, we determined the frequency of individuals with VLs <2000 copies per milliliter for at least 6 months.

Results: We report a prevalence of 0.26% (38/14,492) of HIV controllers in the clinic. We identified 36 ECs and 2 VCs. These individuals were middle-aged with an average CD4 count of 858 ± 172 (mean ± SD, 95% confidence interval: 795 to 921). Their average duration in HIV care was 7.4 ± 2.1 years (mean ± SD, 95% confidence interval: 6.6 to 8.1). The majority of EC/VCs were women (87%, 33/38), reflecting the demographics of the urban clinic.

Conclusions: For the first time, this study demonstrates the frequency of EC/VCs in a large urban clinic in Uganda. Further study of these East African subjects may provide insights into how some individuals are able to control HIV in the absence of medications.

Key Words: HIV, elite controllers, viremic controllers, nonprogressors, HIV cure, Uganda

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INTRODUCTION

Before the availability of antiretroviral therapy (ART), the median time from HIV seroconversion to AIDS-defining illness was 8–10 years.[1,2] Without ART, in the majority of HIV-infected individuals, seropositivity is associated with an inevitable loss of CD4$^+$ T cells, resulting in the development of AIDS.[3] In a few, rare individuals, however, there is little or no clinical progression. These individuals typically maintain elevated CD4$^+$ T-cell counts. Based on these immunological criteria, they are termed as long-term nonprogressors (LTNPs).[2] In the Western world, these individuals comprise about 3%–5% of the HIV-infected population.[4] With recent developments in viral load (VL) detection to levels as low as 20 copies per milliliter,[5] researchers in the Western countries have reclassified LTNPs based on virologic criteria.[6] Two phenotypes have been defined: the first includes individuals able to fully suppress HIV-1 replication, with undetectable plasma VLs (below 20–50 copies/mL) for at least 6–12 months; these are referred to as elite controllers (ECs).[6] The second includes

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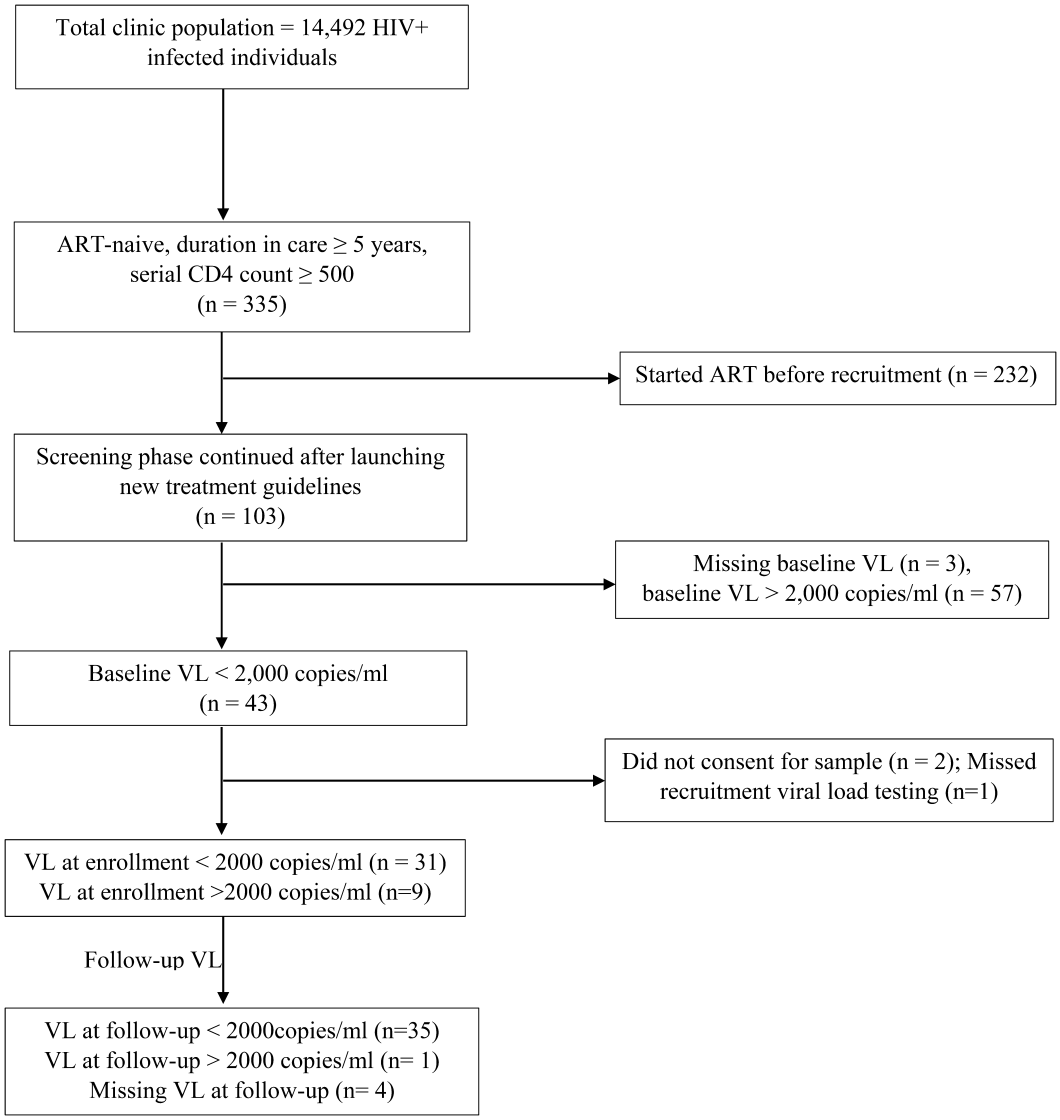

