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Chronic and Early Antiretroviral Therapy Impact Human Immunodeficiency Virus (HIV) Serological Assay Sensitivity, Leading to More False-Negative Test Results in HIV Diagnosis

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This retrospective study evaluated the reactivity of 3 human immunodeficiency virus (HIV) confirmatory assays (INNO-LIA, Geenius, and MP) and 7 HIV rapid tests on samples from 2 different study populations in Belgium. For the early-treated cohort (83 HIV-1 adult patients treated within 3 months after infection), HIV-1 diagnosis was not obtained in at least 1 confirmatory assay in 12.0% (10/83) and in an HIV rapid test in 31.3% (26/83). Confirmation assay sensitivities ranged from 87.5% to 95.2%, whereas rapid test assay sensitivities ranged from 75.9% to 100%. The time to treatment initiation or the length of time on treatment did not have a statistical influence on the probability to obtain a false-negative test result. The fastest reversion was demonstrated after 4 months of treatment. Among the long-term treated cohort (390 HIV-1 patients with ≥ 9 years of undetectable viral load), false-negative test results were found in at least 1 HIV confirmatory assay for 2.1% (8/390) of the patients and in a HIV rapid test for 4.9% (19/390). Confirmation assay sensitivities ranged from 98.1% to 99.5%, whereas rapid test sensitivities ranged from 96.2% to 100%. Longer treatment increased nonreactivity of the HIV rapid tests ($P = .033$). Undetectable viral load decreases the sensitivities of HIV diagnostic tests, and further monitoring of the performance of serological assays is advised.

Keywords. HIV; HIV diagnosis; HIV confirmatory assays; HIV rapid tests.

Depending on the test used, the human immunodeficiency virus (HIV) window period can range from a few days to 3 weeks [1]. In Belgium, a low-HIV-prevalence country, HIV screening is performed in clinical laboratories by enzyme immunoassay (EIA), and in some local healthcare centers and nongovernmental organizations by rapid tests as point-of-care (POC) testing. Samples from all patients with a reactive screening test result are further analyzed by specialized AIDS reference laboratories. In resource-constrained settings or remote places, HIV diagnosis is usually established by an algorithm of solely rapid tests [2]. In both situations, confirmation of a true infection in patients on antiretroviral therapy (ART) is challenging. ART may suppress virus replication for years and reduced antigen presence may result in waning of the host's antibody production [3, 4]. This might lead to a partial or complete loss of antibody detection, which is well known for HIV-infected newborns or young children taking ART [5–8]. However, only a few clinical cases of seroreversion or incomplete seroconversion have been reported in patients with HIV type 1 (HIV-1) starting ART as adult [9–12]. Shortening the period to which the patient is exposed to replicating and disseminating viruses tends to diminish the immune response [4, 13]. Indeed, most cases of seroreversion at adult age are documented for patients initiating ART during the acute phase of HIV infection [13–18]. The implementation of continuous HIV preexposure prophylaxis (PrEP) presents diagnostic settings with even more of a challenge to prove an established HIV infection, as immunoglobulin-based assays would remain nonreactive or become delayed reactive due to the interrupted antibody response. Viral load testing could only confirm the HIV infection in cases of viral escape due to treatment cessation or to PrEP resistance.

Even though the World Health Organization (WHO) does not recommend retesting for diagnosis once a patient is on ART

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[19], it can occur in cases of nondisclosure when visiting a new healthcare facility. In 2017, 7.2% (386/5331) of the requested confirmation tests in Belgium were not performed because the patient was already registered as HIV infected in the addressed AIDS reference laboratory (unpublished data). In the past 10 years, at least 4 cases were recorded as having an indeterminate confirmation result due to years of ART intake in routine HIV clinical diagnostic settings in Belgium. This triggered an in-depth investigation on a national level to assess the current situation. In this retrospective study, the sensitivity of the HIV confirmation assays currently used in Belgium was investigated, as well as some rapid tests used worldwide, using samples from adult patients on fully suppressive therapy for at least 9 years. These assays were also evaluated using samples from adults who started HIV treatment during the acute or early phase of infection.

