



Acquisition of wild-type HIV-1 infection in a patient on pre-exposure prophylaxis with high intracellular concentrations of tenofovir diphosphate: a case report

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Summary

Background Pre-exposure prophylaxis (PrEP) with emtricitabine and tenofovir disoproxil fumarate is highly effective against acquisition of HIV infection, and only two cases of infection with a multidrug-resistant virus have been reported under adequate long-term adherence, as evidenced by tenofovir diphosphate concentrations in dried blood spots. We report a case of wild-type HIV-1 infection despite consistent use of emtricitabine and tenofovir disoproxil fumarate.

Methods The patient participated in the Amsterdam PrEP project, a demonstration project of daily and event-driven PrEP. We did extensive testing for HIV, including plasma HIV RNA and nested PCR on bulk peripheral blood mononuclear cells (PBMCs) and sigmoid biopsies after seroconversion.

Findings A 50-year-old man who has sex with men and had been on daily emtricitabine and tenofovir disoproxil fumarate for 8 months presented with fever, urinary tract infection caused by *Escherichia coli*, anal lymphogranuloma venereum infection, and a positive fourth-generation HIV test. We found an atypical seroconversion pattern, with initially only gp160 antibodies detected in the western blot. HIV RNA could not be detected in plasma, and nested PCR for HIV RNA and DNA on bulk PBMCs and sigmoid biopsies were negative. PrEP was discontinued; 3 weeks later HIV RNA was detected in plasma. No drug-resistant mutations were detected. Tenofovir diphosphate concentrations in dried blood spots were stable and high.

Interpretation To our knowledge, this is the first detailed case report suggesting wild-type HIV-1 infection despite good adherence, evidenced by repeatedly high concentrations of tenofovir diphosphate in dried blood spots. PrEP providers need to be aware that infection can occur despite good adherence. Regular HIV testing and awareness of atypical patterns of seroconversion is highly recommended.

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Introduction

Pre-exposure prophylaxis (PrEP) with emtricitabine and tenofovir disoproxil fumarate is highly effective against acquisition of HIV infection.¹⁻⁴ In PrEP users in whom infection has occurred, reliable evidence of long-term adherence is not available^{1,4-6} or transmission is attributed to insufficient adherence as was evidenced by low concentrations of antiretroviral drugs in bulk peripheral blood mononuclear cells (PBMCs) or dried blood spots.^{3,7} Worldwide, only two cases^{8,9} of PrEP failure with high tenofovir diphosphate concentrations in dried blood spots have been reported, which reflects long-term adherence. Both individuals were infected with a multidrug-resistant virus.^{8,9}

The Amsterdam PrEP project (AMPrEP) is a demonstration project that aims to show the acceptability and feasibility of daily and event-driven use of emtricitabine and tenofovir disoproxil fumarate for PrEP, including long-term adherence, among men who have

sex with men (MSM) and transgender people. The project started in August, 2015, at the Public Health Service of Amsterdam (Amsterdam, Netherlands) with approval from the ethics committee of the Academic Medical Center, Amsterdam, Netherlands; the protocol is available online and the trial is registered with the Netherlands Trial Register, number NTR5411.

PrEP is offered as part of a comprehensive HIV infection prevention programme in the AMPrEP project at the Sexually Transmitted Infection (STI) Clinic of the Public Health Service of Amsterdam. After written informed consent was obtained, we offered participants a choice between daily or event-driven PrEP according to the IPERGAY schedule: two tablets (245 mg tenofovir disoproxil fumarate and 200 mg emtricitabine per pill) between 24 h and 2 h before sex, followed by a third tablet 24 h after the first drug intake and a fourth tablet 24 h later.¹ At PrEP initiation, we tested HIV RNA in combined blood samples from six participants. We monitored

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For the **online protocol** see
<http://www.ggd.amsterdam.nl/infectieziekten/soa-hiv-sense/prep/amprep/>