FIGURE 1. Screening and enrollment flow chart for study participants.

individuals who have detectable plasma VLs <2000 copies per milliliter, referred to as viremic controllers (VCs).[6]

Mechanisms resulting in virologic control have not been fully uncovered. For EC/VCs in the developed world, approximately 20% of the phenotype can be explained by protective HLA alleles[7]; other immune and host genetic mechanisms may also be operational.[8] However, there is paucity of literature regarding adult LTNPs in sub-Saharan Africa, and the prevalence of the phenotype is unknown, mainly due to differences in definitions used.[9] In sub-Saharan Africa, most studies have characterized LTNPs based on immunologic criteria, making it difficult to compare findings with the Western cohorts that use virologic criteria.[6] Recent data from Rakai HIV cohort in Uganda reported a prevalence of 9.1% LTNPs and 1.4% ECs. Their definition of EC was a one-time undetectable VL,[9] and the prevalence of VCs was not reported. Here, we characterize a cohort of adult HIV+ Ugandan ECs/VCs attending a large ambulatory clinic in Kampala, Uganda. Further study of these East African subjects may provide insights into how some individuals are able to control HIV in the absence of ART.

METHODS

Study Design and Population

A cross-sectional study was conducted between August 2016 and February 2018 by reviewing the medical records of HIV+, ART-naive individuals aged 18 years and older, who were under care at Makerere University Joint AIDS Program (MJAP), Mulago ISS clinic in Kampala, Uganda for at least 5 years. Patients were enrolled after a baseline or initial VL of less than 2000 copies per milliliter, with serial CD4 counts ≥ 500 cells/μL. An arbitrary zero time point corresponding to

the initial VL was set for all participants. At recruitment, peripheral blood sample was obtained and HIV VL was analyzed by quantitative reverse transcriptase–polymerase chain reaction using Abbott RealTime HIV-1 assay (Abbott Molecular, Des Plaines, IL). The time interval between initial and enrollment VL was determined and recorded in days. To confirm EC/VC status, a follow-up VL was performed and the time interval between baseline and follow-up VL was also calculated. For safety reasons, we excluded any individuals with a hemoglobin of <10 mg/dL, or active opportunistic infection. Before November 2016, HIV-infected individuals were started on ART when their $CD4^+$ T-cell count dropped below 500 cells/μL. Following the Test and Treat policy launched by the Ugandan Ministry of Health on the November 29, 2016, which was operational at the ISS clinic beginning in March 2017, we excluded participants who were started on ART, irrespective of CD4 count. Sociodemographic and clinical characteristics of the study participants