MATERIALS AND METHODS

A retrospective study on 2 HIV-1–infected study groups was performed in Belgium. The first study group consisted of HIV-1–infected patients treated long-term. The inclusion criteria were patients with all plasma HIV-1 RNA viral load results below the quantification limit of the routine viral load test used at the time of sample collection for at least 9 years, and with a maximum interval of 18 months between 2 consecutive determinations. The patient's age at start of the undetectable viral load period was set at a minimum of 17 years. The second group was composed of HIV-1–infected adults initiating fully suppressive ART at early infection. The inclusion criteria were HIV-1 diagnosis in 2010 or later, laboratory-documented acute HIV-1 infection (ie, HIV confirmation test negative or indeterminate, but with a positive p24 antigen test or detectable RNA viral load), start of ART within 3 months of diagnosis and no viral load blips once undetectable viral load was achieved. For both groups, the most recent sample collected from each patient was evaluated by the routine confirmation tests used in Belgium and by a selection of frequently used rapid tests. Collection dates were between 2011 and 2018 with 80% collected in 2016 or 2017. When an indeterminate, HIV-negative, or nonreactive result was observed in any of the evaluated assays, look-back samples (1 sample per year) were tested until an HIV-1–positive result was obtained. Where possible, the evolution was further investigated with look-forward samples (collected in 2019). Any possible immune dysfunction of the patient was excluded using other laboratory test results and clinical data for each patient with an HIV test reversion. All tests were performed according to the manufacturer's instructions and in compliance with the clinical laboratory's quality regulations ISO15189. The confirmation tests analyzed in this study were INNO-LIA HIV I/II Score (FujiRebio), Geenius HIV 1/2 Confirmatory Assay (Bio-Rad) using the Reader interpretation (Geenius), and HIV Blot 2.2 (MP Biomedicals), hereafter referred to as INNO-LIA, Geenius, and MP Blot 2.2, respectively. Rapid tests were selected based on their use in Belgian help centers and on global use (data provided by the WHO): ABON HIV1/2/O Tri-Line Rapid Test Device (hereafter "Abon"; ABON Biopharm Hangzhou Co Ltd), Alere Determine HIV 1/2 (hereafter "Determine"; Abbott), First Response HIV 1.2.0 Card Test (hereafter "First Response"; Premier Medical Co Ltd), INSTI HIV-1/HIV-2 AntibodyTest (hereafter "INSTI"; BioLytical Laboratories), SD Bioline HIV 1/2 3.0 (hereafter "SD Bioline"; Abbott), HIV 1/2 STAT-PAK Assay (hereafter "StatPak"; Chembio Diagnostic Systems), and Wantai HIV 1 + 2 Rapid Test (hereafter "Wantai"; WANTAI Bio-Pharm). Except for Abon and Wantai, all were Conformité Européenne In Vitro Diagnostic (CE IVD) labeled. False-negative test results were defined as negative or indeterminate for the HIV confirmation assays and nonreactive for the rapid tests. HIV-1 viral load was determined with the automated systems from Roche Cobas, Abbott m2000 RealTime System, or Siemens VERSANT, depending on the site where the patient was followed. Subtype was determined by consensus with COMET HIV-1 version 2.3 [20], and REGA HIV subtyping tool version 3.41 [21] based on protease and reverse transcriptase sequences from the Pol region by in-house techniques, ViroSeq HIV-1 Genotyping System (Abbott), or TRUGENE HIV-1 Genotyping Assay (Siemens).

Statistical analysis was performed on the comparison of the following groups: HIV-1 result in both INNO-LIA and Geenius assays vs indeterminate or negative result in at least 1 of these 2 confirmatory assays. For the rapid tests, sample comparison groups were identified as HIV reactive in all 7 rapid tests vs a nonreactive result in at least 1 rapid test. Statistical significance was set at $<.05$ and depending on sample size and statistical distribution, the Fisher exact test, Mann–Whitney U test, or χ^2 test was retained using SPSS (IBM SPSS Statistics for Windows, version 23.0, IBM). Data concerning time periods were grouped into quartiles for analysis. Univariate logistic regression was used to investigate the influence of treatment duration in the long-term treated group and multivariate logistic regression to investigate whether treatment duration or time to ART initiation influenced the serological test outcomes in the early-treated group.

This study is representative for the whole of Belgium as all 7 AIDS reference laboratories eligible for HIV confirmation and follow-up in Belgium participated. The study was performed on encoded remnant samples, excluding patients who opted out for sample use in scientific research. Ethics committee approval was obtained at the leading investigator's center, University Medical Center St-Pieter in Brussels (number M.007), and was registered as approval CE/17-11-11.

RESULTS

Long-term ART: HIV Confirmatory Assays

A total of 390 patients were included in the first group of patients with a long period of undetectable HIV-1 viral loads, ranging

between 8.6 and 20.9 years, with an average of 13.0 years. HIV-1 infection could not be confirmed on the most recent sample available in 1.5% (6/390) and 0.5% (2/390) for INNO-LIA and Geenius, respectively. All samples with an indeterminate result in INNO-LIA were reported as HIV-1 positive by Geenius, and vice versa. A completely negative profile (ie, none of the HIV-1–specific bands positive) was not observed. MP HIV Blot 2.2 was evaluated on 54 of these 390 samples, of which 1.9% (1/54) scored as indeterminate (Table 1). This sample had an indeterminate test result in INNO-LIA and an HIV-1–positive result in Geenius. In total, 8 different patients (8/390 [2.1%]) could not be confirmed as HIV-1 infected by at least 1 of these 3 confirmatory assays.

