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No Time to Delay! Fiebig Stages and Referral in Acute HIV infection: Seattle Primary Infection Program Experience

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Abstract

There has been increasing recognition of the importance of diagnosing individuals during the earliest stages of human immunodeficiency virus (HIV) infection. Sera from individuals referred to a primary HIV infection research program were screened using the IgG-sensitive Vironostika HIV-1 Microelisa System, IgG/IgM-sensitive GS HIV-1/HIV-2 Plus O antibody enzyme immunoassay (EIA), or Abbott ARCHITECT HIV antigen (Ag)/antibody (Ab) Combo assay and confirmed by the Bio-Rad Multispot and Western blot. A subset of participants was co-enrolled in a study designed to compare the ability of point-of-care tests to detect early infection. We calculated time within primary infection laboratory stages using actual observed transitions and with an expectation-maximization algorithm. Three hundred and sixty participants contributed data to this analysis. Of 123 persons referred with EIA-negative/RNA-positive test results (Fiebig stage I–II) or for concern for symptoms, 24 (20%) were still in stages I–II, and 99 (80%) were in stages III or later at their screening visit. Participants were estimated to spend a median of 13.5 days in stages I and II, 2.3 days in stage III, and 7.8 days in stage IV. OraQuick performed on oral fluids detected 53% of 17 participants in stage V. The durations of stages we observed are consistent with previous publications. Most persons referred for research no longer had acute infection at their first visit. Programs wishing to identify persons in the very earliest stages of infection need to expedite referrals or develop targeted screening programs.

Keywords: HIV testing, acute HIV infection, Fiebig staging

Research in Context

Evidence before this study

Multiple, nonspecific terms have been used to describe the time period following human immunodeficiency virus type 1 (HIV-1) acquisition. In 2003, Fiebig *et al.* first described laboratory-defined stages of infection in plasma donors, beginning with the detection of HIV RNA, p24 antigen, and then anti-HIV antibodies.

Added value of this study

Our study validates prior estimates of Fiebig stages in a larger population infected through sexual transmission, supports findings that the stages may be prolonged with antiretroviral therapy, and describes the challenges of identifying persons in the very earliest stages of HIV infection.

Implications of all the available evidence

There is a need to identify HIV-infected persons as soon as possible after HIV acquisition and refer them expeditiously to treatment research programs, if available, or to HIV care. The current recommendations for universal treatment and changing HIV diagnostic algorithm (including the discontinuation of the Western Blot) have implications for future HIV staging.

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Introduction

Recognition of human immunodeficiency virus type 1 (HIV-1) infection in the period of time immediately after HIV acquisition is important from multiple perspectives. Early diagnosis allows clinical and public health providers to initiate treatment of newly HIV-infected persons and interrupt transmission networks. The understanding of HIV transmission, viral dynamics, and early immunological response is also important for HIV vaccine and eradication research. To ensure focus on persons in the very earliest stages of HIV infection, it is critical for HIV screening programs to be able to detect acute HIV infection (AHI) and for research programs to use accurate staging once such individuals are identified.

The first HIV test approved by the U.S. Food and Drug Administration (FDA) in 1985 was able to detect IgG antibodies directed against HIV 4–6 weeks after infection, and subsequent generations of HIV antibody tests gradually shortened this ''window period.'' However, it was not until after 2001, when a handful of public health departments created pooled HIV nucleic acid amplification testing (NAAT) programs,[1–4] that AHI began to be routinely detected. In 2010, the FDA approved the first 4th generation antigen (Ag)/antibody (Ab) combination assay that can detect HIV p24 antigen, present in blood plasma within a week after HIV RNA can first be detected.[5,6] This approval led to updated recommendations from the Centers for Disease Control and Prevention[7] to use Ag/Ab combination assays for initial HIV screening. As a consequence, although we would want providers to think about AHI as a possible diagnosis for a multitude of reasons, medical care providers in the United States (and other locations where laboratory-based Ag/Ab combination assays are standard) who order an HIV test do not need to consider the diagnosis of AHI specifically under most testing circumstances. Most cases of AHI are detectable by laboratory-based Ag/Ab testing at a fraction of the cost and time that the prior testing algorithm would have entailed, if the diagnosis had even been considered.[8–10]