TABLE 1. Demographic and Clinical Characteristics of Participants

Participant	Age	Sex	Baseline CD4 Count	Duration in Care (yrs)	Baseline or Initial Viral Load (Day 0)	Enrollment Viral Load (Days From Initial Viral Load)	Follow-up Viral Load (Days From Initial Viral Load)	BMI*
EC 1	53	F	1245	10	UD	UD (254)	UD (593)	33.9
EC 2	40	M	920	6	UD	10,500 (151)	UD (518)	27.2
EC 3	32	F	1008	5	UD	UD (261)	UD (368)	31.8
EC 4	38	F	919	9	UD	UD (361)	UD (754)	18.9
EC 5	51	F	895	10	UD	1300 (302)	UD (368)	29.5
EC 6	38	F	1162	6	UD	75 (253)	UD (350)	25.3
EC 7	36	F	1188	7	UD	UD (250)	UD (380)	38.5
EC 8	41	F	1192	6	UD	2840 (315)	UD (360)	37.5
EC 9	40	F	940	5	UD	10,800 (125)	189 (580)	26.3
EC 10	37	F	805	5	UD	155 (458)	UD (496)	24.5
EC 11	56	M	833	7	UD	UD (274)	Missing	17.2
EC 12	54	F	669	8	UD	782 (156)	UD (372)	25.3
EC 13	42	F	909	5	UD	UD (372)	UD (647)	31.8
EC 14	30	F	1050	5	UD	UD (304)	UD (340)	29.3
EC 15	34	F	918	8	UD	1490 (104)	UD (388)	24.2
EC 16	29	F	747	5	UD	14,800 (138)	UD (392)	Missing
EC 17	27	F	609	6	UD	209 (127)	UD (374)	27.8
EC 18	37	F	728	6	UD	UD (273)	UD (366)	23.9
EC 19	39	F	650	9	UD	UD (160)	UD (436)	Missing
EC 20	27	F	716	7	UD	97 (291)	UD (402)	30.7
EC 21	32	F	698	5	UD	280 (409)	UD (747)	30
EC 22	56	F	652	8	UD	1380 (228)	UD (465)	25.3
EC 23	47	F	1322	9	UD	UD (299)	UD (388)	31.2
EC 24	38	F	589	5	UD	75,100 (301)	UD (362)	21.4
EC 25	42	F	1120	10	UD	UD (308)	160 (525)	Missing
EC 26	42	F	1021	8	UD	5250 (180)	UD (451)	21.3
EC 27	37	F	772	10	UD	243 (278)	UD (584)	29
EC 28	35	F	1055	12	UD	12,500 (284)	UD (388)	20.4
EC 29	40	F	994	10	UD	UD (272)	UD (366)	32.3
EC 30	41	M	778	9	UD	UD (366)	UD (434)	25.2
EC 31	37	F	1063	6	UD	UD (252)	UD (316)	26.1
EC 32	40	M	732	6	UD	388 (255)	UD (393)	28.9
EC 33	38	F	719	6	UD	113 (248)	UD (383)	Missing
EC 34	45	F	1036	6	UD	UD (325)	UD (425)	Missing
EC 35	42	F	836	6	UD	99 (286)	UD (362)	35.5
EC 36	28	F	653	8	UD	UD (279)	UD (370)	Missing
VC 1	41	F	852	10	UD	285 (221)	Missing	30.2
VC 2	56	M	897	11	UD	1220 (429)	Missing	25.6
Progressor 1	43	F	781	8	UD	2310 (137)	10,200 (394)	32.7
Progressor 2	37	F	631	11	UD	10,200 (426)	Missing	27.4

*BMI, body mass index; EC, elite controller; UD, Undetectable; VC, viremic controller.