A full HIV-1 profile (ie, all HIV-1–specific bands positive) was observed in 56.4% (220/390), 17.2% (67/390), and 3.7% (2/54) of the samples in INNO-LIA, Geenius, and MP Blot 2.2, respectively. The band capturing the gp41 antibodies was the only 1 detected in all assay test results (Table 2). Overall weakening of the band strength was observed over time. For 37.9% (148/390) of the samples, the INNO-LIA result of the most recent sample could be compared with the original INNO-LIA result around the time of diagnosis (mean of 12 years of fully suppressive ART). The band score weakened with a score of 1.4 on average over all 5 bands taken together (Table 3). Looking at the duration of undetectable viral load for all 390 samples, the INNO-LIA indeterminate samples were spread over the 4 quartiles, while for Geenius, both indeterminate samples were found in Q4 (14.3–20.9 years). Taking both assays together, comparison between the HIV-1 group and the indeterminate group (in either INNO-LIA or Geenius) did not reveal a statistical significant influence of treatment duration ($P = .707$) or any other parameter (age, year of start of treatment). The HIV-1 subtype could not be compared because only 30.3% (118/390) of the population was subtyped, of which only 2 samples were from the indeterminate group.

Long-term ART: HIV Rapid Tests

From the 7 evaluated rapid tests, only 3 tests were able to detect all samples as HIV reactive: Abon, Determine, and Wantai (Table 1). The sensitivity of StatPak was the lowest with 3.8% (15/390) false-negative test results, of which 33.3% (5/15) were sampled in Q2 (after 11.3–12.3 years of undetectable viral load) and 46.7% (7/15) in Q4 (after 14.3–20.9 years). Taking all rapid tests together, treatment duration showed a statistical significant influence on the probability to obtain a false-negative test result in at least 1 of the 7 rapid tests ($P = .033$).

Long-term ART: Analysis Over Time

Yearly look-back samples could be analyzed for 18 of the 19 patients with at least 1 false-negative test result. The first reversion was identified after a mean of 9.5 (range 5.8–14.5), 11.75 (7.7–15.8), and 12.6 (6.7–19.2) years of undetectable plasma viral load for INNO-LIA (5/18), Geenius (2/18), and rapid tests (15/18), respectively (Figure 1).

Early ART Initiation After Infection: HIV Confirmatory Assays

The second study group consisted of 83 adults with acute HIV-1 infection treated within 3 months of diagnosis. ART was started at a mean age of 37.3 years and between 0 and 88 days after diagnosis (mean, 23.5 days; median, 14.0 days). The studied samples were collected after 2.9 years on ART on average and detection of HIV-1 infection failed for 4.8% (4/83) and 8.4% (7/83) of the patients using INNO-LIA and Geenius, respectively (Table 4).

Table 1. Sensitivity of Human Immunodeficiency Virus (HIV) Confirmatory Assays and HIV Rapid Tests in Patients With Long-term Treated HIV-1 and Early-Treated HIV-1 Seroconverters

Assay/Test	CE IVD Label	Long-term Treated HIV-1 Patients[a]	Early-Treated HIV-1 Seroconverters[b]
HIV confirmatory assays			
INNO-LIA HIV I/II Score (FujiRebio)	Yes	98.5% (384/390)	95.2% (79/83)
Geenius HIV 1/2 Confirmatory Assay (Bio-Rad)	Yes	99.5% (388/390)	91.6% (76/83)
HIV Blot 2.2 (MP Biomedicals)	Yes	98.1% (53/54)[c]	87.5% (14/16)[d]
HIV rapid tests			
ABON HIV1/2/O Tri-Line Rapid Test Device (ABON Biopharm Hangzhou Co Ltd)	No	100.0% (390/390)	100.0% (83/83)
Alere Determine HIV 1/2 (Abbott)	Yes	100.0% (390/390)	100.0% (83/83)
HIV 1 + 2 Rapid Test (WANTAI Bio-Pharm)	No	100.0% (390/390)	100.0% (83/83)
First Response HIV 1.2.0 Card Test (Premier Medical Co Ltd)	Yes	99.7% (389/390)	96.4% (80/83)
SD Bioline HIV 1/2 3.0 (Abbott)	Yes	99.5% (388/390)	92.8% (77/83)
INSTI HIV-1/HIV-2 AntibodyTest (BioLytical Laboratories)	Yes	98.7% (385/390)	91.6% (76/83)
HIV 1/2 STAT-PAK Assay (Chembio Diagnostic Systems Inc)	Yes	96.2% (375/390)	75.9% (63/83)

Abbreviations: CE IVD, Conformité Européenne In Vitro Diagnostic; HIV, human immunodeficiency virus.