Fiebig *et al.* were first to describe specific laboratory-defined stages of HIV infection among 51 seroconverting plasma donors to elucidate this time course of detection of viremia and antibody seroconversion following HIV acquisition.[5] Individuals were seen to transition in a systematic manner from the eclipse phase, when no markers of HIV could be identified, to stage I (HIV RNA positive), II (HIV RNA and p24-antigen positive), III [Ab positive/Western blot (WB) negative], IV (Ab positive/WB indeterminate), V (WB positive, but missing p31), and finally to stage VI (WB positive and including p31). Investigators at the CDC later attempted to validate this work and included point-of-care (POC) HIV tests to establish the ''window periods'' for all FDA-approved HIV screening and supplemental tests.[6,11,12] This project aimed to characterize laboratory staging for participants enrolled in the University of Washington Primary Infection Clinic (UWPIC), compare the estimated duration of Fiebig stages to what has been previously reported, determine the ability of POC tests to identify different stages, and report on whether the transition from pooled HIV RNA to Ag/Ab combination testing in the community shortened the interval from clinical presentation to referral to our research program.

Materials and Methods

Population

Individuals with primary HIV infection have been enrolled into an observational cohort at the UWPIC since 1992.[13–16] At the time of cohort entry, all participants were either HIV antibody negative with detectable HIV RNA or HIV antibody positive with a (1) negative or indeterminate WB, (2) negative ''detuned'' antibody test (indicating likely recent infection),[17–19] or (3) negative HIV test within 1 year of screening. All participants were enrolled within 240 days after ''HIV infection,'' estimated to be the date of onset of seroconversion symptoms[13] or, for asymptomatic participants only, the midpoint between the last negative and first positive HIV test. The midpoint was considered to be the date of testing for participants who were concurrently antibody negative and RNA or p24 Ag positive. Five participants whose infections could not be dated with precision were included; these participants had asymptomatic HIV infection, no prior negative HIV test, and either a negative ''detuned'' antibody test ($n=2$) or indeterminate or evolving WB ($n=3$). The UW Institutional Review Board approved these studies, and all participants gave written consent for participation.

From September 2010 to July 2014, UWPIC participants were offered co-enrollment in a prospective, cross-sectional study designed to compare the ability of different POC and laboratory-based HIV tests to detect early HIV infection.[20,21] Enrollment was offered to all UWPIC enrollees as long as POC research staff were available. Participants at the UWPIC could participate repeatedly until all POC tests were reactive. The UW Institutional Review Board also approved this study, and participants gave verbal consent for the additional HIV testing procedures.

Procedures

At the screening visit, we recorded details of the types of HIV tests and results that prompted referral; we considered this the ''eligibility stage.'' At screening, sera were tested using the IgG-sensitive (first generation) Vironostika HIV-1 Microelisa System (bioMerieux) until May 2004, the IgG-/IgM-sensitive (third generation) GS HIV-1/HIV-2 Plus O antibody enzyme immunoassay (EIA; Bio-Rad) from May 2004 until May 2011, and the ARCHITECT HIV Ag/Ab Combo assay (Abbott Laboratories) thereafter. Participants with a reactive ARCHITECT result had supplemental testing using the Multispot HIV-1/HIV-2 Rapid Test (Bio-Rad) to confirm antibody versus presumptive p24 antigen detection. All participants had additional testing, including the Genetic Systems HIV-1 WB (Bio-Rad) and plasma HIV RNA testing using contemporary assays regardless of EIA or ARCHITECT result. Participants were enrolled in the cohort as soon as possible after evaluations confirmed research eligibility; the number of study visits varied with time over the course of the cohort, but participants could have been seen as frequently as weekly for 1 month, then monthly for 3 months, and then at 8-week intervals thereafter. Serial samples underwent repeated HIV diagnostic testing until two consecutive WB confirmed HIV infection; a subset of participants had serial WB testing until they reached stage VI.