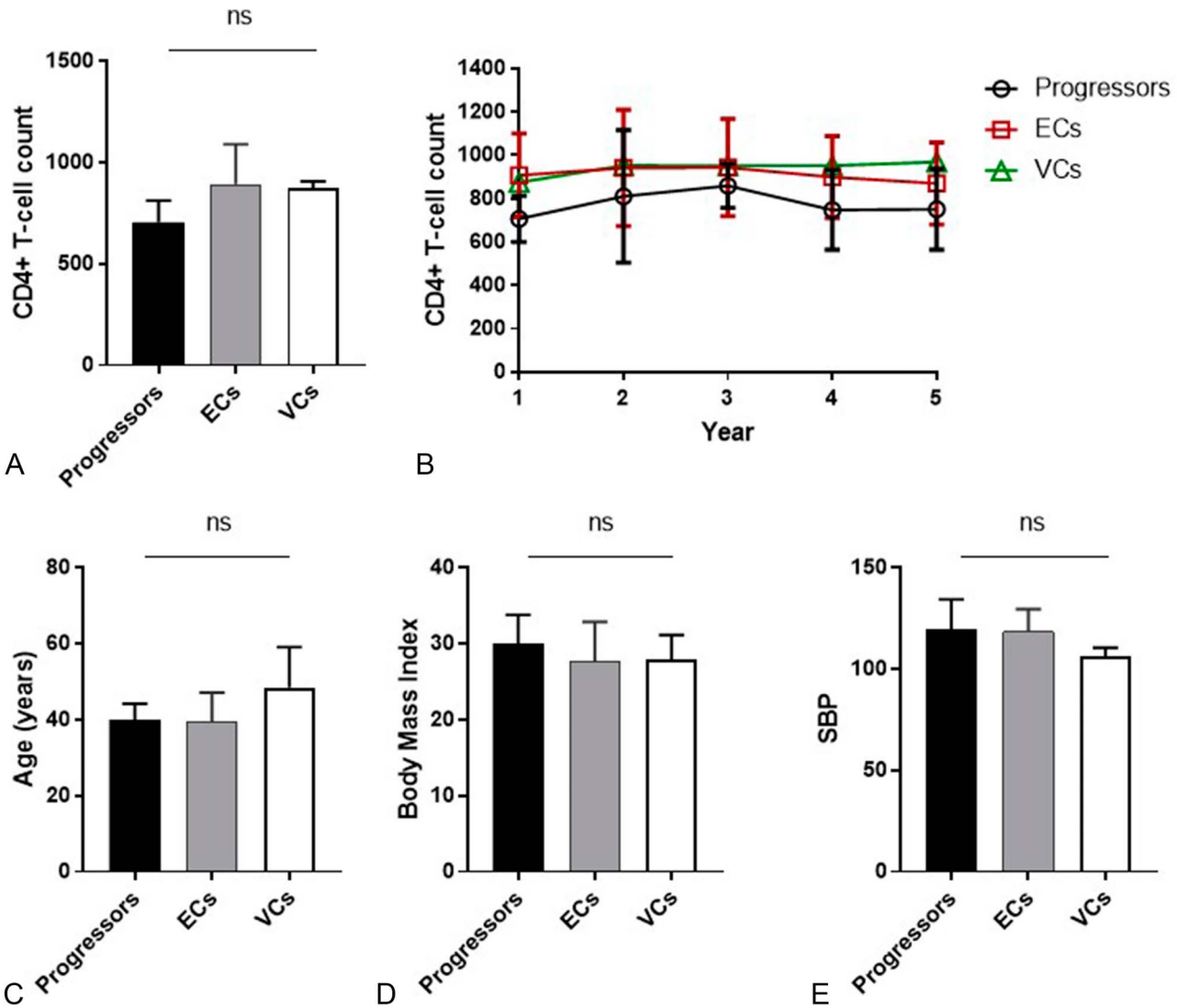

FIGURE 2. Select characteristics of study participants. Comparison of demographic and clinical characteristics of ECs, VCs, and progressors. A, baseline CD4 counts, (B) serial CD4 counts, (C) age, (D) BMI, and (E) systolic blood pressure or SBP. Data were analyzed for each variable using the Mann–Whitney test. Shown are mean and SD for B; medians, 25th and 75th percentiles, and range for other figures, n.s., not significant or $P > 0.05$.