[a]HIV-1 test result from patients with undetectable HIV-1 viral load for ≥9 years.

[b]HIV-1 test result from patients with acute HIV-1 infection treated within 3 months.

[c]Only 54 samples from the 390 selected were analyzed.

[d]Only 16 samples from the 83 selected were analyzed.

Table 2. Patients With Long-term Treated Human Immunodeficiency Virus Type 1: Clinical and Performance Characteristics

Characteristic	INNO-LIA HIV I/II Score (FujiRebio)		Geenius HIV 1/2 Confirmatory Assay (Bio-Rad)		All 7 HIV Rapid Tests		
	HIV-1	Indeterminate	HIV-1	Indeterminate	Reactive	Nonreactive in at Least 1 Rapid Test	Total
No. of samples	98.5% (384/390)	1.5% (6/390)	99.5% (388/390)	0.5% (2/390)	95.1% (371/390)	4.9% (19/390)	390
Mean age, y, at start undetectable VL period	40.9	37.9	40.9	30.3	40.8	41.7	40.9
Time, y, between first undetectable VL and most recent sample tested							
Mean	13.0	12.0	13.0	15.3	12.9	14.3	13.0
Q1	…	…	…	…	…	…	11.3
Q2 (median)	12.3	12.3	12.3	15.3	12.3	13.8	12.3
Q3	…	…	…	…	…	…	14.3
Q4 (max)	…	…	…	…	…	…	20.9
Band profile confirmatory assay	…	…	…	…	NA	NA	…
Presence of gp160	NA	NA	100.0% (388/388)	0.0% (0/2)	…	…	99.5% (388/390)
Presence of gp120	95.3% (366/384)	0.0% (0/6)	NA	NA	…	…	93.8% (366/390)
Presence of gp41	100.0% (384/384)	100.0% (6/6)	100.0% (388/388)	100.0% (2/2)	…	…	100.0% (780/780)
Presence of p31	70.6% (271/384)	16.7% (1/6)	22.7% (88/388)	0.0% (0/2)	…	…	46.2% (360/780)
Presence of p24	91.4% (351/384)	0.0% (0/6)	53.4% (207/388)	0.0% (0/2)	…	…	71.5% (558/780)
Presence of p17	80.7% (310/384)	16.7% (1/6)	NA	NA	…	…	79.7% (311/390)

Abbreviations: HIV, human immunodeficiency virus; NA, not applicable; VL, viral load.

Analysis of look-back and supplementary look-forward samples showed the first reversion in 4 cases for the INNO-LIA assay after an average of 1.4 years of treatment. For the Geenius assay, the first reversion was observed in 6 cases (including 2 INNO-LIA reversions) after a mean of 2.4 years of ART, with further evolution toward a negative result in 1 case while it remained indeterminate in the INNO-LIA assay (Ac-07). Three additional cases never evolved to an HIV-1–positive result and remained indeterminate. The fastest reversion was demonstrated after 13 and 11 months of ART with the INNO-LIA and Geenius assays, respectively (Table 5). One sample was HIV negative in INNO-LIA while indeterminate in Geenius (Ac-02). Taking both assays together with 9 of 83 patients with at least 1 false-negative test result, time on ART ($P = .460$) or time to ART initiation ($P = .727$) did not influence the confirmatory test outcomes in this early-treated study group. Mean viral load at diagnosis for the samples with an indeterminate or negative confirmatory test result (5.92 log copies/mL) was significantly lower compared to the mean viral load of HIV-1–positive confirmations (6.63 log copies/mL) ($P = .031$, Mann–Whitney U test). Other parameters were not found to be statistically significant (age, CD4 count at diagnosis, subtype B vs non-B, integrase strand transfer inhibitor dolutegravir or elvitegravir in first-line treatment). The gp41 capturing band was most frequently present. Recent samples from 16 patients from this cohort were additionally tested by MP Blot 2.2, of which 12.5% (2/16) did not result in a HIV-1 diagnosis. In total, 10 different patients (10/83 [12.0%]) could not be confirmed as HIV-1 infected by at least 1 of the 3 confirmatory assays after a mean of 2.2 years of treatment.

