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Low-level Viremia Early in HIV Infection

Iris Chen, BS,
Dept. of Pathology, Johns Hopkins University School of Medicine, Baltimore, MD, USA
ichen@jhmi.edu

Vanessa Cummings, BS MT (ASCP),
Dept. of Pathology, Johns Hopkins University School of Medicine, Baltimore, MD, USA
vcummin1@jhmi.edu

Jessica M. Fogel, PhD,
Dept. of Pathology, Johns Hopkins University School of Medicine, Baltimore, MD, USA
jfogel@jhmi.edu

Mark A. Marzinke, PhD,
Dept. of Pathology, Johns Hopkins University School of Medicine, Baltimore, MD, USA
mmarzin1@jhmi.edu

William Clarke, PhD,
Dept. of Pathology, Johns Hopkins University School of Medicine, Baltimore, MD, USA
wclarke@jhmi.edu

Matthew B. Connor, MS,
Vaccine and Infectious Disease Division, Fred Hutchinson Cancer Research Center, Seattle, WA, USA mconnor@fhcrc.org

Sam Griffith, MAT,

*__Address correspondence and reprint requests to:__ Susan H. Eshleman, MD/PhD Dept. of Pathology, Johns Hopkins University School of Medicine Ross Building, Room 646 720 Rutland Avenue Baltimore, MD 21205 Phone: (410) 614-4734 Fax: (410) 502-9244 seshlem@jhmi.edu. __Alternate contact:__ Iris Chen Dept. of Pathology, Johns Hopkins University School of Medicine Ross Building, Room 646 720 Rutland Avenue Baltimore, MD 21205 Phone: (410) 614-6498 Fax: (410) 502-9244 ichen@jhmi.edu.

Conflicts of Interest
None of the authors has a conflict of interest.

Author contributions
All authors contributed to manuscript preparation. Additional author roles are listed below.
Iris Chen Coordinated the study; performed HIV genotyping; analyzed the data; wrote the manuscript
Vanessa Cummings Coordinated HIV diagnostic and viral load testing
Jessica M. Fogel Assisted with analysis of ARV drug data
Mark A. Marzinke Coordinated and reviewed results for ARV drug testing
William Clarke Coordinated and reviewed results for ARV drug testing
Matthew B. Connor Data analyst for HPTN 061
Sam Griffith Study Coordinator for HPTN 061
Susan Buchbinder Site PI for HPTN 061
Steven Shoptaw Site PI for HPTN 061
Carlos del Rio Site PI for HPTN 061
Manya Magnus Site PI for HPTN 061
Sharon Mannheimer Site PI for HPTN 061
Darrell P. Wheeler Protocol Co-Chair for HPTN 061
Kenneth H. Mayer Protocol Co-Chair for HPTN 061, Site PI
Beryl A. Koblin Protocol Chair for HPTN 061, Site PI
Susan H. Eshleman Designed the study; reviewed study data; wrote the manuscript

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FHI 360, Research Triangle Park, NC, USA sgriffith@fhi360.org

Susan Buchbinder, MD,
Bridge HIV, San Francisco Department of Public Health, San Francisco, CA, USA susan.buchbinder@sfdph.org

Steven Shoptaw, PhD,
Department of Family Medicine, University of California Los Angeles, Los Angeles, CA, USA sshoptaw@mednet.ucla.edu

Carlos del Rio, MD,
Department of Global Health, Emory University Rollins School of Public Health, Atlanta, GA, USA cdelrio@emory.edu

Manya Magnus, PhD, MPH,
Department of Epidemiology and Biostatistics, The George Washington University, Washington, DC, USA manyadm@gwu.edu

Sharon Mannheimer, MD,
Department of Medicine, Harlem Hospital, Columbia University, Mailman School of Public Health, New York, NY, USA sbm20@columbia.edu

Darrell P. Wheeler, PhD, MPH, ASCW,
Graduate School of Social Work, Loyola University Chicago, Chicago, IL, USA dwheeler@luc.edu MPH, ASCW

Kenneth H. Mayer, MD,
The Fenway Institute, Beth Israel Deaconess Medical Center/Harvard Medical School, Boston, MA, USAk mayer@fenwayhealth.org

Beryl A. Koblin, PhD, and
Laboratory of Infectious Disease Prevention, Lindsley F. Kimball Research Institute, New York Blood Center, New York, NY, USA bkoblin@nybloodcenter.org

Susan H. Eshleman, MD, PhD
Dept. of Pathology, Johns Hopkins University School of Medicine, Baltimore, MD, USA

Abstract

HIV RNA levels are usually high early in HIV infection. In the HPTN 061 study, men were tested for HIV infection every six months; six (21.4%) of 28 men who acquired HIV infection during the study had low or undetectable HIV RNA at the time of HIV diagnosis. Antiretroviral drugs were not detected at the time of HIV diagnosis. False-negative HIV test results were obtained for two men using multiple assays. Antiretroviral drug resistance mutations were detected in HIV from one man. Additional studies are needed to identify factors associated with low HIV RNA levels during early HIV infection.