If initial diagnostic testing was not sufficient to resolve the Fiebig stage, further testing was performed retrospectively when stored specimens were available. Specimens

Table 1. Demographic and Other Baseline Characteristics of Study Participants

Characteristics	*Observed in >1 stage and eligibility stage <5 (N = 199),* n *(%) or median (IQR)*	*Observed in >1 stage and eligibility stage 5+ (N = 161),* n *(%) or median (IQR)*	*Observed in single stage (N = 35),* n *(%) or median (IQR)*
Eligibility year	2005 (1999–2008)	1999 (1996–2001)	2004 (1997–2007)
Days from estimated date of ''HIV infection'' to screening[a]	21 (14–34)	79 (48–104)	52 (24–71)
Age, years	33 (27–40)	32 (28–37)	31 (24–40)
Male	194 (97)	159 (99)	31 (89)
Race[b]			
White	158 (80)	143 (89)	27 (77)
Hispanic	17 (9)	10 (6)	5 (14)
African American	14 (7)	1 (1)	0 (0)
Other	9 (5)	7 (4)	3 (9)
Education, years[c]	16 (13–16)	15 (13–16)	15 (13–16)
HIV risk group			
MSM	185 (93)	145 (90)	30 (86)
MSM/IDU	7 (4)	8 (5)	0 (0)
IDU	1 (1)	1 (1)	0 (0)
Heterosexual	6 (3)	4 (2)	4 (11)
Other/unknown	0 (0)	3 (2)	1 (3)
Symptoms at seroconversion			
Yes	183 (92)	123 (76)	30 (86)
No	15 (8)	38 (24)	5 (14)
Unknown	1 (1)	0 (0)	0 (0)
Referral			
PCP/ED/ambulatory	88 (44)	46 (29)	13 (37)
Health department/STD clinic	76 (38)	47 (29)	6 (17)
AIDS clinic	10 (5)	8 (5)	0 (0)
Other research study	14 (7)	41 (25)	8 (23)
Self/friend/internet	5 (3)	6 (4)	2 (6)
Partner/UWPIC enrollee	3 (2)	2 (1)	5 (14)
Other	3 (2)	11 (7)	1 (3)
Fiebig stage at eligibility			
I	10 (5)	—	—
I/II	104 (52)	—	—
II	13 (7)	—	—
III	10 (5)	—	—
IV	60 (30)	—	—
V	—	50 (31)	15 (43)
V/VI	—	106 (66)	3 (9)
VI	—	0 (0)	9 (26)
Missing/unavailable	2 (1)	5 (3)	8 (23)
Fiebig stage at screening			
I	10 (5)	—	—
I/II	3 (2)	—	—
II	15 (8)	—	—
III	7 (4)	—	—
IV	30 (15)	—	—
V	97 (49)	52 (32)	19 (54)
V/VI	4 (2)	5 (3)	4 (11)
VI	33 (17)	104 (65)	12 (34)
No antiretroviral therapy	43 (22)	43 (27)	11 (31)
Non-HAART antiretroviral therapy	4 (2)	7 (4)	0
First HAART regimen			
PI based	80 (53)	48 (43)	8 (33)
NNRTI based	33 (22)	48 (43)	7 (29)
Integrase inhibitor based	29 (19)	4 (4)	5 (21)
NNRTI/PI based	9 (6)	10 (9)	2 (8)
Entry inhibitor based	0 (0)	0 (0)	1 (4)
3TC/AZT/ABC	0 (0)	1 (1)	1 (4)
HAART of unknown type	1 (1)	0 (0)	0 (0)

(*continued*)

Table 1. (Continued)

Characteristics	*Observed in >1 stage and eligibility stage <5 (N = 199),* n *(%) or median (IQR)*	*Observed in >1 stage and eligibility stage 5+ (N = 161),* n *(%) or median (IQR)*	*Observed in single stage (N = 35),* n *(%) or median (IQR)*
Days from estimated date of infection to HAART[d]	42 (21–211)	118 (79–721)	74 (40–193)
First available on or after screening visit			
CD4 count (cells/mm^3)—calculated[e]	515 (400–693)	601 (469–792)	643 (371–735)
HIV RNA (viral load), $\log_{10}$ copies/mL	5.38 (4.57–6.08)	4.39 (3.70–4.90)	4.52 (3.52–5.23)