Early ART Initiation After Infection: HIV Rapid Tests

Again, rapid tests Abon, Determine, and Wantai showed a 100% HIV detectability while the StatPak assay was the least performant, with a sensitivity of 75.9% (Table 1). Overall, the same percentage of HIV-1–nonreactive specimens was found in all quartiles, ranging from 0 to 88 days until treatment initiation after acute diagnosis. The length of time on ART ($P = .974$) or the time to ART initiation ($P = .967$) did not influence the rapid test outcomes. All other investigated parameters did not reveal statistical significant differences between the group with at least 1 nonreactive test and the group with a consistent reactive test result (CD4 count at diagnosis, viral load at diagnosis, age, subtype B vs non-B, use of integrase strand transfer inhibitor

Table 3. Patients With Long-term Treated Human Immunodeficiency Virus Type 1: Weakening of Band Intensities in INNO-LIA Confirmatory Assays After a Mean of 12 Years of Antiretroviral Therapy

Average Band Score, INNO-LIA	gp120 ENV1	gp41 ENV1	p31 POL	p24 GAG	p17 GAG	Complete HIV-1 Band Profile, %[a]	Average Presence of the HIV-1 Profile, %[b]
A. Around time of diagnosis	2.9	3.5	2.4	2.9	2.5	87.8% (130/148)	97.1
B. After average 12 y ART	2.0	2.5	1.3	2.0	1.7	57.4% (85/148)	89.5
Difference in band score between A and B	–1.27	–1.22	–1.54	–1.46	–1.52	–30.4%	–7.6

Abbreviations: ART, antiretroviral therapy; HIV, human immunodeficiency virus.

[a]Number of samples with a complete HIV-1 band profile in test result (all HIV-1 bands positive).

[b]Overall average of the HIV-1 profile test result: 100% = all 5 bands positive, 80% = 4/5 bands positive, etc.

dolutegravir or elvitegravir in first-line treatment). Look-back samples demonstrated the HIV rapid test reversion for 14 patients after mean of 2.4 years of treatment (13/14 StatPak, 4/14 INSTI, 1/14 SD Bioline, and 1/14 First Response). Additionally, samples from 6 patients never evolved to a reactive test result for StatPak (6 patients), INSTI (1/6), and SD Bioline (1/6).

DISCUSSION

Monitoring of the diagnostic HIV assays is essential as antibody response fades on continuous, efficient ART [3, 4]. The combination of highly efficient new ART molecules, the "test and treat" strategy, PrEP and increased effective treatment duration may influence the performance of HIV antibody-based diagnostic assays [22, 23]. Long-term suppression of plasma viral load is an easy marker for sample selection in the surveillance of possible negativation of HIV diagnostic assays. It must, however, be kept in mind that other processes might continue stimulating immune responses, for example, virus release in lymph nodes due to lack of penetration of the administered ART to the lymphatic tissues, while maintaining undetectable plasma viral load [24]. This may be an explanation for the fact that we only could observe a statistical link between length of treatment and test result reversion for the rapid tests in the chronic treated group. As at least 1 confirmatory assay (INNO-LIA or Geenius) had a reactive gp41 band for each tested sample in this study, the more sensitive and gp41-based EIA screening tests are assumed to have a reactive result on the study samples (not assessed). The quantification of HIV-1 DNA could not be tested in the samples with confirmatory reversion because whole blood or buffy coat was not available. In any case, HIV-DNA testing is not a valid alternative as it is not cost-effective enough to be performed in routine analysis for all negative or indeterminate confirmation test results. As long as routine EIA screens remain reactive after many years of suppressive treatment, a negative confirmatory assay result might be disputed by the clinician who could further question the patient (eg, initial nondisclosure by the patient), but nevertheless the danger of missed diagnosis remains. Moreover, seroreversion on third- and fourth- generation EIA has already been proven on samples from patients initiating ART in the early acute infection stage [17, 25]. The risk of having low HIV-1 antibody levels due to ART uptake during early HIV-1 infection entails a high risk for misdiagnosis of these patients [26, 27].

In this evaluation, the early-treated HIV seroconverters in particular were prone to false-negative results in HIV confirmatory assays and HIV rapid tests. A baseline factor associated with nonpositivity of the confirmatory assays was the lower viral load at time of diagnosis, but not the CD4 count, differing from de Souza et al, where both factors were statistically significant in the evaluation of EIAs and Western blot [17]. In contrast, initial viral load in nonreactive rapid tests was slightly higher than in the reactive group ($P = .283$ Mann–Whitney U test).