Research in context

Evidence before this study

We searched PubMed and conference abstracts on April 29, 2017, for articles or abstracts in English, using the search terms “pre-exposure prophylaxis” OR “PrEP” AND “HIV” AND “infection” AND “seroconversion”. Earlier pre-exposure prophylaxis (PrEP) trials showed that breakthrough HIV-1 infections are a rare occurrence and have been reported in the absence of adequate measurements that show long-term adherence. Transmission in PrEP trials was attributed to insufficient adherence, as evidenced by concentrations of antiretroviral medication in bulk peripheral blood mononuclear cells (PBMCs) or dried blood spots. The two cases of PrEP failure that have been previously reported with high tenofovir diphosphate concentrations in dried blood spots, reflecting long-term adherence, were shown to be because of infection with a multidrug-resistant virus.

Added value of this study

In this report we illustrate a case of wild-type HIV-1 infection in a participant of the Amsterdam PrEP (AMPrEP) demonstration project, despite consistent use of daily PrEP, as was shown by high intracellular concentrations of tenofovir diphosphate in

dried blood spots. At seroconversion, while still on PrEP, HIV RNA was not detected in plasma; 12 days after stopping PrEP, PCR for HIV RNA and integrated HIV DNA on bulk PBMCs was negative. We also report an atypical seroconversion pattern, with initially only gp160 antibodies detected in the western blot. Regarding the mechanism of wild-type HIV acquisition, we hypothesise that frequent condomless anal sex with the related possibility of repeated exposure to HIV, repeated gut-localised sexually transmitted infections, and the pharmacokinetics of tenofovir diphosphate and emtricitabine triphosphate in rectal mucosa might all have contributed.

Implications of all the available evidence

HIV infection during consistent PrEP use is extremely rare, and PrEP is a highly effective intervention to prevent HIV infection in individuals who are at increased risk for acquiring HIV infection. However, our findings show that infection can occur even if long-term adherence is optimal, and emphasise the importance of regular HIV testing and awareness of atypical patterns of seroconversion in PrEP users.

participants after 1 month and subsequently every 3 months, which included testing for HIV and STIs. To measure tenofovir diphosphate concentrations we collected blood samples to generate dried blood spots every 3 months in the first year, followed by annual blood samples for the duration of the project (up to 3 years in total).

We report the case of an individual who participated in the AMPrEP project and acquired wild-type HIV-1 infection while having documented stable and high concentrations of tenofovir diphosphate in dried blood spots.

Case report

A 50-year-old white MSM started daily PrEP on Sept 23, 2015. He reported anal sex with 50 partners in the 3 months before enrolment, and had receptive anal sex without a condom with 37 of them. While on PrEP he reported condomless anal sex with between 12 and 75 partners per month (table 1), and he often was the receptive partner. At baseline (ie, on the day he started PrEP) he tested negative for HIV antibody and antigen, HIV RNA, and hepatitis C virus (HCV) RNA. During follow-up while on PrEP, pill counts and daily diary information indicated adherence to the use of seven pills per week. We diagnosed and treated several STIs (table 2). While on PrEP, he reported the use of drugs during sex, including amphetamine, cocaine, GHB or GBL, mephedrone, and ketamine. He injected ketamine twice, both times intramuscularly, but insisted clean injection equipment was used on both occasions. HIV antibody and antigen tests were done at months 1, 3,

and 6 after starting PrEP; the results were negative on all occasions.