[a] $n = 2$ in eligibility <F5 and $n = 3$ in eligibility ≥F5 could not have an estimated date of ''HIV infection'' established with precision and are excluded here.
[b] $n = 1$ in eligibility <F5 had missing race.
[c] $n = 4$ in eligibility <F5, $n = 6$ in eligibility ≥F5, and $n = 2$ in only one stage missing years education.
[d] $n = 1$ in eligibility ≥F5 could not have an estimated date of HIV infection established with precision and is excluded here.
[e] $n = 1$ in eligibility <F5 and 1 in eligibility ≥F5 missing CD4 count.
3TC, lamivudine; ABC, abacavir; AZT, zidovudine; ED, emergency department; HAART, highly active antiretroviral therapy; HIV, human immunodeficiency virus; IDU, injection drug use; IQR, interquartile range; MSM, men who have sex with men; NNRTI, non-nucleoside reverse transcriptase inhibitor; PCP, primary care provider; PI, protease inhibitor; UWPIC, University of Washington Primary Infection Clinic.

from antibody-negative/RNA-positive participants (Fiebig stage I/II) were submitted for p24 antigen testing using the HIV-1 p24 enzyme-linked immunosorbent assay (ELISA) kit (PerkinElmer Life and Analytical Sciences, Inc., Boston, MA). Specimens from ARCHITECT-positive/Multispot-negative participants (Fiebig II/III) were first submitted for EIA testing using the IgG-/IgM-sensitive GS HIV-1/HIV-2 Plus O antibody EIA and then for p24 Ag testing if the EIA was nonreactive.

Participants who co-enrolled in the POC testing study were evaluated by one POC test performed on oral fluids (OraQuick ADVANCE Rapid HIV-1/2 Antibody Test; OraSure Technologies) and three POC tests each performed on separate fingerstick whole blood specimens: OraQuick, Determine Combo (Alere, Inc.), and either the Uni-Gold Recombigen HIV Test (Uni-Gold; Trinity Biotech) or INSTI HIV-1 Rapid Antibody Test (INSTI; bioLytical).[20,21] The switch from Uni-Gold to INSTI occurred in spring 2013. Determine Combo was not FDA approved at the start of the study; the manufacturer provided devices for investigational use beginning 10 months after the start of enrollment, and there were occasional interruptions in supply. When a release of information was signed and records obtained for ''eligibility''-indicated participation in this POC testing study at one of the two other local research sites, we also recorded these results and included them in our analysis.

Highly active antiretroviral therapy (HAART) became readily available after February 1996 and was provided to study participants through a variety of research protocols and clinical care. We considered antiretroviral regimens to be HAART if they included three or more agents representing at least two classes of antiretroviral medications; the triple nucleoside regimen of zidovudine, lamivudine, and abacavir was also considered HAART.

Statistical analysis

We first calculated the median duration within observed stages using the time between actual visit dates and then repeated this calculation using the midpoint between visit dates. If we did not have results of p24 Ag testing and specimens were not available for testing, we considered EIA-negative/RNA-positive participants to be stage I/II. Similarly, if we did not have detailed WB results, we considered participants with a positive WB to be stage V/VI. Initially, we evaluated all stages separately (I, I/II, II, III, IV, V, V/VI, and VI). We then pooled stages I, I/II, and II due to small numbers of participants and evaluated the time until participants reached stage V or later, given that WB assays were not routinely performed once participants reached stage V.

For those participants evaluated in at least one stage before stage V and for whom the ''HIV infection'' date was known or could be estimated, following methods by Fiebig *et al.*,[5,22,23] an expectation-maximization (E-M) algorithm was used to estimate the mean time spent within each stage: the time origin was the date of HIV transmission, which was a known date for 26 participants, occurring a median of 14 days before the onset of symptoms (or the day of an EIA-negative/RNA-positive HIV test result, for 1 asymptomatic participant). For the remainder of participants, the date of transmission was estimated as 14 days before their ''HIV infection'' date. The stages after transmission were ''HIV infection'' (symptom onset or midpoint of last negative and first positive tests if asymptomatic) and then Fiebig stages I through II (I, I/II, and II combined), III, IV, and V. Using observed stage transitions, the mean (''expected'') times (days) and rates (1/mean time) were first calculated and used to simulate 100 sets of exponentially distributed times that were consistent with the left and right interval endpoints in the subject's observed data. The log-likelihood was maximized to identify updated mean rates (with the exception of the hazard between transmission and ''HIV infection'' date, which was fixed at 1/14 day^{-1} after each iteration), and the process was iterated until convergence criteria for rates were obtained (defined as <0.01 day^{-1} for each of the rates for waiting times in combined stage I through II, III, and IV). Confidence intervals (CIs) were obtained using the jackknife, a resampling method in which each observation