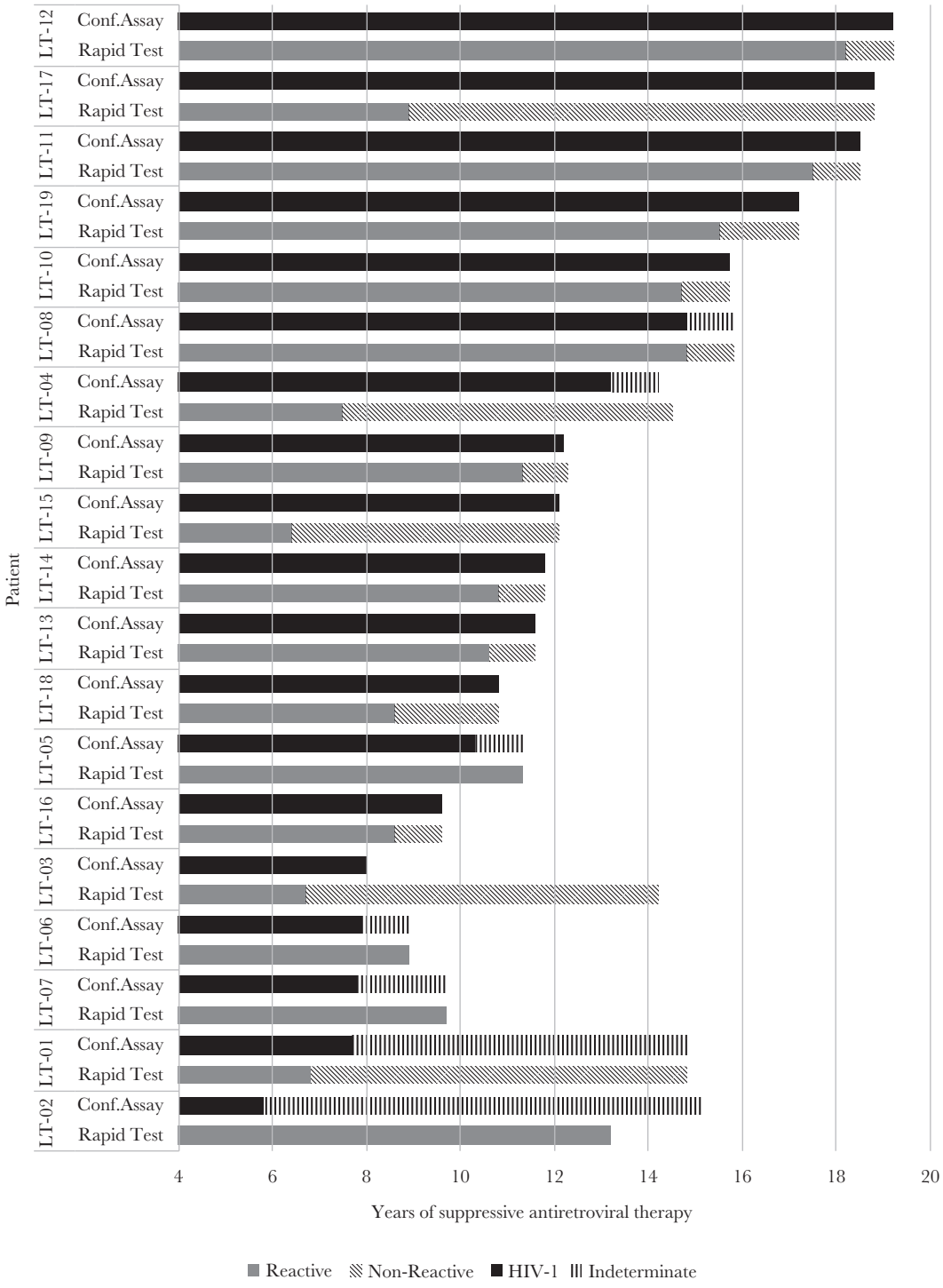

Figure 1. Long-term treated: reversion time identified by analysis of look-back samples (1 sample per year). Abbreviation: HIV, human immunodeficiency virus.

