Novel Criteria for Diagnosing Acute and Early HIV Infection in a Multi-National Study of Early Antiretroviral Therapy Initiation

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Summary: Criteria for acute and early HIV that incorporated the ARCHITECT signal-to-cutoff ratio were used in a multinational cohort study, facilitating antiretroviral therapy initiation on the day of enrollment in 171 (87.7%) participants and 24 (12.3%) the next day.

ABSTRACT

Background: Antiretroviral therapy (ART) initiation during acute and early HIV infection (AEHI) limits HIV reservoir formation and may facilitate post-ART control but is logistically challenging. We evaluated the performance of new AEHI diagnostic criteria from a multi-national prospective study of ART initiation during AEHI.

Methods: ACTG 5354 enrolled adults at 30 sites in the Americas, Africa, and Asia who met any one of six criteria based on combinations of results of HIV RNA, HIV antibody, Western blot or Geenius assay, and/or the signal-to-cutoff (S/CO) ratio of the ARCHITECT HIV Combo Ag/Ab CMIA or GS HIV COMBO Ag/Ab EIA. HIV infection and Fiebig stage were subsequently confirmed by centralized testing.

Results: From 2017-2019, 195 participants were enrolled with median age 27 (interquartile range 23-39) years. Thirty (15.4%) were female. ART was started by 171 (87.7%) on the day of enrollment and 24 (12.3%) the next day. AEHI was confirmed in 188 (96.4%) participants after centralized testing, four (2.0%) participants were retrospectively found to have chronic infection, and three (1.5%) found not to have HIV discontinued ART and were withdrawn. Retrospectively, a nonreactive or indeterminate HIV antibody on the Geenius assay combined with ARCHITECT S/CO ≥10 correctly identified 99 of 122 (81.2%) Fiebig II-IV AEHI cases with no false-positive results.

Conclusions: Novel AEHI criteria incorporating ARCHITECT S/CO into diagnostic algorithms facilitated rapid and efficient ART initiation without waiting for an HIV RNA result. These new criteria may facilitate AEHI diagnosis, staging, and immediate ART initiation in future research studies and clinical practice.

Key Words: Acute HIV infection; Antigen/antibody assays; Antiretroviral Agents; Same day therapy; Signal-to-cutoff ratio

Prior Presentation: This work was presented, in part, at the 27th Conference on Retroviruses and Opportunistic Infections, 8-11 March 2020.

INTRODUCTION

Early antiretroviral therapy (ART) is associated with decreased risk of HIV-related illnesses, other serious illnesses, and death as compared to deferred ART initiation [1, 2] and immediate ART initiation upon HIV diagnosis is now recommended by most international guidelines [3-5]. Despite this, HIV is routinely diagnosed during the chronic phase of infection, after establishment of HIV reservoirs that represent the major barrier to HIV cure [6]. In contrast, ART initiation during acute and early HIV infection (AEHI) limits the initial establishment of HIV reservoirs [7], enhances reservoir decay [8], restricts viral genetic diversification [9], may facilitate post-treatment control [10, 11], and reduces the risk of onward transmission [12]. For these reasons, individuals who initiate ART during AEHI constitute an ideal population in which to test novel strategies to achieve durable HIV suppression without ART.

Although there is a strong rationale for initiating ART during AEHI, early diagnosis and treatment is challenging and therefore mostly limited to research settings [13]. The window of opportunity is small, with rapid evolution of diagnostic markers that includes detectability of HIV RNA (Fiebig I), followed by p24 antigen (Fiebig II), HIV IgM antibody (Fiebig III), indeterminate Western blot or Geenius HIV-1/2 antibody assay (Fiebig IV), and positive Western blot or Geenius HIV-1/2 antibody assay with negative p31 band (Fiebig V) [14]. Fiebig stages I-IV each last about 3-5 days or less [14-16].

