NOVEL CRITERIA FOR DIAGNOSING ACUTE HIV IN A MULTINATIONAL ART-INITIATION STUDY

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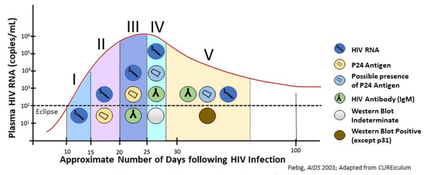
BACKGROUND

- Antiretroviral therapy (ART) initiation during acute HIV infection (AHI) limits HIV reservoirs, enhances reservoir decay, restricts viral genetic diversification and may facilitate post-ART control.
- Identifying and treating persons with AHI is desirable but logistically challenging.
  - Individuals with AHI may have no or non-specific symptoms.
  - Antibody-based assays may fail to detect infection during its earliest stages and rapid diagnostic tests intended to detect the HIV-1 p24 antigen may perform poorly when used on whole blood at point-of-care.
  - Confirmatory laboratory testing, such as Geenius antibody assay and/or nucleic acid testing, can delay diagnosis and ART initiation.
- We describe the performance of new AHI diagnostic criteria for an ongoing multinational study of ART initiation during AHI.

METHODS

- The ACTG A5354 study, Early ART to Limit Infection and Establishment of Reservoir (EARLIER), is an open-label study to evaluate the impact of early ART initiation on the establishment of HIV reservoirs and development of HIV-specific immune responses.
- Participants 18 years and older with AHI (Fiebig stages I-V, **Figure 1**) were enrolled at 30 sites in the Americas, Africa, and Southeast Asia.
  - Local strategies for identifying AHI varied by site and included:
    - Local strategies for identifying AHI varied by site and included frequent HIV-1 testing of at-risk individuals, outreach to providers administering pre- and post-exposure prophylaxis, HIV-1 testing soon after suspected exposures, and use of AHI screening algorithms with combinations of immunoassays and/or nucleic acid testing.
  - Participants had to satisfy one of six study-specific AHI criteria prior to enrollment, each associated with an estimated Fiebig stage **(Table 1)**.
    - These criteria were designed to support HIV diagnosis, establish relative recency of infection, and accommodate differences in testing procedures across sites.
    - Where available, signal-to-cutoff (S/CO) ratios from the ARCHITECT HIV Ag/Ab Combo assay (Abbott Diagnostics) or GS HIV Combo Ag/Ab EIA (Bio-Rad Laboratories) could be used to support recency of infection.
- Participants were encouraged to initiate ART on the day of enrollment, at which time samples were sent for centralized testing for HIV infection and to determine actual Fiebig stage.
- Centralized testing included HIV-1 RNA (Abbott m2000sp/rt), ARCHITECT Ag/Ab, Bio-Rad HIV-1/2 Ab (IgM sensitive), and Geenius HIV-1/2 lateral flow antibody assays.
- Participants found to be HIV-uninfected discontinued ART and were withdrawn.
- Participants found to be in Fiebig stage VI exited the study by week 24.

Figure 1. Evolution of Laboratory Findings during Fiebig Stages of Acute HIV Infection



Fiebig, *AIDS* 2003; Adapted from CUREiculum

Acute HIV infection can be assigned stages (Fiebig stages I-V) based on the evolution of diagnostic laboratory findings that include detectability of HIV RNA (Fiebig I), followed by p24 antigen (Fiebig II), HIV IgM antibody (Fiebig III), indeterminate Western blot or Geenius-HIV/12 antibody assay (Fiebig IV), and Western blot or Geenius-HIV/12 antibody assay that is positive for all bands except p31 (Fiebig V). A Western blot or Geenius-HIV/12 antibody assay that is fully positive indicates that an individual is no longer considered to be in the acute phase of infection (Fiebig VI).

Table 1. Diagnostic Criteria for Acute HIV Infection in the A5354/EARLIER Study

	Criterion to Diagnose HIV Infection		Criterion to Establish Recency	Estimated Fiebig Stage
A	Detectable HIV-1 RNA within 28 days	AND	Non-reactive HIV-1 antibody within 7 days	I/II
B	Detectable HIV-1 RNA or reactive HIV-1 antibody within 28 days	AND	Negative/indeterminate WB or Geenius HIV-1/HIV-2 Supplemental Assay within 7 days	III/IV
C	Reactive HIV-1 antibody, positive WB that is negative for p31 band, or positive Geenius HIV-1/HIV-2 Supplemental Assay that is negative for p31 band within 7 days	AND	Non-reactive HIV-1 antibody or negative HIV-1 RNA within 90 days prior to study entry	V
D	ARCHITECT or GSCOMBO S/CO ≥10 within 7 days	AND	Non-reactive HIV-1 antibody within 7 days	I/II
E	ARCHITECT or GSCOMBO S/CO ≥1 within 7 days AND prior S/CO <0.5 within 90 days prior to entry	AND	Non-reactive HIV-1 antibody within 7 days	I/II
F	Detectable HIV-1 RNA within 7 days	AND	Non-reactive HIV-1 antibody within 7 days AND ARCHITECT or GSCOMBO S/CO 0.5-9.9 within 7 days	I/II

RESULTS

- From January 2017 through December 2019, a total of 195 participants were enrolled and had centralized confirmatory HIV testing completed **(Table 2)**.
  - ART was started by 171 (87.7%) participants on the day of enrollment and 24 (12.3%) the next day, mostly with study-provided elvitegravir/cobicistat/emtricitabine/tenofovir alafenamide fumarate, n=151 (77.4%).
- AHI was confirmed in 188 (96.4%) participants and varied in Fiebig stage according to the AHI criteria used **(Table 3)**.
  - Centralized testing identified 3 (1.5%) uninfected participants.
    - All three were enrolled due to a specimen mix up at the local laboratories.
    - Participants received ART for 2, 13, and 35 days before discontinuation and withdrawal from the study.
    - Centralized testing identified 4 (2.0%) participants with Fiebig VI (chronic) infection who were followed for up to 24 weeks prior to being withdrawn and referred for treatment through local standard of care.
- Rapid progression through Fiebig stages was observed, with discrepancies between estimated Fiebig stage and actual Fiebig stages directly correlated with time between first HIV screening test and enrollment into the study **(Figure 2)**.
- Centralized ARCHITECT S/CO ≥10 combined with nonreactive or indeterminate HIV antibody on the Geenius assay at time of enrollment confirmed 99 of 121 (82%) Fiebig II-IV AHI cases **(Figure 3)**.

