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THE AUTHORS REPLY: Gerhard raises an important question. Iron overload and advanced liver disease have been associated with *V. vulnificus* infection, specifically with septicemia,[1] and experimental models support enhanced vibrio replication in iron-rich environments.[2] Isolated cases of hemochromatosis diagnosis after *V. vulnificus* wound infection have been reported.[3] However, we are aware of no epidemiologic studies of *V. vulnificus* wound infection and iron overload. Given the recognized association of *V. vulnificus* infection with liver disease, which is often coincident with iron overload, we agree that it is prudent for patients with liver disease or iron overload to take precautions with shellfish exposure. In the absence of other indicators of hemochromatosis, the usefulness of screening patients with *V. vulnificus* infection for this diagnosis remains uncertain.

In our patient, iron studies revealed a depressed serum iron level of 16 μg per deciliter (normal range, 40 to 159) and a calculated transferrin saturation of 7% (normal range, 25 to 45). These findings were consistent with hypoferremia induced by acute inflammation,[4] and the values subsequently normalized.

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Since publication of their article, the authors report no further potential conflict of interest.

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Multidrug-Resistant HIV-1 Infection despite Preexposure Prophylaxis

TO THE EDITOR: Preexposure prophylaxis with emtricitabine (FTC)–tenofovir disoproxil fumarate (TDF) has been shown to be efficacious in preventing human immunodeficiency virus type 1 (HIV-1) infection in men who have sex with men and in whom adherence to treatment is high, as measured by levels of tenofovir diphosphate (TFV-DP) in dried blood spots.[1] We describe a case of HIV-1 infection despite FTC-TDF–based preexposure prophylaxis.[2]

A 43-year-old man in Toronto who reported having sex with men began to receive oral daily FTC-TDF in April 2013 and had seven nonreactive fourth-generation HIV screening tests over the next 21 months. Pharmacy dispensation records provided support for his report of "perfect" adherence to preexposure prophylaxis over 24 months.

On day 0, the initial result of a combination assay to detect antibodies to HIV types 1 and 2 was antigen/antibody-reactive, p24 antigen–reactive, and negative on Western blot testing. This test was followed by screen-positive, p24 antigen–negative, Western blot–negative results 7 days later. Since the level of p24 antigen peaks 3 to 4 weeks after infection and becomes nonreactive at 5 to 6 weeks, this change provided support for the clinical suspicion of HIV acquisition during the patient's reported receptive anal sex with multiple partners without the use of condoms 2 to 6 weeks before day 0.[3] In addition, the infection date estimated by means of viral deep-sequencing analysis (BEAST software, version v1.8.3) was within the exposure period. Details are provided in the Supplementary Appendix, available with the full text of this letter at NEJM.org.

Liquid chromatographic–tandem mass spectrometric analysis of a plasma sample obtained on day 0 revealed a tenofovir concentration of 152 ng per milliliter; this finding was consistent with recent administration of the drug. Dried blood spots were obtained on day 24 to assess long-term adherence, at which time the expected TFV-DP concentration (±SD) would have been 722±217 fmol or more per dried blood–spot punch had the patient not been receiving FTC-TDF before learning of his infection status on day 4. Steady-state TFV-DP concentrations after 8 weeks of daily FTC-TDF administration are usually 1560±468 fmol per punch.[4] The observed TFV-DP concentration, 2297 fmol per punch, was consistent with long-term adherence.

Genotypic and phenotypic testing of a plasma

Table 1. Results of Genotypic and Phenotypic Drug-Resistance Testing of the Patient's Plasma Sample on Day 7.*

Drug Class and Drug	Drug Resistance on Genotypic Testing	Relative Drug Susceptibility on Phenotypic Testing
Nucleoside or nucleotide reverse-transcriptase inhibitors		
Abacavir	Intermediate	Susceptible (3.9 × IC_{50})
Lamivudine	High	Resistant (more than maximum IC_{50})
Emtricitabine	High	Resistant (more than maximum IC_{50})
Tenofovir	Low	Sensitive (0.6 × IC_{50})
Nonnucleoside reverse-transcriptase inhibitors		
Efavirenz	Intermediate	Sensitive (0.56 × IC_{50})
Etravirine	Intermediate	Sensitive (0.19 × IC_{50})
Nevirapine	High	Resistant (19 × IC_{50})
Rilpivirine	Intermediate	Sensitive (0.53 × IC_{50})
Protease inhibitors: all agents	Susceptible	Susceptible
Integrase strand-transfer inhibitors		
Raltegravir	Intermediate	Reduced response (9.6 × IC_{50})
Elvitegravir	High	Resistant (>100 × IC_{50})
Dolutegravir	Low	Reduced response (2.7 × IC_{50})