Diagnosis is complicated by the fact that AEHI may cause no or non-specific symptoms [17-21]. Antibody-based assays may not detect HIV during its earliest stages and rapid diagnostic tests that detect the p24 antigen may perform poorly when used on whole blood in field conditions [22-24]. In resource-limited settings, AEHI diagnoses may be missed because testing algorithms only include these types of tests for reasons of cost and feasibility [25]. Where included in testing algorithms, confirmatory tests such as Western

blots or nucleic acid testing for HIV RNA can delay diagnosis and ART initiation, particularly when testing is performed off-site or in batches [15]. Delays of only a few days may allow progression through Fiebig stages and expansion of HIV reservoirs [26]. Therefore, there is a need to improve upon current diagnostic strategies for AEHI that leverage compatible clinical syndromes to identify candidates for intensive testing and generally require detectable HIV RNA as a prerequisite for diagnosis [27].

Fourth-generation immunoassays are commonly used screening tests that simultaneously detect HIV antigens and antibodies. Though results are often dichotomized as reactive or non-reactive, the assays actually report a signal-to-cutoff (S/CO) ratio that, when high, is strongly predictive of confirmatory HIV test results and can be rapidly communicated to clinicians without adding another step to diagnostic algorithms [28, 29]. The magnitude of the S/CO ratio may also be useful for distinguishing AEHI from chronic HIV infection, particularly in combination with other markers of recent infection such as an undetectable HIV antibody [30]. S/CO-based criteria from fourth-generation HIV immunoassays may therefore facilitate rapid AEHI diagnosis and ART initiation without a need for confirmatory HIV RNA or other off-site testing.

We evaluated the performance of new AEHI diagnostic criteria from an ongoing multi-national prospective study of ART initiation during AEHI.

METHODS

Study Design and Participants

The AIDS Clinical Trials Group (ACTG) A5354 study ("Early ART to Limit Infection and Establishment of Reservoir" [EARLIER]; clinicaltrials.gov NCT02859558) 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 AEHI were enrolled at 30 sites in the Americas, Africa, and Southeast Asia.

Local strategies for identifying AEHI varied by site and included serial HIV testing of behaviorally vulnerable individuals; outreach to providers administering pre-exposure and post-exposure prophylaxis; performance of HIV testing soon after suspected exposures and in patients with symptoms consistent with acute retroviral syndrome; and use of AEHI screening algorithms with combinations of antigen/antibody (Ag/Ab) immunoassays, antibody-only immunoassays, and/or nucleic acid testing.

Participants presented with community-based testing results and had to satisfy one of six study-specific AEHI criteria prior to enrollment (Table 1). These criteria were designed to support HIV diagnosis in early stages of infection, establish relative recency of infection, accommodate differences in testing procedures at various study sites, and estimate AEHI stage according to protocol-defined groupings. HIV RNA results had to be reported from an FDA-approved assay. Where available, S/CO ratios from the fourth-generation ARCHITECT HIV Ag/Ab Combo CMIA (Abbott Diagnostics, Chicago, IL) or GS HIV Combo Ag/Ab EIA (Bio-Rad Laboratories, Redmond, WA) could be used to support recency of infection [30]. Both assays report a single S/CO without distinguishing HIV p24 antigen from antibody reactivity; an S/CO value ≥1 is considered reactive according to manufacturer instructions.

Participants were encouraged to initiate ART on the day of enrollment, either with study-provided single-tablet elvitegravir/cobicistat/emtricitabine/tenofovir alafenamide fumarate (EVG/COBI/FTC/TAF) or another regimen at participant or provider discretion. Study-provided single-tablet bictegravir/emtricitabine/tenofovir alafenamide fumarate (BIC/FTC/TAF) became available as a second option after enrollment was completed and ART changes were allowed at participant or provider discretion. Participants were followed longitudinally with a primary objective of evaluating the impact of early ART on cell-associated HIV DNA in CD4+ T-cells after 48 weeks.

All participants provided written informed consent prior to enrollment. The study was approved by ethics committees and institutional review boards at all participating institutions.