were obtained from electronic medical records. The participants agreed and signed informed consent forms to take part in the study. The research was approved by School of Biomedical Sciences, Higher Degrees Research and Ethics committee (SBS HDREC), Makerere University, and the National Council of Science and Technology (UNCST).

Statistical Analysis

Analysis was performed using GraphPad Prism version 7.0. Continuous variables were summarized using mean values and SDs or medians and interquartile range [confidence interval (CI)]. The frequencies of ECs and VCs were expressed as percentages of the total population analyzed. *P* values <0.05 were consider significant.

RESULTS

The medical records of 14,492 HIV+ individuals followed in the ISS clinic were reviewed (Fig. 1). Of those, 335 were ART-naive, followed for more than 5 years, with multiple CD4 counts not less than 500/μL. Approximately 2/3rds of those (232/335) had begun on ART before recruitment and were thus excluded from further study. Of the remaining 103, 3 had no baseline or initial VL and 57 had VL >2000. Forty-three subjects thus had an initial VL performed, which in all cases was undetectable, and of those, 2 refused consent for the study. Forty of 41 consented participants had an enrollment VL. Out of those 40 subjects, 9 had enrollment VL >2000, and 31 had VL <2000. At follow-up VL testing, 36 had VL <2000, 1 had VL >2000, and 3 were missing a follow-up VL. Based on the 3 serial VLs, 36 were classified

as ECs, 2 as VCs, and 2 as progressors (Table 1). Demographic and clinical characteristics of those patients are shown in Table 1. These subjects had a mean age of 40.8 ± 8.2 years (mean ± SD, 95% CI: 37.5 to 43.5) and an average CD4 count of 858 ± 172 (mean ± SD, 95% CI: 795 to 921). Their average duration in HIV care was 7.4 ± 2.1 years (mean ± SD, 95% CI: 6.6 to 8.1), and 87% (27/31) were women. The female predominance reflects the sex disparity of the ISS clinic. None, including those defined as progressors, developed any AIDS-defining illness during the course of the study.

In comparing ECs, VCs, and progressors, there were no differences in peripheral CD4 T-cell counts (*P* value = ns, Fig. 2A). There was also no significant difference in the trends of CD4 count over a 5-year period between those groups, suggesting that CD4 count alone is not a distinguishing feature of controller status (Fig. 2B). In addition, there was no difference in average age between EC/VC and progressors (40.29 ± 8.48 vs. 38.6 ± 4.142, Fig. 2C), in body mass indices, or systolic blood pressure measurements (Figs. 2D, E). In reviewing the medical records of these EC/VCs, we found no history of tuberculosis, other opportunistic infections, or any AIDS-defining illnesses. Similarly, the progressors did not have a history of any AIDS-defining illnesses. Thus, for those individuals who maintained high CD4 counts over time, there were no distinguishing demographic or clinical features to predict whether they were EC/VC or would become progressors, at least for the relatively short duration of this study. We uncovered no evidence that any of these EC/VCs were ever prescribed or took any ART.