On May 18, 2016, almost 8 months after starting PrEP, the patient was tested for HIV by a sexual health clinic elsewhere in the Netherlands because of symptoms of fever and dysuria; he had no symptoms of proctitis. On May 23, he was informed that the fourth-generation HIV antibody and antigen test was positive (table 2). Moreover, he had an anal lymphogranuloma venereum infection; no other STIs were diagnosed (table 2). *Escherichia coli* was cultured from his urine (table 2). The lymphogranuloma venereum and urinary tract infections were treated, and he was referred to the Public Health Service of Amsterdam for confirmation of the HIV test. On May 24, we confirmed HIV-1 seroconversion with the fourth-generation HIV antibody and antigen test, which was negative for antigen (p24) and positive for antibodies (table 2). The western blot showed an atypical pattern, characterised by the presence of antibodies detecting a single gp160 band (figure). We did a qualitative point-of-care HIV RNA test and real-time PCR with a lower limit of detection of 40 copies per mL, both were negative. We could not detect proviral HIV DNA in bulk PBMCs (in total 2.5×10^6 PBMCs) obtained on May 30, using a nested PCR that detected a conserved region in the *pol* gene.¹⁰ Furthermore, we did a sigmoidoscopy on June 6. In three 1–1.5 mm³ sigmoid biopsy samples taken during this procedure, we could not detect HIV DNA and HIV RNA in bulk mononuclear cells.¹⁰

Although we could not confirm the diagnosis of an acute HIV infection at this point, given the seroconversion

	Month 1	Month 2	Month 3	Month 4	Month 5	Month 6	Month 7	Month 8*
Anal sex partners†	75	56	56	50	38	49	66	12
Days condomless anal sex was reported per month†	21/31	12/30	13/31	15/31	15/29	19/31	17/30	3/20
Sex partners per day if condomless anal sex was reported†	3 (1–8)	5 (2–9)	4 (2–6)	4 (1–5)	2 (1–5)	3 (1–4)	5 (2–6)	5 (1–5)

Data are n and median (IQR). PrEP=pre-exposure prophylaxis. *Until May 24, 2016. †Data were collected from daily diary via application for mobile phone.

Table 1: Sexual risk behaviour of a PrEP user who seroconverted for HIV with high tenofovir diphosphate concentrations

	Sept 23, 2015	Oct 19, 2015	Dec 7, 2015	March 7, 2016	May 18, 2016	May 24, 2016	May 30, 2016	June 6, 2016	June 14, 2016	June 23, 2016	July 18, 2016
PrEP use	Start	Yes	Yes	Yes	Yes	Stop
Sexually transmitted infection diagnosed*	Anal chlamydia and gonorrhoea	Anal chlamydia and gonorrhoea	Anal lymphogranuloma venereum
Fourth-generation antibody and antigen test	Non-reactive	Non-reactive	Non-reactive	Non-reactive	Reactive†	Reactive†
Tenofovir diphosphate in dried blood spot (fmol per punch)	2234	..	2258
HIV RNA qualitative	Negative‡	ND	Negative§
HIV RNA quantitative¶	ND	<40 copies per mL	<40 copies per mL	..	12 882 copies per mL	101 156 copies per mL	..
Western blot	ND	gp120/160 positive	gp120/160 positive; p24 weak positive	gp120/160 positive; p24 positive; p17 positive
HIV cDNA in PBMCs	Negative
HIV cDNA in sigmoid biopsies	Negative
ART use	Start	Yes

PrEP=pre-exposure prophylaxis. ND=not done. PBMCs=peripheral blood mononuclear cells. ART=combination antiretroviral therapy. *Anal chlamydia and gonorrhoea were also diagnosed and treated in November, 2015. †Antibody positive and antigen negative. ‡Pooled HIV RNA using COBAS TaqScreen MPX version 2.0. §Analysed with the Xpert HIV-1 Qual test. ¶Analysed with the Abbott RealTime HIV-1 Viral Load Assay. ||No resistance mutations detected.