HIV Confirmatory Testing and Staging

Samples from the day of enrollment underwent retrospective, centralized HIV confirmatory testing and Fiebig staging [14]. Centralized testing included plasma HIV RNA quantification using the Abbott m2000rt RealTi*m*e HIV-1 RNA Viral Load assay (Abbott Molecular, Des Plaines, IL) as well as the ARCHITECT HIV Ag/Ab Combo CMIA, the GS HIV Combo Ag/Ab EIA, and the third-generation IgM-sensitive Bio-Rad Genetic Systems™ HIV-1/HIV-2 PLUS O EIA (Bio-Rad Laboratories, Redmond, WA). The Geenius HIV-1/2 Supplemental Assay (Bio-Rad Laboratories, Redmond, WA) was used for HIV-1/2 IgG-antibody discrimination, facilitating Fiebig stage assignment through detection of antibodies against gp41, gp160, p24 and p31. All testing was conducted per manufacturer instructions.

Statistical Methods

Demographic and diagnostic data from the screening and enrollment visits for all enrolled participants were used for these analyses. Data were summarized by descriptive statistics, including median and interquartile range (IQR) for continuous measures. Rank-based Spearman correlation coefficient was used to assess associations between outcomes. Exact Wilcoxon rank-sum test was used for comparisons between groups and Jonckheere-Terpstra test was used for assessing trends across Fiebig stages. Fisher's exact test was used for comparisons of two proportions. All analyses were performed using SAS version 9.4 (SAS Institute Inc., Cary, NC).

RESULTS

Study Population and Antiretroviral Therapy Initiation

From January 2017 through December 2019, a total of 195 participants enrolled and completed centralized confirmatory HIV testing, including 133 (68.2%) from sites in the United States and 62 (31.8%) from other countries (Supplementary Table 1). Enrolled participants had median age 27 (IQR 23-39) years, median body mass index 24.0 (IQR 21.2-28.0) kg/m^2, and 30 (15.4%) were female. The interval between the first specimen used for HIV screening and study enrollment was a median of 5 (IQR 3-7) days for U.S. sites and 6 (IQR 2-12) days for other countries. ART was started by 171 (87.7%) participants on the day of enrollment and 24 (12.3%) the next day, mostly with study-provided EVG/COBI/FTC/TAF (n=151; 77.4%) or dolutegravir/lamivudine/tenofovir disoproxil fumarate (n=37; 19.0%). Same-day ART initiation was more common at U.S. sites than other countries (92.5% vs. 78.0%, p=0.005).

Centralized testing identified 3 (1.5%) participants without HIV, all of whom were enrolled based on erroneous HIV RNA determinations due to local specimen processing errors. These participants received ART for 2, 13, and 35 days before discontinuation and study withdrawal. Centralized testing identified 4 (2.0%) participants with Fiebig VI (chronic) HIV who were followed for up to 24 weeks before study withdrawal and referral for standard local care.

Among the 192 participants confirmed to be living with HIV, 136 (70.8%) were symptomatic at diagnosis. Sixty-eight (35.4%) were enrolled while hospitalized.

HIV-Related Laboratory Characteristics

Median CD4+ T-cell count at enrollment was 403 (IQR 292-563) cells/mm³, CD8+ T-cell count 617 (IQR 322-1030) cells/mm³, and HIV RNA 6.3 (IQR 5.3-6.8) log_{10}copies/mL. HIV RNA was lower among female as compared to male participants (median 5.21 [IQR 4.34-6.49] vs 6.36 [IQR 5.58-6.88] log_{10}copies/mL, p=0.001) and among asymptomatic as compared to symptomatic participants (median 5.39 [IQR 4.59-6.64] vs 6.41 [IQR 5.68-6.88] log_{10}copies/mL, p<0.001). HIV RNA results were available at a median of 8 (IQR 4-11) days after sample collection.

Two participants with HIV RNA <40 copies/mL at enrollment were in Fiebig stages V and VI with no history of ART use or participation in a candidate HIV vaccine trial. The participants were enrolled based on a reactive third-generation EIA on the day of enrollment combined with a non-reactive Alere Determine HIV-1/2 Ag/Ab Combo assay 8 and 34 days prior to enrollment, respectively (AEHI criterion C). Results from the Abbott Architect HIV Ag/Ab Combo assay and Bio-Rad Geenius HIV-1/2 assay, run retrospectively on samples from the day of enrollment at a centralized laboratory, confirmed both HIV diagnoses despite undetectable HIV RNA.