was omitted and the E-M algorithm was refit. Resulting values were then used to estimate standard errors. To evaluate the impact of antiretroviral therapy on stage durations, the analysis was repeated using only observations before initiation of a HAART regimen. Intervals were considered off HAART if participants remained untreated or HAART was started after the midpoint between visit dates; intervals for which HAART initiation occurred earlier than the midpoint between visit dates were considered to be on HAART. The E-M algorithm and jackknife were performed using R v3.2.2 (R Foundation for Statistical Computing; Vienna, Austria). All other analyses were performed using Stata SE v14.2 (StataCorp, College Station, TX).

Results

From 1992 to 2015, 397 participants were enrolled in the UWPIC cohort. We excluded two participants who were co-enrolled in HIV vaccine trials because their WB results were uninterpretable in the setting of continued study blinding. Demographic and baseline characteristics of the remaining 395 participants are shown in Table 1. Thirty-five (8.9%) participants were only observed in a single Fiebig stage. Of the remaining 360 participants, 199 (55.3%) were first observed in stages I–IV. The median time from the estimated date of ''HIV infection'' to the study screening visit was 38 (interquartile range [IQR] 19–79) days.

There were 127 participants enrolled who were in Fiebig stage I–II at the time of HIV diagnosis (i.e., ''eligibility,'' Table 2); 123 of these participants had been referred to the UWPIC from outside sources, either because of their laboratory results or because of concern for symptoms consistent with the acute retroviral syndrome. One hundred and fourteen (93%) of these 123 persons had symptoms consistent with the acute retroviral syndrome and obtained eligibility HIV testing a median of 5 (IQR 3–8) days following the start of symptoms. At the time of their UWPIC screening visit, 7 (6%) remained in stage I, 3 (2%) were in stage I/II (i.e., specimen was not available for retrospective p24 Ag testing), 14 (11%) were in stage II, and 99 (80%) were in stages III or later. The median time between the initial testing and screening visit for the 123 individuals referred from outside sources was 14 (IQR 8–20) days. For symptomatic and asymptomatic persons, the median times from the initial testing to the screening visit were 13 (7–19 IQR) and 21 (20–24 IQR), respectively. For asymptomatic persons, this duration was longer before Ag/Ab combination HIV testing became routinely available in May 2011 [median 21 (20–24 IQR days for six participants] compared to after the availability of Ag/Ab combination testing [median 16 (14–34 IQR) days for three participants], although the difference was not statistically significant (Wilcoxon rank-sum test, $p = .4$).

The 360 participants observed in more than 1 stage contributed a total of 497 intervals, 215 of whom were in consecutive stages (Table 3). Using the midpoint between visit dates, participants were estimated to spend a median of 4 days in stages I and II combined, 4 days in stage III (EIA positive/WB negative, data not shown in table), 8 days in stage IV (WB indeterminate), and 35 days in stage V (WB without p31). After pooling stages I, I/II, and II and evaluating only until participants reached stage V or later, 199 participants contributed a total of 241 intervals (107 consecutive). Participants