Table 2: HIV test results and tenofovir diphosphate concentrations in dried blood spot samples of a PrEP user who seroconverted for HIV with high tenofovir diphosphate concentrations

we strongly suspected an infection and therefore decided to interrupt PrEP on the day of confirmed seroconversion to minimise the chance of selecting for resistant virus, should our patient indeed have acquired HIV infection. After stopping PrEP, the patient did not report condomless anal sex. Plasma HIV RNA analysis was repeated 12 days and 27 days after the seroconversion date (figure). In a plasma sample taken at day 27 after seroconversion, HIV RNA was detected (12 882 copies per mL; table 2). When the HIV RNA test result became available, and pending the results of resistance testing, we immediately started combination antiretroviral therapy (emtricitabine 200 mg and tenofovir disoproxil fumarate 245 mg once a day, with ritonavir-boosted darunavir [darunavir 800 mg and ritonavir 100 mg] once a day, and dolutegravir 50 mg twice a day) on June 23, resulting in an undetectable plasma viral load after 1 month. He developed fever 2 days before starting combination antiretroviral therapy, presumably as a result of HIV viraemia.

Methods

Virological assays

Before the start of PrEP and during follow-up, we used the fourth-generation LIAISON XL test to test for HIV, which detects both HIV antigen and HIV antibodies (LIAISON XL Murex HIV Ag/Ab; Diasorin, Saluggia, Italy). Before the start of PrEP, we excluded the presence of HIV RNA in plasma using the COBAS TaqScreen MPX Test version 2.0 (Roche Diagnostics, Mannheim, Germany). At confirmed seroconversion, we did a qualitative point-of-care HIV RNA test (Xpert HIV-1 Qual test, lower limit of detection [LOD] of test: 50 copies per mL; Cepheid, Solna, Sweden), followed by a sensitive HIV RNA assay with an LOD of 40 copies per mL in plasma (Abbott RealTime HIV-1 Viral Load Assay; Abbott Laboratories, Lake Bluff, IL, USA).

We isolated total DNA from PBMCs with the QIAamp DNA Mini Kit (Qiagen, Hilden, Germany). Total DNA and RNA were isolated from sigmoid biopsies using the AllPrep DNA/RNA Mini Kit (Qiagen). We used the

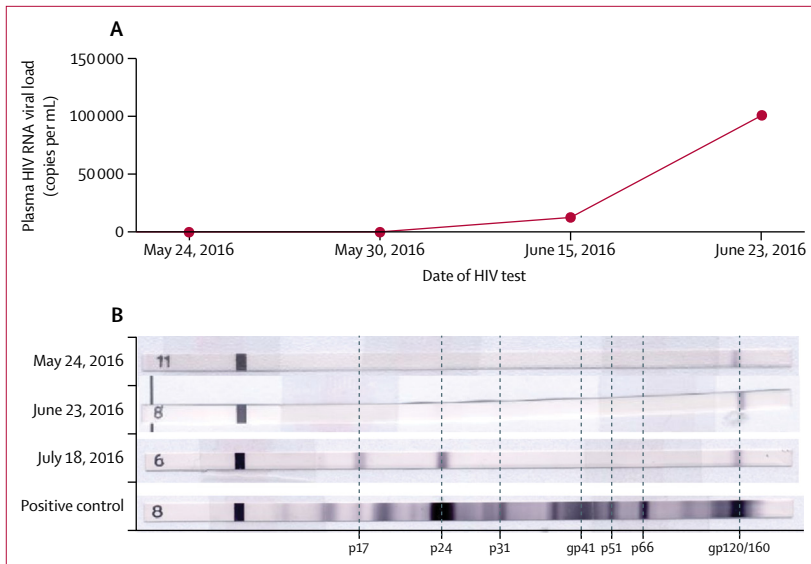


Figure: Results of HIV test (A) and sequential western blot (B) in a PrEP user who acquired wild-type HIV-1 infection while high intracellular tenofovir diphosphate concentrations were documented
PrEP=pre-exposure prophylaxis.