Estimated and Actual Fiebig Stages

AEHI was confirmed in 188 (96.4%) participants with a variety of Fiebig stages (Table 2). The most used criterion used by sites to enroll participants was a combination of detectable HIV RNA and non-reactive HIV antibody (n=113, 57.9%). Criteria that incorporated the S/CO ratio were used for prospective enrollment of 26 (13.3%) participants, all of whom were confirmed to be in Fiebig stages I-V via retrospective centralized testing.

A total of 102 (53.1%) participants had a later actual Fiebig stage at enrollment than had been estimated based on the AEHI criterion used for enrollment. For these cases, a

median of 5 (IQR 4-7) days had elapsed between the initial specimen collection for HIV screening and study enrollment. When stratified by estimated Fiebig stage group, discrepancies were directly correlated with time between first HIV screening test and study enrollment (Figure 1).

ARCHITECT Signal-to-Cutoff Ratio

Centralized retrospective testing showed that the ARCHITECT S/CO ratio increased overall with actual Fiebig stage, with participants in Fiebig I (n=6) having median 0.4 (IQR 0.4-0.6), Fiebig II (n=43) 41.9 (IQR 11.0-114.9), Fiebig III (n=56) 35.7 (17.0-108.3), Fiebig IV (n=23) 44.0 (IQR 18.3-116.4), Fiebig V (n=60) 58.6 (IQR 22.0-90.1), and Fiebig VI (n=4) 147.2 (IQR 63.5-346.5; p=0.033). ARCHITECT S/CO was directly correlated with enrollment HIV RNA (unadjusted Spearman correlation coefficient 0.62, p<0.001; adjusted for Fiebig stage 0.71, p<0.001). Retrospectively, ARCHITECT S/CO ≥10 combined with nonreactive or indeterminate HIV antibody on the Geenius assay at enrollment confirmed 99 of 122 (81.2%) Fiebig II-IV AEHI cases (Figure 2). By definition, Fiebig I was associated with S/CO <1 and Fiebig V with a reactive HIV antibody [14].

DISCUSSION

This study demonstrated the feasibility of diagnosing HIV and initiating ART during AEHI across a wide variety of clinical research sites, including both resource-rich and resource-limited settings. Criteria that can be broadly applied in this way are necessary to undertake research that reflects the heterogeneity of both the virus and affected populations, including geographic diversity of HIV subtypes that may affect disease pathophysiology and outcomes [31, 32].

Importantly, the AEHI criteria in this study allowed for ART initiation before receipt of HIV RNA or other confirmatory HIV testing. Despite recent scale-up of HIV RNA testing worldwide, transportation of specimens to centralized laboratories and batch testing on automated machinery introduce delays in the process of getting HIV RNA results to healthcare providers and research participants [33-36]. Point-of-care HIV RNA testing could eliminate such delays and facilitate same-day ART initiation but is not widely available [37]. ART initiation on the day of HIV diagnosis improves engagement in care in many settings [38-42], produces rapid and durable viral suppression for most individuals [43, 44], and decreases HIV transmission [45]. In our study, enrolling participants without HIV RNA results available to confirm their HIV diagnosis facilitated rapid ART initiation. Despite potential concern for false-positive HIV diagnoses, we found that the few cases without HIV were due to erroneous results from local HIV RNA tests and not failures of the AEHI criteria. These three participants were exposed to days or weeks of ART with a well-established and favorable safety profile. Risks and benefits of potentially exposing individuals without HIV to investigational agents would need to be considered if these criteria are used in future studies of novel interventions during AEHI.

Participants were also enrolled before AEHI staging could be determined. The novel AEHI criteria utilized in this study were mapped to estimated Fiebig stages with actual Fiebig stages only determined retrospectively via centralized testing. This strategy, paired with study-provided ART options, facilitated early identification and treatment of AEHI. Understanding that progression through Fiebig stages can occur rapidly [14, 15], it is not surprising that discrepancies between estimated and actual Fiebig stages in this study were directly associated with increased duration between initial HIV testing and study enrollment. This was particularly true among participants whose initial testing indicated the earliest stages of HIV. Delays between initial testing and enrollment were due to multiple factors

such as turnaround time for initial test results, need for follow-up testing to satisfy criteria, and site-specific administrative issues. Our findings underscore the urgency to identify AEHI so that ART can be initiated as early as possible in order to achieve its potential maximal benefit.