TABLE 2. DURATION OF TIME IN DAYS SPENT IN FIEBIG STAGES I-VI, FROM ELIGIBILITY TO SCREENING VISITS

	Screenstage	*Fiebig eligibility stage*								*Missing/unavailable*	N
		I	*I/II*	*II*	*III*	*IV*	*V*	*V/VI*	*VI*		
Actual: median (IQR)	I	—									10
N		10									
Actual: median (IQR)	I/II	—	—								3
N		0	3								
Actual: median (IQR)	II	—	6 (3–8)	—							15
N		0	9	6							
Actual: median (IQR)	III	—	6 (3.5–9.5)	7	—						7
N		0	4	1	2						
Actual: median (IQR)	IV	—	13 (6.5–15.5)	10	11.5 (2–21)	—				—	
N		0	20	1	2	5				2	30
Actual: median (IQR)	V	—	17.5 (13–21.5)	—	14 (13–15)	15 (9–22)	—			—	
N		0	60	0	5	32	62			9	168
Actual: median (IQR)	V/VI	—	—	46 (36–56)	—	67 (8–126)	—	—		—	13
N		0	0	2	0	2	0	8		1	
Actual: median (IQR)	VI	—	22 (13.5–27)	63 (39–90)	18	34 (21–56)	33 (21–55)	23 (15–35)	—	—	
N		0	8	3	1	21	3	101	9	3	149
N	All	10	104	13	10	60	65	109	9	15	395

Days between visits, among 395 UWPIC subjects.

Table 3. Duration of Time in Days Between Fiebig Stage (t) and Subsequent Stage (t+1) Among All Study Visits

		Stage at time t							
	Stage at time +1	*I*	*I/II*	*II*	*III*	*IV*	*V*	*V/VI*	N
	I								
Midpoint: median (IQR)	I/II	3 (2–7)							
Actual: median (IQR)		6 (4–10)							
N		3							3
Midpoint: median (IQR)	II	3	3 (2–5)						
Actual: median (IQR)		6	6 (2–8)						
N		1	11						12
Midpoint: median (IQR)	III	—	4 (3–6)	3.5 (3–4)					
Actual: median (IQR)		—	7 (5–8)	6 (5–7)					
N		0	5	2					7
Midpoint: median (IQR)	IV	18.5 (5–32)	7 (4–8)	6.5 (5–10.5)	4 (1–12)				
Actual: median (IQR)		24 (9–39)	13 (7–16)	12 (8.5–16.5)	7 (2–18)				
N		2	22	8	3				35
Midpoint: median (IQR)	V	12 (7.5–13.5)	9 (7–11)	7 (6–11)	10.5 (7–13)	8 (5–12)			
Actual: median (IQR)		17 (10.5–20)	17 (13–21)	11 (6–21)	14.5 (12–23.5)	10 (7–17)			
N		4	61	11	12	69			157
Midpoint: median (IQR)	V/VI	—	—	10.5 (10–18.5)	—	7 (4–21)	—		
Actual: median (IQR)		—	—	20.5 (19–36)		13 (7–41)	—		
N		0	0	4	0	13	0		17
Midpoint: median (IQR)	VI	—	11 (7–13.5)	—	12 (9–15)	12 (8–17)	35 (21–79)	14 (8–24)	
Actual: median (IQR)		—	22 (13.5–27)	—	20 (18–22)	21 (12–31)	40 (25–103)	24 (16–41)	
N		0	8	0	2	15	122	119	266
N	Total	10	107	25	17	97	122	119	497

Days between earlier stage (t) and subsequent stage ($t+1$) transitions, among 360 UWPIC subjects.

TABLE 4. COMPARISONS OF DURATION OF TIME SPENT IN FIEBIG STAGES I–VI IN TWO PRIOR REPORTS AND THE UNIVERSITY OF WASHINGTON PRIMARY INFECTION CLINIC COHORT

	Fiebig[5]		*Delaney*[12]		*UWPIC midpoint*[a]		*UWPIC actual*[a]		*UWPIC E-M algorithm*		*UWPIC E-M algorithm*		
											Pre-HAART intervals		
Stage	*Phase*	*Cumulative*	*Median*	*Cumulative*	*Median*	*Cumulative*	*Median*	*Cumulative*	N[b]	*Mean (95% CI)*	*Cumulative*	N[c]	*Mean*	*Cumulative*
I, I/II, or II	10.3 (7.1–13.5)	10.3 (7.1–13.5)	11.9[d]	11.9	4	4	7	7	197	13.5 (10.7–18.1)	13.5	186	13.0	13.0
III	3.2 (2.1–4.8)	13.5 (10.0–17.0)			4	8	7	14	197	2.3 (1.3–11.2)	15.8	183	1.8	14.8
IV	5.6 (3.8–8.1)	19.1 (15.3–22.9)	12.9[e]	24.8	9	17	13	27	197	7.8 (6.1–10.7)	23.6	171	7.8	22.6