Although second-generation HIV integrase strand transfer inhibitor administration during acute HIV infection might accelerate seroreversion, this could not be demonstrated for HIV confirmatory assays ($P = .312$) or HIV rapid tests ($P = .132$). Future monitoring is, however, recommended, based on the recent observation that 2 seroconverting patients with plasma viral loads of > 7.0 log copies/mL obtained undetectable viral load at their follow-up consultation only 12 and 35 days after bictegravir/emtricitabine/tenofovir alafenamide initiation, with a positive but incomplete Geenius profile and thus a suspected

Table 4. Early-Treated Human Immunodeficiency Virus Type 1 Seroconverters: Clinical and Performance Characteristics

Characteristic	INNO-LIA HIV I/II Score (FujiRebio)		Geenius HIV 1/2 Confirmatory Assay (Bio-Rad) Using Reader		All 7 HIV Rapid Tests		Total
	HIV-1	Indeterminate or Negative	HIV-1	Indeterminate	Reactive	Nonreactive in at Least 1 Rapid Test	
No. of samples	95.2% (79/83)	4.8% ([3 Ind + 1 Neg] / 83)	91.6% (76/83)	8.4% (7/83)	68.7% (57/83)	31.3% (26/83)	83
Mean age at start ART, y	37.7	29.5	37.6	34.4	37.3	37.4	37.3
Mean viral load at diagnosis, log copies/mL plasma	6.61	5.50	6.61	6.02	6.65	6.38	6.56
Mean CD4 count at diagnosis, cells/μL	447	571	438	601	439	483	453
Start of ART after diagnosis, d							
Mean	23.6	21.8	23.6	22.1	23.8	22.8	23.5
Q1	…	…	…	…	…	…	7.0
Q2 (median)	14.0	9.5	14.0	12.0	14.0	13.5	14.0
Q3	…	…	…	…	…	…	38.5
Q4 (max)	…	…	…	…	…	…	88.0
Time between start of ART and most recent sample tested, y							
Mean	2.9	2.6	2.9	2.4	2.9	2.9	2.9
Q1	…	…	…	…	…	…	1.3
Q2 (median)	2.5	2.6	2.7	1.8	2.6	2.2	2.5
Q3	…	…	…	…	…	…	4.1
Q4 (max)	…	…	…	…	…	…	8.7
HIV-1 subtype B[a]	52.1% (38/73)	66.6% (2/3)	53.6% (37/69)	42.9% (3/7)	53.8% (28/52)	50.0% (12/24)	52.6% (40/76)
Band profile confirmatory assay					NA	NA	
Presence of gp160	…	…	100.0% (76/76)	0.0% (0/7)	…	…	91.6% (76/83)
Presence of gp120	62.0% (49/79)	0.0% (0/4)	…	…	…	…	59.0% (49/83)
Presence of gp41	100.0% (79/79)	75.0% (3/4)	100.0% (76/76)	100.0% (7/7)	…	…	99.4% (165/166)
Presence of p31	16.5% (13/79)	0.0% (0/4)	3.9% (3/76)	0.0% (0/7)	…	…	9.6% (16/166)
Presence of p24	94.9% (75/79)	0.0% (0/4)	31.6% (24/76)	0.0% (0/7)	…	…	59.6% (99/166)
Presence of p17	43.0% (34/79)	0.0% (0/4)	…	…	…	…	41.0% (34/83)

Abbreviations: ART, antiretroviral therapy; HIV, human immunodeficiency virus; Ind, indeterminate; NA, not applicable; Neg, negative.

[a]Based on protease and reverse transcriptase sequences from Pol region; 96.4% (80/83) of the samples could be subtyped.

Table 5. Early-Treated Human Immunodeficiency Virus Type 1 Seroconverters: Reversion of the Confirmation Test Result and Their STAT-PAK Test Results