Of the six AEHI criteria allowed by the study protocol, by far the most utilized was a combination of detectable HIV RNA by PCR with a non-reactive HIV antibody. A number of prior and ongoing studies of AEHI have used this strategy successfully, sometimes leveraging pooled PCR to decrease costs [46-49]. While S/CO-based criteria were used relatively less frequently for enrollment into this study, the retrospectively-evaluated combination of an ARCHITECT Ag/Ab Combo assay S/CO ≥10 with a nonreactive or indeterminate HIV antibody by the Geenius assay performed well in identifying AEHI during Fiebig stages II-IV. By definition, Fiebig I was associated with S/CO <1 and Fiebig V with detectable HIV antibody. In resource-limited settings, transportation of specimens to centralized laboratories and the need for trained technicians may create barriers to using S/CO ratio in AEHI diagnostic algorithms [50]. These barriers mirror those observed with centralized HIV RNA testing, but Ag/Ab assays return results faster than high-throughput HIV RNA assay systems and may be useful in the setting of failed pre-exposure prophylaxis that can suppress viremia [51, 52]. As such testing platforms become more commonplace, S/CO may prove a useful tool for identifying AEHI in the absence of HIV RNA testing, thereby facilitating rapid ART initiation in individuals with a high pre-test probability of AEHI.

Most participants in this study were symptomatic at the time of AEHI diagnosis and approximately one-third were hospitalized. While prior studies have reported a similar prevalence of symptoms during AEHI [17-19], this may be at least partly an artefact of symptomatic patients being more likely to seek care and diagnostic testing. When participants

are screened prospectively for AEHI with serial HIV testing, clinical manifestations tend to be less common at diagnosis [20, 21]. Symptoms were not considered in the AEHI criteria for this study, which sought to capture a wide range of testing practices at participating research sites. However, symptom-based scoring systems have been proposed to identify individuals at highest risk for AEHI in order to optimize screening yields [53, 54] and some participating sites may have considered symptoms when deciding who to test for HIV.

These analyses leveraged centralized laboratory testing from a global network of participating sites to evaluate the performance of novel criteria for AEHI diagnosis. By design, strategies for identifying candidates for HIV screening varied by site and enrollment into this study only occurred after the initial diagnosis was made, so we are unable to assess diagnostic yield of screening strategies at participating sites. Similarly, the relative frequency of each of the six criteria used for enrollment largely reflects site-level differences in procedures and does not necessarily reflect the relative usefulness of each criterion. It should be noted that the Abbott m2000rt platform, used for centralized viral load quantification in this study, is not FDA-approved for HIV diagnostic testing. Although S/CO ratio is an intrinsic read-out from fourth-generation HIV immunoassays, use of the S/CO to diagnose AEHI is not an FDA-approved approach nor is it included in manufacturer instructions. Some participating sites reported difficulty obtaining this result from commercial laboratories, but our findings suggest a potential value of routinely reporting S/CO ratio. In lower prevalence populations, S/CO-based criteria for AEHI may yield false-positive diagnoses that were not observed in this study [30]; symptom-based scores [53, 54] or other risk-stratification methods could be considered to focus S/CO assessment on individuals with a high pre-test probability of AEHI. The Geenius assay used for Fiebig staging may not correlate perfectly with the standard Western blot due to differences such as the gp160 band detected by each assay and timelines for positivity of other bands, though there is evidence that the p31 band

critical for determining progression out of Fiebig V becomes positive at the same time or earlier on the Geenius assay [55]. The Ag/Ab assays used in this study did not differentiate the reactive component per se; however, testing platforms that do differentiate Ag/Ab may have added value in evaluating AEHI and should be further evaluated for this purpose. The study was carried out at experienced clinical research centers and there may be additional barriers to utilizing these criteria in routine clinical care centers.