Days between stage transitions, among 199 UWPIC subjects with eligibility assessed before Fiebig stage V.
[a] $n = 7$ intervals for stage I, I/II, or II; 3 intervals for stage III; and 97 intervals for stage IV.
[b] $n = 2$ subjects could not have HIV infection date estimated and were excluded from E-M algorithm estimates.
[c] $n = 12$ subjects were on HAART before their attaining Fiebig stage 3, 15 before stage 4, and 26 total before stage 5.
[d] Combines stage I (5.9 days) and stage II (6.0 days).
[e] Combines stages III and IV.
CI, confidence interval; E-M, expectation-maximization.

were estimated to spend a median of 4 days in stages I and II, 4 days in stage III, and 9 days in stage IV (Table 4). Using an E-M algorithm for 197 participants who had an estimated date of ''HIV infection,'' participants were estimated to spend a mean of 13.5 (95% CI 10.7–18.1) days in stages I–II, 2.3 (95% CI 1.3–11.2) days in stage III, and 7.8 (95% CI 6.1–10.7) days in stage IV (Table 4).

The majority of the cohort (287, 72.7%) initiated a HAART regimen during study follow-up. The main analysis described above was conducted without regard to HAART. A comparative analysis was done in which the 26 (9.1%) participants who initiated HAART in stages I–IV were excluded from the E-M algorithm to estimate stage durations only for people not on HAART and therefore infer the impact that HAART may have had on stage transitions. An additional 15 (5.2%) participants initiated HAART on the same day as testing in stage V or before reaching stage V, but after the midpoint between visits, and they therefore did not impact the analysis. When only intervals before antiretroviral treatment initiation were included, mean durations in stages calculated using the E-M algorithm appeared to be somewhat shorter compared to durations estimated by the entire dataset (13.0 days in stages I–II, 1.8 days in stage III, and 7.8 days in stage IV), suggesting that mean durations while on antiretroviral treatment would likely be prolonged.

Of 44 HIV-positive UWPIC participants enrolled between September 2010 and July 2014, 33 (75%) participants co-enrolled in the POC testing study, including 8 participants who were evaluated both at the UWPIC and a second POC testing study research site (Table 5). POC tests performed on fingerstick whole blood detected HIV in 25%–50% of participants in stage III, 0%–100% of participants in stage IV, 88%–100% of participants in stage V, and all participants in stage VI. OraQuick performed on oral fluids detected HIV infection in 53% of participants in stage V and 90% of participants in stage VI.

Discussion

This is one of the first projects to validate the widely used laboratory-based (Fiebig) staging system for HIV infection and has an important conclusion: the critical period of time between HIV acquisition, detection of viral markers, and development of the systemic immune response is very short. For research and clinical programs wishing to identify, study, and treat persons in the very earliest stages of HIV infection, the important corollary to that conclusion is that delay in referrals or scheduling of the initial research visit created a situation where only one in five persons who were referred following suspected or confirmed acute infection was still in Fiebig stage I or II at the time of their first research visit.

In many circumstances, newer assays, such as antigen-antibody combination assays, are likely to facilitate more rapid detection and expedite referral compared to pooled NAAT,[2,8,10] as was suggested by our results. Unfortunately, the Determine Combo, the only POC Ag/Ab combination assay currently FDA-approved for use in the United States, identifies few antibody-negative persons with AHI,[21,24–27] as described again in this analysis. One strategy to identify large numbers of persons with acute HIV is to implement screening programs with very frequent testing in the highest risk populations. In one recent report of such a program,[28] 2,276 high-

Table 5. Proportion of Subjects with Reactive Point-of-Care HIV Test Results, by Fiebig Stage