Transcriptor First Strand cDNA Synthesis Kit (Roche, Basel Switzerland) for cDNA synthesis of the total RNA. DNA and cDNA were subjected to a nested PCR, which amplified a conserved region in the HIV-1 polymerase (*pol*) gene with GoTaq DNA polymerase (Promega, Madison, WI, USA) and primer pairs: outer primer sets were POL-F (5'TTAGTCAGTGCTGGAATCAGG3', HIV-1 genome [HXB2] positions 4199–4219) and POL-D (5'CCA-CTGGCTACATGAACTGCTAC3', HXB2 positions 4473–4450), and inner primer sets were POL-E (5'GAT-TTTAACCTGCCACCTGTAGTAGC3', HXB2 positions 4302–4327) and POL-B (5'ATGTGTACAATCTAGTTGCC3', HXB2 positions 4429–4410).¹⁰

For confirmation of HIV antibodies, we used the HIV blot 2.2 western blot assay (MP Biomedicals, Geneva, Switzerland). For interpretation of antibody patterns, we defined patterns as negative (no reactivity to HIV proteins detectable), positive (reactivity to one or more gag, one or more pol, and one or more env proteins detectable), or indeterminate (any other pattern of reactivity).

See Online for appendix

To analyse resistance mutation, we did standard HIV-1 genotyping (appendix p 1) and subtyping; mutations associated with drug resistance were identified on the first sample with detectable HIV RNA, according to the International AIDS Society-USA drug resistance mutations in HIV-1.¹¹ The standard method is based on population sequencing, and minor mutations can be detected with a threshold of 20%. To increase this sensitivity, we cloned the RT-PCR product with the TOPO TA Cloning Kit (Thermo Fisher Scientific, Waltham, MA, USA) and we sequenced 48 colonies with the Big-Dye Terminator Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA), resulting in detection of minor variants with a threshold of around 2%.

We isolated PBMCs from 21 mL of whole blood collected 12 days after seroconversion, according to a standard protocol with density-gradient centrifugation, and cryopreserved the sample in liquid nitrogen until further use.

For the proliferation assay, we labelled PBMCs with the CellTrace Violet Proliferation Kit (CTV; Thermo Fisher Scientific) and resuspended them in RPMI supplemented with 10% human pooled serum and 100 U/mL penicillin and 100 µg/mL streptomycin (Thermo Fisher Scientific). Cells were stimulated with HIV-1 consensus B gag Peptide Pool (2 µg/mL; National Institute of Health [NIH] AIDS Reagent Program, Division of AIDS, National Institute of Allergy and Infectious Diseases, NIH, Rockville, MD, USA; catalogue number 12425) and cultured for 6 days at 37°C and 5% CO₂. As positive controls, we stimulated CTV-labelled cells with αCD3 (0.2 µg/mL) and αCD28 (2 µg/mL) or HCMV pp65 Peptide Pool (2 µg/mL; NIH AIDS Reagent Program; catalogue number 11549). We stained cells for 30 min at 4°C in the dark, with directly conjugated monoclonal antibodies: CD3 FITC, CD4 PerCP Cy5.5, and CD8 PE-Cy7 (BD Biosciences, Breda, Netherlands), and determined the fraction of proliferating CD4 and CD8 cells.

We measured tenofovir diphosphate concentrations in red blood cells in dried blood spots, obtained at regular intervals during PrEP use and at 6 days after seroconversion, with a previously described liquid chromatography-tandem mass spectrometry method.¹²

HCV testing

We tested for HCV RNA with COBAS Taqscreen MPX Test version 2.0 before the start of PrEP and 6 days after HIV-1 seroconversion, and with COBAS AmpliPrep/COBAS TaqMan HCV-test (Roche Diagnostics, Pleasanton, CA, USA) at 3 months after HIV-1 seroconversion.