In summary, novel AEHI criteria incorporating ARCHITECT S/CO into diagnostic algorithms facilitated rapid ART initiation in individuals with a high pre-test probability of AEHI. ART initiation during AEHI was feasible even in situations where HIV RNA testing remained pending, with minimal ART exposure observed for the few participants in whom centralized testing did not confirm HIV. Importantly, retrospectively applied S/CO-based criteria yielded no false-positive HIV diagnoses. These new criteria may facilitate AEHI diagnosis, staging, and immediate ART initiation in future research studies that will test novel interventions to achieve durable HIV suppression with products administered during AEHI or after ART initiation during AEHI [56].

NOTES

ACKNOWLEDGMENTS

We would like to thank the study participants who made this research possible. We gratefully acknowledge the following members of the ACTG A5354 Study Team: Oladapo Alli (DAIDS Pharmacist), Deborah Anisman-Posner (laboratory specialist), LuAnn Borowski (laboratory technologist), Benjamin Chi (investigator), Susan E. Cohn (investigator), Ann Collier (investigator), Morgan Gapara (international site specialist), Sonya Heath (investigator), Tydie Higgins (data manager), Brenda Hoagland (investigator), Andrew Kaytes (community scientific subcommittee representative), Dimas Kliemann (field representative), Eugène Kroon (investigator), Susan Little (investigator), Gonasagrie Nair (investigator), Deborah Persaud (investigator), Sharon Riddler (investigator), James Rooney (industry representative), Scott Sieg (immunologist), Magdalena Sobieszczyk (investigator), Jennifer Tiu (clinical trials specialist), Kyle Whitson (laboratory data manager). The T A5354 study was conducted at the following clinical research sites: Alabama (Sonya Heath), Barranco (Javier Lama), Brigham and Women's Hospital Therapeutics (Paul Sax), Chapel Hill (Cynthia Gay, Brittney Soderman, Mandy Tipton), Columbia Physicians and Surgeons (Magdalena E. Sobieszczyk), Cincinnati (Carl Fichtenbaum), Greensboro (Cornelius Van Dam), Harbor-UCLA (Eric Daar), Hospital Nossa Senhora da Conceição (Breno Santos), Houston AIDS Research Team (Roberto Arduino), Instituto de Pesquisa Clinica Evandro Chagas (Sandra Cardoso), Joint Clinical Research Centre/Kampala (Cissy Kityo), Kenya Medical Research Institute/Walter Reed Project (Deborah Langat), Malawi (McNeil Ngongondo, Mina Hosseinipour), Massachusetts General Hospital (Rajesh Gandhi, Amy Sbrolla, Teri Flynn), Milton Park (Wadzanai Samaneka, Rachel Mahachi), Miriam Hospital (Karen Tashima), New Jersey Medical School Clinical Research Center (Shobha Swaminathan), Northwestern University (Babafemi Taiwo, Johnny Perez, Shannon Galvin),

Ohio State University (Susan Koletar), Penn Therapeutics (Pablo Tebas), Ponce de Leon Center (Jeffrey Lennox, Albert Anderson, Ericka Patrick), Rush University (Beverly Sha), San Miguel (John Thays), Thai Red Cross AIDS Research Centre (Kiat Ruxrungtham), Trinity Health and Wellness Center (Roger Bedimo), UCLA CARE Center (Raphael Landovitz), UCSD Antiviral Research Center (Constance Benson), University of Pittsburgh, University of Washington (Janine Maenza), Washington University Therapeutics (Rachel Presti), Weill Cornell Chelsea (Timothy Wilkin), Whitman-Walker Health (Sarah Henn).

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FUNDING

This work was supported by the National Institute of Allergy and Infectious Diseases of the National Institutes of Health (grant numbers UM1 AI068634, UM1 AI068636, UM1 AI068618, UM1 AI106701 and P30 AI027757). Antiretroviral therapy for the study was donated by Gilead Sciences.

CONFLICTS OF INTEREST

JJE reports research grants from ViiV Healthcare, Gilead Sciences, and Janssen as well as personal fees from ViiV Healthcare, Gilead Sciences, Janssen, and Merck. JWM reports research grants from Gilead Sciences; consultant fees from Merck, Accelevir Diagnostics and Xi'an Yufan Biotechnologies; and financial interests in Abound Bio and CoCrystal Pharmaceuticals.