Keywords

HIV; seroconversion; viral load; HIV controllers; diagnosis

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INTRODUCTION

HIV infection is classified into different stages by the presence of HIV RNA, viral antigens, and HIV-specific antibodies.[1] HIV RNA levels tend to be high during acute HIV infection and then decline in response to the development of HIV-specific antibodies, eventually stabilizing at a viral load set point. Low-level viremia in the absence of antiretroviral (ARV) drugs has been observed in elite and viremic HIV controllers who are able to maintain undetectable (<50 copies/mL) or low (50-2,000 copies/mL) viral loads for at least one year without ARV therapy (ART).[2] Studies suggest that elite controllers represent <1% of HIV-infected individuals, while viremic controllers represent up to 7%.[2-4] Other studies have shown that virologic suppression among HIV controllers is usually established within the first year of HIV infection.[3,5,6] In a recent study, over half of the individuals identified as HIV controllers had undetectable viral loads at seroconversion, and 25% achieved viremic control within six months.[6]

Relatively little is known about the frequency of viral suppression early in HIV infection. Viral suppression may complicate HIV diagnosis, since some diagnostic testing algorithms include HIV RNA assays.[7] Viral suppression has also been associated with false-negative results using serologic HIV assays.[8-10] In this report, we describe six individuals who acquired HIV infection during a clinical study and had low or undetectable HIV RNA at their first HIV-positive study visit.

METHODS

Study Cohort

Individuals described in this report were enrolled in the HIV Prevention Trials Network (HPTN) 061 study (NCT 0095129). The HPTN 061 study enrolled Black men who have sex with men in six cities in the US.[11,12] Men who were HIV uninfected at enrollment were tested for HIV infection 6 and 12 months after enrollment. HIV screening was performed at study sites using a single HIV rapid test; if the rapid test was reactive, a Western blot was performed at a local laboratory. Stored plasma samples were tested retrospectively at the HPTN Laboratory Center for quality assurance, to identify men who had acute or recent HIV infection at enrollment, to confirm cases of HIV seroconversion, and to characterize viral and host factors related to HIV acquisition.

Laboratory Methods

Test results presented in this report were obtained retrospectively at a centralized laboratory using plasma samples collected at study enrollment and at the 6- and 12-month follow-up visits. The following assays were included in these analyses: the OraQuick ADVANCE Rapid HIV-1/2 Antibody Test (OraSure Technologies, Bethlehem, PA); a third-generation enzyme immunoassay (EIA; VITROS Anti-HIV 1+2 Test, Ortho Clinical Diagnostics, Raritan, NJ); a fourth-generation chemiluminescent microparticle immunoassay (ARCHITECT HIV Ag/Ab Combo assay, Abbott Laboratories, Wiesbaden, Germany); a fourth-generation EIA (GS HIV Combo Ag/Ab EIA, Bio-Rad Laboratories, Redmond, WA), a discriminatory assay (the Multispot HIV-1/HIV-2 Rapid Test, Bio-Rad Laboratories,

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Redmond, WA); and a Western blot assay (Genetics System HIV-1 Western Blot, Bio-Rad Laboratories, Redmond, WA). HIV RNA testing was performed using a qualitative HIV RNA assay (the APTIMA HIV-1 RNA Qualitative Assay, Gen-Probe Inc., San Diego, CA) and a viral load assay (the COBAS AmpliPrep/COBAS TaqMan HIV-1 Test, version 2.0, Roche Molecular Diagnostics, Indianapolis, IN). HIV genotyping was performed for samples with >400 copies/mL HIV RNA using the ViroSeq HIV-1 Genotyping System (Celera Corporation, Alameda, CA).