Role of the funding source

The funders of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

Tenofovir diphosphate concentrations in dried blood spots were 2234 fmol per punch at 6 months after the start of PrEP and 2258 fmol per punch at seroconversion, indicative of consistent adherence between the visits, and consistent dosing in the 4–8 weeks before the dried blood spots sampling. The mean concentration associated with daily dosing is 1560 fmol per punch (SD 468).¹³ The HIV subtype was B. Using routine sequencing, we could not detect mutations in reverse transcriptase gene analysis associated with resistance against tenofovir disoproxil fumarate, emtricitabine, or any other antiretroviral

agents. Furthermore, no mutations were observed in the 48 clonal sequences. These findings endorse the conclusion of infection with a wild-type virus. Because no viral load could be detected at seroconversion, we sought for HIV-induced immune responses. We investigated the presence of HIV-1 gag-specific T cells (CD4 and CD8) because gag is highly conserved and has a presumed high concentration in virions. We observed a small expansion of CD4 cells (3.55% of proliferating cells) and CD8 cells (2.77% of proliferating cells) in the gag-stimulated sample compared with the RPMI medium control (1.19% of proliferating cells). These findings suggest the presence of an HIV-induced T-cell response at the moment of seroconversion (appendix p 2).

HCV RNA was negative in samples taken at the start of PrEP, and 6 days and 3 months after seroconversion.

Discussion

We describe acquisition of infection with wild-type HIV-1 that occurred while on daily PrEP in an MSM who reported frequent condomless anal sex, indicating possibly repeated exposure to HIV. Another PrEP study² showed that HIV-1 infection is a rare occurrence. Our case differs from reports of HIV-1 infections in previous PrEP studies by the documentation of long-term adherence, as evidenced by repeated high tenofovir diphosphate concentrations in dried blood spots^{5,6} and from the two HIV-1 infections in fully adherent PrEP users by the absence of drug-resistant mutations.^{8,9} Although periods of non-compliance were possible, we believe this was unlikely to have happened because the dried blood spots concentrations were stable over time and high, suggesting consistent adherence.

PrEP efficacy is high if taken regularly: in a subgroup analysis among PrEP users of tenofovir diphosphate concentrations in dried blood spots compatible with the use of four to seven tablets per week, the hazard ratio for HIV infection was 0.00 (95% CI 0.00–0.17).⁷ The underlying mechanism of HIV-1 acquisition in our case remains speculative. Perhaps the sheer volume of viral inoculum coupled with the amount of mucosal injury related to sexual activity and STIs led to repetitive localised infection in the gut, and overwhelmed the effect of PrEP.^{14,15} Variable pharmacokinetics of emtricitabine and tenofovir disoproxil fumarate in rectal mucosa might also have contributed. Although we cannot exclude penile acquisition of infection, our patient reported that when he had sex, it was often with several partners on the same day, often he was the receptive partner, and most sex acts were condomless. Moreover, he reported injecting and non-injecting drug use during sex, which is associated with high-risk condomless anal sex and acquisition of STIs among HIV-positive MSM.^{16,17} Indeed, we documented several anal STIs in this patient while on PrEP, and around the time of seroconversion we diagnosed lymphogranuloma venereum infection, an ulcerative STI that is associated

with infections such as hepatitis B, hepatitis C, and HIV.¹⁸ Additionally, tenofovir diphosphate and emtricitabine triphosphate concentrations in rectal mucosa are presumably important determinants of protection against HIV infection in MSM. Although tenofovir diphosphate reaches higher concentrations in rectal mucosal mononuclear cells than in PBMCs, emtricitabine triphosphate concentrations can be lower in rectal mucosal cells.^{19,20} Moreover, two MSM acquired HIV infection while taking tenofovir disoproxil fumarate alone for treatment of hepatitis B virus infection,²¹ indicating that this drug does not offer full protection against HIV infection. The observation that plasma viraemia became detectable after PrEP withdrawal might be suggestive of a mucosally contained infection that had not disseminated systemically. This notion would support the hypothesis of insufficient tenofovir diphosphate concentrations rectally, allowing an initial localised and disseminating infection after withdrawal of emtricitabine and tenofovir disoproxil fumarate. We could not show HIV DNA or HIV RNA in gut biopsies, but the small number of biopsies might have led to a sampling error. Finally, in non-human primates, breakthrough infections during adequate PrEP have been documented.^{22,23} We deemed HIV-1 acquisition less likely to be through injecting drugs while on PrEP, as the participant reported the use of clean injecting equipment for intramuscular injection on two occasions. Moreover, in a randomised controlled trial among injecting drug users, the efficacy of PrEP to prevent infection was similar to that in MSM.^{3,24}