KR reports receipt of the Senior Research Scholarship from the Thailand Research Fund (TRF); honoraria or consultation fees from Merck, Roche, Jensen- Cilag, Tibotec, Mylan and the Thai Governmental Pharmaceutical Organization (GPO; and speaker fees from Abbott, Gilead Sciences, Bristol- Myers Squibb, Merck, Roche, Jensen- Cilag, Thai Governmental Pharmaceutical Organization, GlaxoSmithKline and GPO.

JA reports honoraria from ViiV Healthcare, Gilead Sciences, Merck, Roche, and AbbVie. ESD reports research grants from Gilead Sciences and Merck as well as consultant fees from Gilead Sciences, Merck, and Genentech. T.C. reports ACTG CTU Award from Division of AIDS, NIH. The remaining authors declare no relevant conflicts of interest.

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Table 1. Diagnostic Criteria for Acute HIV Infection Used at Clinical Research Sites for Enrollment of Participants in the A5354/EARLIER Study

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

F	Detectable HIV RNA within 7 days	AND	Non-reactive HIV antibody within 7 days AND ARCHITECT or GS HIV Combo S/CO 0.5-9.9 within 7 days	I/II

Abbreviations: S/CO, signal-to-cutoff ratio (from ARCHITECT HIV Antigen/Antibody Combo assay or GS HIV Combo Antigen/Antibody enzyme immunoassay)

Eligibility for enrollment was assessed using available test results to satisfy one of six study-specific criteria for acute and early HIV infection, each of which was mapped to an estimated Fiebig stage group (defined by protocol as I/II, III/IV, or V). Criteria were designed to support HIV diagnosis in early stages of infection and establish relative recency of infection (including minimizing the risk of progression to Fiebig VI by the time of enrollment) while accommodating differences in testing procedures at study sites. Time periods refer to the number of days that a test could be collected prior to screening for study entry. Criteria D-F were designed to capture participants in the earliest stages of AEHI, with low-level viremia and before development of antibody responses to HIV. The S/CO was used as a surrogate for HIV RNA based on the understanding that p24 antigen detection at an S/CO of 0.5-10 (and a negative IgM/IgG immunoassay) corresponds roughly to an HIV RNA of 4.0-5.5 $\log_{10}$copies/mL but is also the most common range for false-positive tests [30]. A recent increase of S/CO from below to within this range was considered indicative of new infection (criterion E). To minimize false-positive diagnoses, participants with this range of S/CO but no prior S/CO assessment required HIV RNA testing prior to enrollment (criterion F). The concomitant requirement for a non-reactive HIV antibody test within the preceding 7 days was designed a priori to exclude participants in Fiebig stages III-V.

Table 2. Actual Fiebig Stage of Participants by Inclusion Criterion for Acute and Early HIV Infection

		Actual Fiebig Stage						
Inclusion AEHI Criterion (Estimated Fiebig Stage)	**Uninfected** (n=3)	**I** (n=6)	**II** (n=43)	**III** (n=56)	**IV** (n=23)	**V** (n=60)	**VI** (n=4)	**Total** (n=195)
A (I/II)	2	5	27	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%)

Abbreviations: AEHI, acute and early HIV infection

Eligibility for enrollment was assessed using available test results to satisfy one of six study-specific criteria for acute and early HIV infection, each of which was mapped to an estimated protocol-defined Fiebig stage group (I/II, III/IV, or V). Actual Fiebig stage was determined by centralized testing of samples collected on the day of enrollment. Please see Table 1 for definitions of inclusion criteria for acute and early HIV infection.

NOTES

Figure 1. Associations Between Timing of Enrollment, Initial HIV Screening Test, and Actual Fiebig Stage.

Actual Fiebig stage at enrollment was plotted against the number of days elapsed since initial HIV testing for all participants (panel A) and separately for participants who enrolled with estimated Fiebig I/II (panel B), estimated Fiebig III/IV (Panel C), and estimated Fiebig V (panel D). The left and right edges of each box in the figure indicate the interquartile range. The line inside the box indicates the median. Points plotted on panel A using the log-scale were binned in order to accurately present the number of data points.