ARV drug testing was performed using a modified version of a qualitative multi-drug assay.[13] This assay uses high resolution mass spectrometry (HRMS) to screen for 15 ARV drugs (non-nucleoside reverse transcriptase inhibitors [NNRTIs], nucleoside reverse transcriptase inhibitors [NRTIs], and protease inhibitors [PIs]). The modified version of the assay is faster and has higher resolution; the lower limit of identification for all 15 ARV drugs is 10 ng/mL. Briefly, samples were processed and injected into a liquid chromatography system equipped with Accela 1250 pumps. Drugs were then separated using a Hypersil Gold PFP ultra performance liquid chromatography column (50x2.1 mm, 1.9 μm) and detected using the QExactive mass analyzer in full scan mode. HRMS equipment was obtained from Thermo Fisher Scientific (Pittsburgh, PA).

Ethical Considerations

The institutional review boards of the participating institutions approved the HPTN 061 study, and study participants provided written informed consent.

RESULTS

In HPTN 061, 28 (2.4%) of the 1,164 men who were HIV uninfected at enrollment acquired HIV infection during the study.[11] The median HIV viral load for these 28 men at the time of HIV diagnosis (6 or 12 months after enrollment) was 25,680 copies/mL (interquartile range: 5,165-153,794 copies/mL). Six (21.4%) of the 28 men had a HIV viral load <1,000 copies/mL at their first HIV-positive visit. The six men were enrolled in Atlanta, Los Angeles, and New York City and had a mean age of 28.2 years (range: 18 to 43 years).

Additional testing was performed retrospectively using a panel of assays (see Methods, Table). In all six cases, HIV tests were non-reactive or negative at study enrollment. These tests included an HIV rapid test, a third-generation EIA (Vitros EIA), two fourth-generation immunoassays (Abbott and Bio-Rad Combo assays), and a qualitative HIV RNA assay (Aptima HIV RNA assay). In Cases #1-4, all of the serologic assays were reactive or positive at the 6-and 12-month visits, including a Western blot assay and an HIV-1/HIV-2 discriminatory assay (Multispot assay). In Case #5, the Vitros EIA and the Multispot assay were reactive at the 6-month visit; other serologic assays were non-reactive and a Western blot was indeterminate. At the 12-month visit, the Abbott Combo assay was reactive and a Western blot was positive. In Case #6, the Vitros EIA and the Abbott Combo assay were reactive at the 6-month visit; other serologic assays were non-reactive and a Western blot was indeterminate. At the 12-month visit, a Western blot was positive and the Multispot assay was reactive.

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HIV RNA was detected at the 6-month visit in Cases #2-4 and at the 12-month visit in Cases #1-4. In Cases #5 and #6, the Aptima HIV RNA assay was non-reactive and the viral load was undetectable (<20 copies/mL) at both follow-up visits. In these two cases, the signal-to-cutoff (S/CO) ratios for the Vitros EIA and the Abbott Combo assay increased between the 6-and 12-month visits and the Western blots evolved from indeterminate to positive. In contrast, the S/CO ratios remained similar for the Bio-Rad Combo assay and the Aptima HIV RNA assay at these two visits.

In all six cases, ARV drugs (NNRTIs, NRTIs, and PIs) were not detected at the 6- or 12-month visits. In one case (Case #1), darunavir and tipranavir were detected at the enrollment visit when the participant was HIV uninfected. HIV drug resistance mutations (K103N and P225H) were detected in one case (Case #4) at the 6- and 12-month visits; these mutations are associated with reduced susceptibility to nevirapine and efavirenz.[14,15]

DISCUSSION

HIV controllers are thought to comprise a small proportion of HIV-infected individuals.[2-4] However, in the HPTN 061 study, six (21.4%) of the 28 men who seroconverted during the study had low or undetectable HIV RNA levels at their first HIV-positive visit (six months after a negative HIV test). HIV RNA levels were still low six months after the first positive HIV test. The follow-up period in the HPTN 061 study was too short to determine whether these men were HIV controllers, since the criteria used to identify HIV controllers usually include having viremic control for at least one year.[2]