Patient ID (REV/NP)[a]	HIV-1 Subtype	Start of Treatment After Diagnosis, d	Time After Treatment Initiation, d	HIV-1 Plasma Viral Load, Copies/mL	CD4 Count, Cells/μL	INNO-LIA HIV I/II Score	Geenius HIV 1/2 Confirmatory Assay Using Reader	HIV 1/2 STAT-PAK Assay
AC-01 (G REV)	B	12	598	< 20	500	HIV-1	Ind	NR
			38	79	671	HIV-1	HIV-1	R
			–12	970 000	587	Neg	HIV-1	R
AC-02 (G REV, I REV)	B	12	1322	< 20	1193	Neg	Ind	NR
			658	< 20	1353	Ind	HIV-1	NR
			332	< 20	1724	HIV-1	Ind	NR
			29	< 20	1251	HIV-1	HIV-1	R
			–12	306 000	867	Ind	Ind	ND
AC-03 (G NP)	A1	8	84–1407	< 20	900–1605	HIV-1	Ind	NR
			27	50	364	HIV-1	Ind	NR
			–8	415 000	527	Neg	Neg	ND
AC-04 (G REV, I REV)	B	7	1125	< 20	791	Ind	Ind	NR
			786	< 20	676	Ind	Ind	NR
			390	< 20	640	Ind	HIV-1	NR
			0	333 000	484	HIV-1	Ind	R
			–7	Unknown (p24 Ag pos)	Unknown	Neg	Neg	ND
AC-05 (G REV)	02_AG	47	672	< 40	1140	HIV-1	Ind	NR
			575	< 40	1362	HIV-1	Ind	ND
			98	< 40	1036	HIV-1	HIV-1	R
			–47	314 000	1004	Ind	Ind	ND
AC-06 (I REV, G NP)	G	7	440–1180	< 40	679–829	Ind	Ind	NR
			29	83	1040	HIV-1	Ind	NR
			–5	Unknown (p24 Ag pos)	316	ND	ND	NR
AC-07 (G REV, I REV)	Unknown	61	1373	< 40	1853	Ind	Neg	ND
			1019	< 40	1112	Ind	Ind	ND
			788	< 40	1174	Ind	HIV-1	NR
			611	< 40	1212	Ind	ND	ND
			123	100	1365	HIV-1	ND	NR
			29	Unknown	618	ND	ND	R
AC-08 (G NP)	B	54	106	< 40	834	HIV-1	Ind	NR
			–48	223 000	727	Neg	Neg	NR
AC-09 (G REV)	01_AE	15	1925	< 20	639	HIV-1	Ind	R
			1526	< 20	848	ND	HIV-1	ND
			–15	> 107	182	Neg	ND	ND

Abbreviations: Ag pos, antigen positive; HIV, human immunodeficiency virus positive; ID, identifier; Ind, indeterminate; Neg, human immunodeficiency virus negative; ND, not done; NR, nonreactive; R, reactive.

[a]G, Geenius HIV 1/2 confirmatory assay using Reader (Bio-Rad); I, INNO-LIA HIV I/II Score (FujiRebio); NP, confirmatory assay never became positive; REV, confirmatory assay using Reversion.

aborted antibody response (unpublished data, authors' experience). Bictegravir/emtricitabine/tenofovir alafenamide was not administered to the patients of the investigated study cohorts.

HIV POC testing is commonly used in resource-constrained and nonclinical settings. While they are easy to use and results are obtained faster than with other serology screening tests, some of the third-generation rapid tests fail to detect HIV-1 antibodies in early HIV infection [23, 28]. The impact of efficient ART was most clearly seen on the StatPak performance in both study groups. Possible inhibition caused by a particular antiretroviral drug was not found, but was only superficially investigated. There was no common factor and the same ART was taken at the time of a reactive and a nonreactive StatPak result. The second-worst-performing POC test was INSTI. In the seroconverters cohort, a nonreactive INSTI result was obtained in 8.4% (7/83) of the samples after an average of 3.3 years of treatment, which is perfectly in line with the 9.1% (4/44) INSTI nonreactivity after 3 years of suppressive ART reported by a French study group [18]. Time from infection to treatment initiation was statistically not linked with nonreactivity of the rapid tests, something that was also observed by the same French research.

The limitations of this retrospective study are the limited number of specimens with an indeterminate, negative, or nonreactive test result and the use of stored frozen specimens even though freeze-thaw cycles were kept to a minimum. Additionally, all 7 different HIV rapid tests were performed with 1 single production lot, impeding assessment of lot-to-lot variation.

Missed (treated) HIV infections by the current diagnostic tests might lead to life-threatening situations, for example, drug–drug interactions or blood/tissue donations to immunocompromised patients. It might also lead to AIDS status if the patient decides to stop all ART after a nonreactive self-test or rapid test, something that has already been observed at least once in Belgium (unpublished data), and such cases could contribute to HIV epidemiological expansion. Other markers to identify an established HIV infection in an easy, fast, and cost-effective way will be required in the upcoming years.

In conclusion, assays used to confirm an HIV infection and distinguish between a real and a false-reactive EIA HIV screening test were not 100% reliable for patients on ART. A false-negative test result was observed in at least 1 of the 3 tested assays (INNO-LIA, Geenius, and MP Blot 2.2) in 2.1% (8/390) of the adult patients with undetectable plasma viral load for at least 9 years and in 12.0% (10/83) of the seroconverters treated within 3 months. At the same time, at least 1 of the 7 rapid tests generated a nonreactive result in 4.9% (19/390) of the patients on long-term efficient ART and in 31.3% (26/83) of the seroconverters. Most nonreactive results were obtained with StatPak. Patient nondisclosure might have a significant clinical impact when entering care in regions where diagnosis is based on an algorithm of rapid tests. Future monitoring is necessary as most samples analyzed in this study were collected in 2016–2017 and new, highly effective ART molecules have become available since then.

Notes

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