Figure 2. 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-1/2 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. Points plotted were binned in order to accurately present the number of data points.

Figure 1

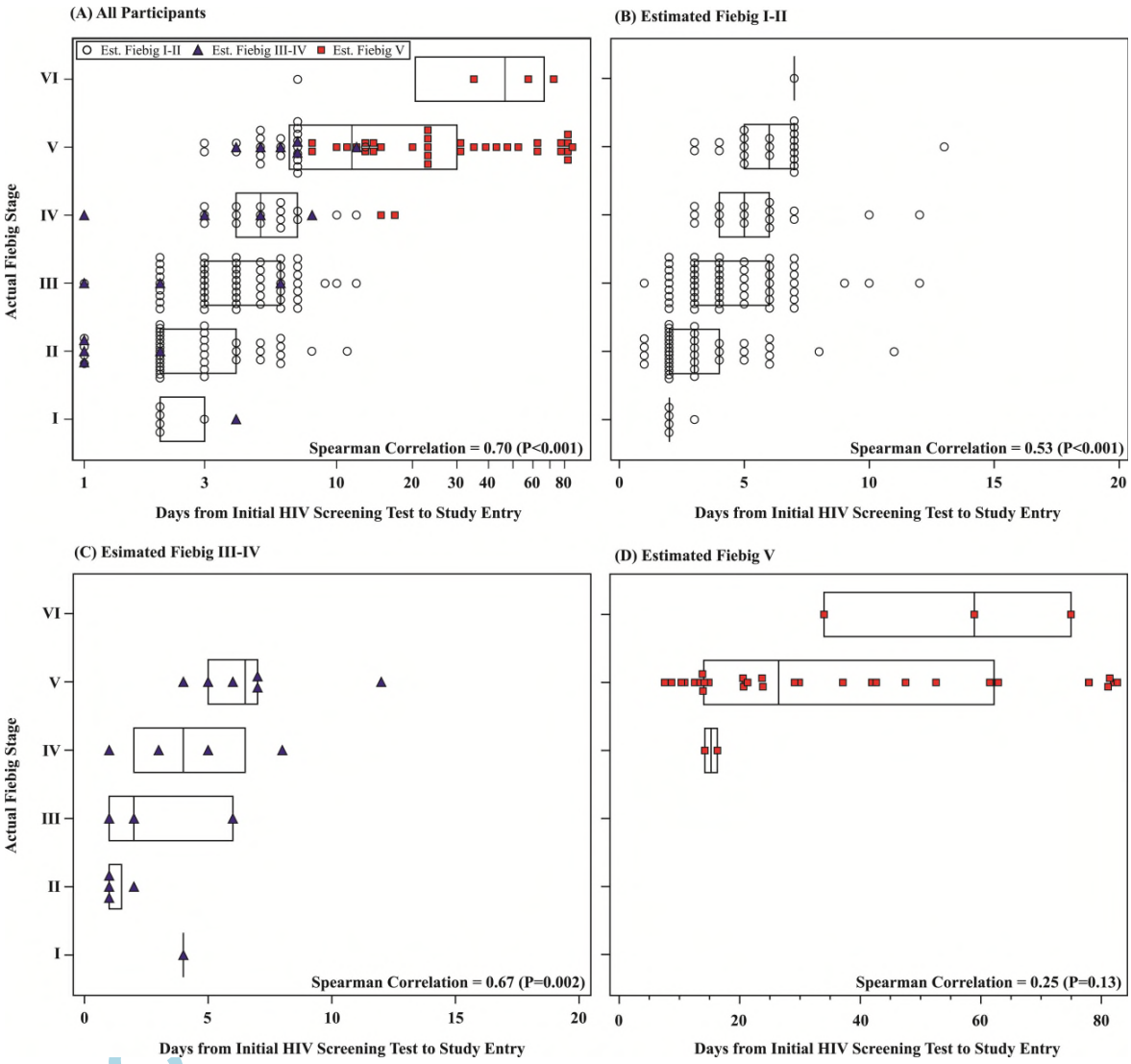

Figure 2