It is not clear why the frequency of low-level viremia in early infection was high in this cohort. Recent studies of viral load dynamics early in infection have focused on small numbers of well-characterized elite or viremic HIV controllers,[3-6,16] and most reports from longitudinal cohort studies do not provide detailed information about viremia early in infection. Some studies have suggested that certain HLA alleles (e.g., HLA-B57) have a protective effect that may contribute to lower viral load set points in HIV controllers.[17-19] The HPTN 061 study did not seek consent for human genetic testing; therefore, it was not possible to perform HLA typing in this study. Viral factors could also contribute to viral suppression in early HIV infection. Viruses from some HIV controllers during acute or early HIV infection have been shown to have reduced replication capacity.[5] In addition, some ARV drug resistance mutations are known to have viral fitness costs;[20] therefore, individuals who are infected with drug-resistant HIV strains might also have lower viral loads early in infection. Drug resistance mutations were detected in HIV from one man in this study (K103N and P225H), but these mutations are associated with little to moderate effects on viral replication capacity.[20]

In this report, we used a qualitative multi-drug assay to assess ARV drug use. Use of an objective biomedical measure of ARV drug use is important, since self-report of ARV drug use may be unreliable.[21-23] ARV drug testing indicated that none of the men were taking ARV drugs at the 6- or 12-month visits; however, PIs were detected in one man at study enrollment (prior to infection). Those drugs may have been used for pre- or post-exposure prophylaxis (PrEP or PEP). The HPTN 061 study collected data on the use of ARV drugs

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for PrEP, PEP, and ART; all six men described in this report denied previous or current ARV drug use. However, some men may have previously used PrEP or PEP but chose not to disclose this to study staff. We recently analyzed enrollment samples from HIV-infected men in the HPTN 061 study who denied both knowledge of their HIV status and ARV drug use but had low or undetectable HIV RNA.[22] ARV drugs were detected in enrollment samples from 85% of those men; in some cases, unusual patterns of ARV drugs were detected. The use of ARV drugs in the HPTN 061 study cohort, including the use of non-suppressive regimens, use of unusual drug combinations, and likely use of ARV drugs for other indications (e.g., PrEP or PEP), may be related to our finding of viral suppression early in infection among many of the men who seroconverted during the study. A limitation of this study is that we only tested for NRTIs, NNRTIs, and PIs. Some of the men may have been taking another class of ARV drug (e.g., an integrase strand-transfer inhibitor).

Finally, we note that viral suppression early in HIV infection can complicate HIV diagnosis. In this report, HIV infection would have been missed in two cases (Cases 5 and 6) by testing algorithms recently proposed by the US Centers for Disease Control.[7] In Case 5, both fourth-generation assays (the Abbott and Bio-Rad Combo assays) were non-reactive at the 6-month visit; HIV infection would have been missed if either test was used as the first step in the HIV testing algorithm. HIV infection would also have been missed in this case at the 12-month visit using the Bio-Rad Combo assay. In Case 6, the Bio-Rad Combo assay was non-reactive at the 6- and 12-month visits; even if a different fourth-generation test were used for screening (e.g., the Abbott Combo assay, which was reactive), this infection would have been missed at the 6-month visit, since the Multispot assay was non-reactive and HIV RNA was undetectable. It is not possible to draw conclusions about the relative sensitivities of the Abbott and Bio-Rad Combo assays in this report due to the small number of cases evaluated. In a previous report, we described two other cases from the HPTN 061 study where HIV infection was missed at all three study visits when a single HIV rapid test was used for screening at study sites; one of those men was likely an elite controller, and the other was virally suppressed on ART.[10] In one case, HIV infection was also missed using other HIV rapid tests and the Abbott Combo assay. Taken together, our findings indicate that low-level viremia in early HIV infection should be considered when HIV RNA assays are included in algorithms for HIV diagnosis. In addition, it is important to recognize that serologic assays, including fourth-generation assays, may also fail to detect HIV infection in this setting. Further studies are needed to identify factors associated with low HIV RNA levels in early HIV infection.

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Table. Test results for six study participants who had low or undetectable HIV RNA at the time of HIV diagnosis.