Fiebig stage	N	*OraQuick Oral fluid* n/N *(%)*	*OraQuick Fingerstick* n/N *(%)*	*Uni-Gold*[a] n/N *(%)*	*INSTI*[a] n/N *(%)*	*Determine Combo* n/N *(%)*
I/II	5	0/4 (0)	0/5 (0)	0/2 (0)	0/2 (0)	0/3 (0)
II	1	0/1 (0)	0/1 (0)	0/1 (0)	—	0/1 (0)
III	4	0/3 (0)	1/4 (25)	—	1/2 (50)	1/3 (33)
IV	4	0/3 (0)	2/4 (50)	0/1 (0)	2/2 (100)	3/3 (100)
V	17	9/17 (53)	15/17 (88)	11/11 (100)	6/6 (100)	16/16 (100)
VI	10	9/10 (90)	10/10 (100)	9/9 (100)	1/1 (100)	7/7 (100)
Total	41	18/38 (47)	28/41 (68)	20/24 (83)	10/13 (77)	27/33 (82)

[a]The study protocol switched from use of Uni-Gold ($N=24$) to INSTI ($N=13$) in Spring 2013.

risk persons underwent twice-weekly HIV testing with Ag/Ab combination testing and pooled NAAT conducted in parallel; over 174,950 tests were performed to identify 112 persons with AHI (an average of 77 HIV tests per subject). There has been no report of the cost of this program. Public health campaigns designed to teach at-risk persons the signs and symptoms of the acute retroviral illness[13] could increase the yield of testing in a more cost-effective manner but have met with mixed results.[29,30] Our results suggest that additional education is needed for diagnosing clinical providers to facilitate immediate treatment[31] or rapid referral of these often symptomatic patients to research programs.

This analysis confirms our prior work regarding the importance of using laboratory-based HIV tests for diagnosis and accurate staging of HIV infection, as persons with negative POC tests may be in Fiebig stage V or later, particularly if oral fluid tests are used. Even persons who test presumptively positive for p24 antigen using the new HIV diagnostic algorithm[7] may be in stage III instead of stage II, as were four of five persons in our cohort when specimens were retested using an IgG-/IgM-sensitive EIA.

Our study has several limitations, including the small numbers of participants who contributed time in the earliest Fiebig stages, requiring grouping of these stages for analyses. We also had a relative lack of diversity in our study participants. We did find evidence supporting findings that Fiebig stage transitions may be prolonged by antiretroviral medications; others have found that persons taking HIV pre-exposure prophylaxis (PrEP) may have a delay in seroconversion,[32] particularly in oral fluid,[33] and people treated very early in HIV infection may never develop a detectable antibody response[34] The rollout of PrEP and universal HIV treatment will have heretofore unrecognized impact on HIV diagnostics and will require that researchers studying AHI pay close attention to treatment history in HIV staging and use consistent terminology in describing these stages.

These results do not accurately account for the eclipse phase because persons may have spent some number of days in Fiebig stage I before seeking diagnosis. Although our application of the E-M method partially accounts for the eclipse phase, it still results in an underestimate of the duration of stage I because the symptom onset date is included as part of the eclipse phase. UWPIC analyses have traditionally used a surrogate date of ''HIV infection,'' the date of symptom onset or midpoint between last negative and first positive HIV tests, whereas others have estimated the date of HIV infection using only the midpoint,[35] a specific number of days before the first antibody-negative/RNA-positive test (usually 10–14 days), or some other combination of clinical and laboratory data[36] to estimate the date of HIV acquisition. In the small number of participants in our cohort, who reported a single sexual exposure that led to HIV acquisition ($N=26$), the median time from that exposure to symptom onset was 14 (IQR 5–19, range 0–50) days. Additional work is needed to develop strategies to estimate the true HIV acquisition date using information gleaned from clinical testing results and to validate such dating strategies.

In conclusion, because of the short durations of stages between HIV acquisition and antibody development, there continues to be a need to identify HIV-infected persons as soon as possible after HIV acquisition to facilitate immediate treatment as well as access to HIV research programs. The possible future FDA approval of POC NAAT[37] could be one reasonable solution to the delays we have observed in diagnosis and referral. The new world of PrEP, universal and potentially immediate treatment, and changing tests in the HIV diagnostic testing algorithm[7] (including the discontinuation of the WB) have major implications for HIV diagnosis, precise staging, HIV care, and clinical trials of acute infection treatment.

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Author Disclosure Statement

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