DISCUSSION

This is the first study in Uganda to characterize HIV+ LTNP in 2 subsets, that is, ECs and VCs, based on virologic criteria. In most studies demonstrating the existence of ECs and VCs in sub-Saharan Africa, the cohorts have been very small, solely based on immunologic criteria, and viral and host genetics have not been explored.[9,10] Due to differences in definitions of HIV long-term nonprogression, the prevalence and clinical characteristics of HIV controllers who have not received ART is largely undefined.[6,9] In contrast to a high frequency of HIV controllers reported based on immunologic criteria by Laeyendecker et al[9] in 2009 (9.1%), we demonstrate a lower prevalence of HIV EC/VCs based on VL (0.26%). This is probably an underestimate due to a variety of reasons, including (1) it is likely that a number of EC/VCs with a peripheral CD4 <500/μL had already been initiated on ART and thus excluded from the study, and (2) the newly launched Test and Treat policy in Uganda in which the majority (here, 69%) of potential HIV controllers with CD4 >500 had already been started on ART and were thus automatically excluded from our study. Thus, we would estimate that the true frequency of EC + VCs in this clinic is greater than 1 in 500 patients.

In addition, it has been shown that healthy HIV-negative Africans have lower CD4 counts compared with those of US and other Western patients, suggesting that strict immunologic criteria may not be fully generalizable or applicable to African populations.[9] Because many EC/VCs in the developed world have CD4 counts <500, setting a threshold CD4 count >500 would have excluded perhaps even a greater number of Ugandan HIV controllers. One concern is that the Test and Treat strategy recommended by the World Health Organization and now being implemented throughout Africa will preclude the in-depth mechanistic study of EC/VCs, who represent functional cures of HIV. Whether more widespread VL testing will temper the World Health Organization Test and Treat policy, coupled with fears regarding limited resources and growing expenses of ART, not to mention increasing virus resistance by giving ART in the absence of VL measurements, remains to be seen.

CONCLUSIONS AND RECOMMENDATIONS

This study demonstrates the existence of HIV+ EC/VCs in Uganda based on very strict, conservative criteria and highlights the importance of defining control of HIV based on VL testing in the developing world. It is hoped that these individuals, as models for functional cure, will help us understand the underlying mechanisms of HIV control in East Africans, which may differ from those in the Western world, including African Americans, whose origins are mainly West African. Further study of Ugandan EC/VCs may inform both the cure agenda and prophylactic vaccine efforts.

REFERENCES

1. Morgan D, Mahe C, Mayanja B, et al. HIV-1 infection in rural Africa: is there a difference in median time to AIDS and survival compared with that in industrialized countries? *AIDS.* 2002;16:597–603.
2. Zaunders J, van Bockel D. Innate and adaptive immunity in long-term non-progression in HIV disease. *Front Immunol.* 2013;4:95.
3. Pantaleo G, Fauci A. Immunopathogenesis of HIV infection. *Annu Rev Microbiol.* 1996;50:825–854.
4. Deeks SG, Walker BD. Human immunodeficiency virus controllers: mechanisms of durable virus control in the absence of antiretroviral therapy. *Immunity.* 2007;27:406–416.
5. Palmer S, Wiegand AP, Maldarelli F, et al. New real-time reverse transcriptase-initiated PCR assay with single-copy sensitivity for human immunodeficiency virus type 1 RNA in plasma. *J Clin Microbiol.* 2003; 41:4531–4536.
6. Gurdasani D, Iles L, Dillon DG, et al. A systematic review of definitions of extreme phenotypes of HIV control and progression. *AIDS.* 2014;28: 149.
7. Walker WE, Kurscheid S, Joshi S, et al. Increased levels of macrophage inflammatory proteins result in resistance to R5-tropic HIV-1 in a subset of elite controllers. *J Virol.* 2015;89:5502–5514.
8. Okulicz JF, Marconi VC, Landrum ML, et al. Clinical outcomes of elite controllers, viremic controllers, and long-term nonprogressors in the US Department of Defense HIV natural history study. *J Infect Dis.* 2009;200: 1714–1723.
9. Laeyendecker O, Redd AD, Lutalo T, et al. Frequency of long-term nonprogressors in HIV-1 seroconverters from Rakai Uganda. *J Acquir Immune Defic Syndr.* 2009;52:316.
10. Patel P, Brooks M, Anabwani G, et al. Control and non-progression of HIV-1 infection in sub-Saharan Africa: a case and review: case report. *South Afr J HIV Med.* 2012;13:152–155.