#	Visit	OraQuick (rapid)	Vitros EIA (3rd gen)[a]	Abbott Combo (4th gen)[b]	Bio-Rad Combo (4th gen)[c]	Multispot (discrim)	Western blot[d]	Aptima RNA (qualitative)[e]	Viral load (copies/mL)[f]
1	Enr	NR	NR	NR	NR			NR	
	6 mo	**R**	**R**	**R**	**R**	**R**	**P**		<100[g]
	12 mo			**R**	**R**	**R**	**P**		595[g]
2	Enr	NR	NR	NR	NR			NR	
	6 mo	**R**	**R**	**R**	**R**	**R**	**P**		82
	12 mo			**R**	**R**	**R**	**P**		660[g]
3	Enr	NR	NR	NR	NR			NR	
	6 mo	**R**	**R**	**R**	**R**	**R**	**P**		502
4	Enr	NR	NR	NR	NR			NR	
	6 mo	**R**	**R**	**R**	**R**	**R**	**P**		837
	12 mo			**R**	**R**	**R**	**P**		2,191
5	Enr	NR	NR (0.05)	NR (0.10)	NR (0.27)			NR (0.15)	
	6 mo	NR	**R** (2.04)	NR (0.13)	NR (0.20)	**R**	IND	NR (0.16, 0.25)	<20
	12 mo	NR	**R** (5.19)	**R** (1.78)	NR (0.23)	**R**	**P**	NR (0.19, 0.12)	<20
6	Enr	NR	NR (0.06)	NR (0.16)	NR (0.23)			NR (0.15)	
	6 mo	NR	**R** (4.71)	**R** (1.54)	NR (0.65)	NR	IND	NR (0.15, 0.27)	<20
	12 mo	NR	**R** (14.10)	**R** (3.20)	NR (0.26)	**R**	**P**	NR (0.16, 0.15)	<20

Laboratory test results are shown for six men in the HPTN 061 study who had low or undetectable HIV RNA at the time of HIV seroconversion. Testing was performed retrospectively at a centralized laboratory. The laboratory tests used for analysis are described in the Methods section. Results are shown for samples collected at the enrollment visit (shaded rows), at the 6-month follow-up visit (seroconversion), and at the 12 month follow-up visit. Reactive/positive results are shown in bold font. In one case (Case #3), no sample was stored at the 12-month visit. Abbreviations: EIA: enzyme immunoassay; gen: generation (indicates a third- or fourth-generation assay); discrim: HIV-1/HIV-2 discriminatory assay; mL: milliliters; Enr: enrollment; mo: months; NR: non-reactive; R: reactive; P: positive; IND: indeterminate.

[a]For the VITROS Anti-HIV 1+2 Test (Vitros EIA), a signal/cutoff ratio <1 is considered non-reactive. Signal/cutoff ratios for Cases #1 and #3 were not available. For Cases #2 and #4, results reported as non-reactive were 0.09 and 0.11, respectively, and results reported as reactive were 36.7 and 56.9, respectively. For Cases #5 and #6, signal/cutoff ratios are shown in parentheses.

[b]For the ARCHITECT HIV Ag/Ab Combo assay (Abbott Combo), a signal/cutoff ratio <1 is considered non-reactive. For Cases #1-4, results reported as non-reactive had signal/cutoff ratios of 0.10 to 0.16, and results reported as reactive had signal/cutoff ratios of 51.72 to 464.15. For Cases #5 and #6, signal/cutoff ratios are shown in parentheses.

[c]For the GS HIV Combo Ag/Ab EIA (Bio-Rad Combo), a signal/cutoff ratio is only reported if the value is <1 (non-reactive); otherwise, the result is reported as reactive. For Cases #1-4, results reported as non-reactive had signal/cutoff ratios of 0.21 to 0.27, and results reported as reactive had maximal signal/cutoff ratios. For Cases #5 and #6, signal/cutoff ratios are shown in parentheses.

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[d]For Case #5, the only Western blot band detected at 6 months was gp160+/–; the Western blot bands detected at 12 months were gp160+, p55/51+, gp41+/–, p40+, p31+/–, p24++, and p18++. For Case #6, the only Western blot band detected at 6 months was p24+; the Western blot bands detected at 12 months were gp160+, p55/51+/–, p40+/–, and p24++.

[e]The lower limit of detection for the APTIMA HIV-1 RNA Qualitative Assay (Aptima RNA) is 30 copies/mL HIV RNA at a 95% detection rate; a signal/cutoff ratio <1 is considered non-reactive. For Cases #1-4, results reported as non-reactive had signal/cutoff ratios of 0.18 to 0.28. For Cases #5 and #6, signal/cutoff ratios are shown in parentheses; in addition, repeat testing was performed on a second aliquot from the 6- and 12-month visits.

[f]The lower limit of detection for the COBAS AmpliPrep/COBAS TaqMan HIV-1 Test, version 2.0, is 20 copies/mL HIV RNA.

[g]These samples were testing using a validated, low-volume modification of the COBAS AmpliPrep/COBAS TaqMan HIV-1 Test, version 2.0, because the amount of stored plasma was insufficient for testing using standard assay procedures. The lower limit of detection for this modified test method is 100 copies/mL HIV RNA.