Diagnosis of HIV infection was not straightforward in our case because results were atypical for acute infection. Diagnosis of acute HIV-1 infection is usually based on the sequential appearance of diagnostic tests: viral RNA measured by PCR, p24 and p31 viral antigens measured by ELISA, HIV-1-specific antibody detected by ELISA, and finally HIV-1-specific antibodies detected by western blot.²⁵ Studies in human beings and non-human primates showed that during PrEP use, without actual infection, HIV-specific T-cell responses can develop in peripheral blood and HIV-specific antibody responses can be induced.^{23,26,27} Therefore, the observation in our patient that seroconversion and induction of HIV-specific T cells occurred without viraemia might reflect infection with viral load suppressed by emtricitabine and tenofovir disoproxil fumarate, but alternatively, the antibody and T-cell responses might reflect early immune priming by multiple HIV-1 exposures without overt infection, with full blown infection only occurring after PrEP is stopped. The 3 week interval between stopping of PrEP and detection of plasma viraemia provides support for this hypothesis. Finally, the patient could have acquired HIV after stopping PrEP; however, as he repeatedly denied having condomless anal sex and injecting drug use after stopping PrEP, we think this is a less likely scenario.

A western blot typically shows a sequential appearance of antibodies during natural infection, starting with the

appearance of antibodies directed against p17 and p24. In the western blot of our patient we initially detected only gp160-specific antibodies, which normally appear late in the course of acute infection. This observation could be explained by the possibility that the gp160 antibodies detected were in fact reacting to a multimer of gp41 (a transmembrane proximal region of the virus). This multimer appears very early during acute infection, around a week after plasma viraemia, and exceeding 100 copies per mL.^{28,29} Importantly, our findings illustrate the possibility that people who acquire HIV while on PrEP might show atypical patterns of seroconversion.

We decided to stop PrEP at diagnosis of seroconversion for fear that we would select for resistance in the case of a true breakthrough infection, which we could not formally confirm at that time. One might argue that if we had continued PrEP or had started combination antiretroviral therapy immediately, infection could have been aborted, and with the knowledge gained from this case, one might consider this in future comparable cases. However, with such a scenario it is possible that viraemia would never be detected, and structured treatment interruption of combination antiretroviral therapy might be indicated as the only way to prove the presence of infection before deciding to expose a person to lifelong treatment.

Taken together, this report emphasises the importance of regular HIV testing of PrEP users and promotes awareness of atypical patterns of seroconversion. Provision of PrEP should be closely monitored by trained health-care providers.

Contributors

EH, HJcDv, and MP participated in the conception and design of the AMPREP study. EH, RCAA, LRW, and GJdB cared for the patient. EH, GJdB, RCAA, MP, HJcDv, LRW, MC, SJ, NAK, PLA, PR, and JMP acquired, analysed, and interpreted the data. EH, GJdB, and JMP drafted the manuscript. All authors critically revised the manuscript.

Declaration of interests

EH's institution received financial reimbursement for time spent serving on advisory boards of Gilead Sciences. MP received independent research support and speakers fees from Gilead Sciences; AbbVie; Merck, Sharp, and Dohme; and Roche, paid to her institution. PR received independent scientific grant support from Gilead Sciences, Janssen Pharmaceuticals Inc, Merck & Co, Bristol-Myers Squibb, and ViiV Healthcare through his institution; he has served on a scientific advisory board for Gilead Sciences and a data safety monitoring committee for Janssen Pharmaceuticals Inc; and he chaired a scientific symposium by ViiV Healthcare, for which his institution received remuneration. PLA received research funding from Gilead Sciences, paid to his institution. GJdB received speakers fees from Gilead Sciences and independent research support from ViiV Healthcare, paid through her institution. All other authors declare no competing interests.

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