Table 2. Characteristics of 195 Participants Enrolled in the A5354/EARLIER Study

Characteristic	n (%)
Age (years)	
18-29	110 (56.4)
30-39	43 (22.0)
40-49	24 (12.3)
50+	18 (9.2)
Sex	
Male	165 (84.6)
Female	30 (14.4)
Gender Identity	
Cisgender	186 (95.4)
Transgender spectrum	8 (4.1)
Not reported	1 (0.5)
Race	
Black/African American	97 (49.7)
White	65 (33.3)
Asian	14 (7.2)
Multiple/Other/Unknown	19 (9.7)
Body Mass Index (kg/m2)	
Underweight (<18)	15 (7.7)
Normal (18-24.9)	95 (48.7)
Overweight (25-29.9)	47 (24.1)
Obese (30+)	38 (19.5)
Country	
United States	133 (68.2)
Brazil	25 (12.8)
Thailand	13 (6.7)
Malawi	11 (5.6)
Zimbabwe	8 (4.1)
Peru	5 (2.6)
HIV-1 RNA* (copies/mL)	
<10,000	11 (5.7)
10,000-99,999	24 (12.5)
100,000-999,999	46 (24.0)
1,000,000-9,999,999	72 (37.5)
10,000,000+	39 (20.3)
CD4 Count* (cells/mm3)	
<200	19 (9.9)
200-499	107 (55.7)
500-749	42 (21.9)
750+	21 (10.9)
Not available	3 (1.6)
Initial Antiretroviral Regimen	
EVG/COBI/FTC/TAF	151 (77.4)
DTG/3TC/TDF	37 (19.0)
Other	7 (3.6)

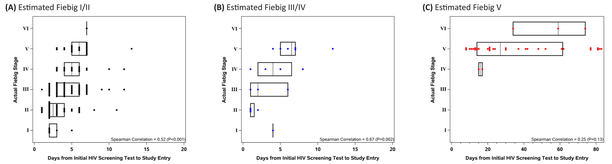
Abbreviations: EVG/COBI/FTC/TAF, elvitegravir/cobicistat/emtricitabine/tenofovir alafenamide fumarate; DTG/3TC/TDF, dolutegravir/lamivudine/tenofovir disoproxil fumarate. *Excludes three HIV-uninfected participants.

Table 3. Actual Fiebig Stage of Enrolled Participants, by Criterion for Acute HIV Infection

Enrollment AHI Criterion (Estimated Fiebig Stage)	Uninfected (n=3)	Actual Fiebig Stage I (n=7)	II (n=42)	III (n=56)	IV (n=23)	V (n=60)	VI (n=4)	Total (n=195)
A (I/II)	2	6	26	47	13	18	1	113 (57.9%)
B (III/IV)	1	1	4	3	4	6	0	19 (9.7%)
C (V)	0	0	0	0	2	32	3	37 (19.0%)
D (I/II)	0	0	12	4	4	4	0	24 (12.3%)
E (I/II)	0	0	0	0	0	0	0	0 (0%)
F (I/II)	0	0	0	2	0	0	0	2 (1.0%)

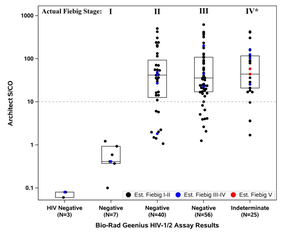
Please see Table 1 for definitions of enrollment criteria for acute HIV infection.

Figure 2. Associations Between Timing of Enrollment Relative to Initial HIV Screening Test and Actual Fiebig Stage at Enrollment.



Actual Fiebig stage at enrollment was plotted against the number of days elapsed since initial HIV testing for participants who enrolled with estimated Fiebig I/II (panel A), estimated Fiebig III/IV (Panel B), and estimated Fiebig V (panel C).

Figure 3. Enrollment ARCHITECT Signal-to-Cutoff (S/CO) Ratios of Participants with Negative/Indeterminate Geenius HIV-1/2 Antibody Results, by Fiebig Stage



ARCHITECT and Geenius-HIV/12 antibody assays were run using samples from the day of enrollment for all participants. The bottom and top edges of each box in the figure indicate the interquartile range. The line inside the box indicates the median. Filled circles represent actual data points. *Two Fiebig II participants had an indeterminate Geenius with cross-reacting IgG antibody to gp140/p31 and a negative 3rd generation HIV-1/2 result.

CONCLUSIONS

- Novel AHI criteria incorporating ARCHITECT S/CO into diagnostic algorithms facilitated rapid ART initiation pending confirmation.
- False-positive diagnoses of AHI (including participants who were HIV-uninfected or were in Fiebig stage VI) were rare.
- Rapid progression through Fiebig stages and discrepancies between estimated and actual Fiebig stages when enrollment was delayed underscore the urgency of ART initiation to achieve maximal benefit.
- These new criteria may facilitate AHI diagnosis, staging, and immediate ART initiation in research studies and possibly in clinical practice.

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