* Genotypic results are from standard consensus sequencing and independent Illumina MiSeq "deep" sequencing on human immunodeficiency virus type 1 (HIV-1) protease, reverse-transcriptase, integrase, glycoprotein 41, and V3 regions, with interpretations from the Stanford University HIV Drug Resistance database. Phenotypic drug-resistance testing was performed with the use of the PhenoSense (Monogram Biosciences) assay for the HIV-1 protease and reverse-transcriptase regions and an in-house recombinant phenotype assay (British Columbia Centre for Excellence in HIV/AIDS) for integrase. Mutations included the following: nucleoside or nucleotide reverse-transcriptase inhibitors: 41L, 67G, 69D, 70R, 184V, and 215E; nonnucleoside reverse-transcriptase inhibitors: 181C; protease inhibitors: 10I; and integrase strand-transfer inhibitors: 51Y and 92Q. IC_{50} denotes 50% inhibitory concentration.

sample obtained on day 7 revealed multidrug resistance (Table 1). The M184V mutation, which compromises FTC activity, in addition to several thymidine analogue mutations, revertant substitutions, or both, which slightly decrease TDF susceptibility, probably explain this failure of preexposure prophylaxis. The multiple thymidine analogue mutations detected are unlikely to have been selected in the short duration of drug exposure; this suggests that resistance was transmitted rather than acquired after drug exposure.[5]

Although data from Toronto are not available, in British Columbia, the proportion of patients with a plasma sample containing circulating virus that was resistant to FTC, TDF, or both was 1.7%, 0.004%, and 0.001%, respectively, in 2014–2015. Continued surveillance of mutations that may affect the efficacy of preexposure prophylaxis is needed.

Incident HIV is possible despite adherence to preexposure prophylaxis when persons are exposed to FTC-resistant virus, TDF-resistant virus, or both. We recommend that patients be counseled regarding the use of preexposure prophylaxis as part of a combination approach to HIV prevention.

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Disclosure forms provided by the authors are available with the full text of this letter at NEJM.org.

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Supplementary Appendix

This appendix has been provided by the authors to give readers additional information about their work.

Supplement to: Knox DC, Anderson PL, Harrigan PR, Tan DHS. Multidrug-resistant HIV-1 infection despite pre-exposure prophylaxis. N Engl J Med 2017;376:501-2. DOI: 10.1056/NEJMc1611639

Multi-Drug-Resistant HIV-1 Infection Despite Pre-Exposure Prophylaxis

Knox DC, Anderson PL, Harrigan PR, Tan DHS

NEJM Online Supplement

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NEJM Online Supplement

1. Clinical Course

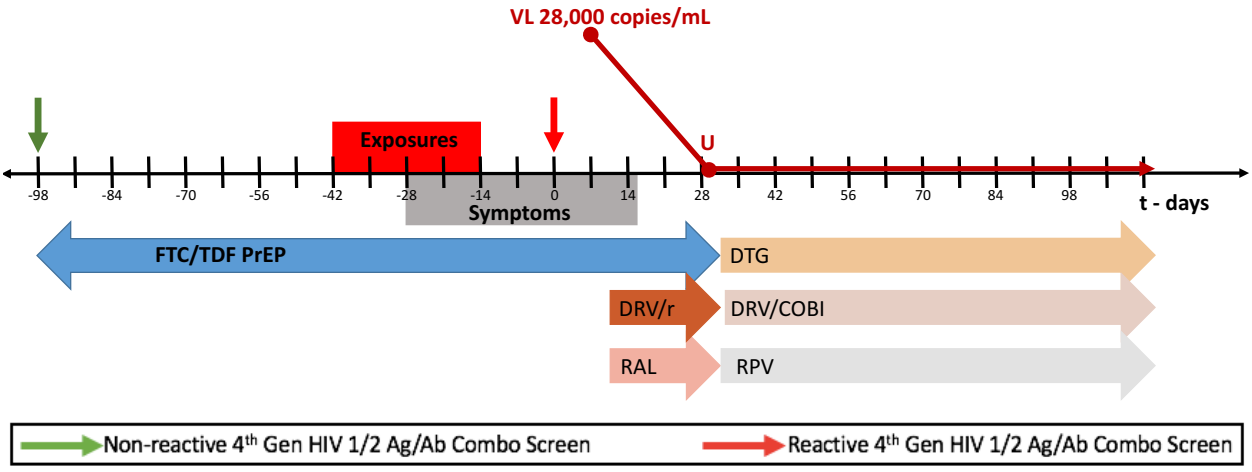
The patient initiated oral daily FTC/TDF in April 2013 nine weeks after a non-reactive 4th generation HIV 1/2 antigen/antibody (Ag/Ab) combination test and after three months of sexual abstinence. During routine follow up, there were seven non-reactive HIV screening tests at 1, 3, 7, 11, 15, 18 and 21 months after FTC/TDF initiation. Twenty-four months after starting daily FTC/TDF, a routine 4th generation HIV 1/2 Ag/Ab combination screen was reactive, HIV-1 p24 antigen reactive (7.6 pmol/L), HIV-1 p24 Ag confirmatory reactive, and HIV-1 Western Blot negative (figure S1). Urine nucleic acid amplification testing, pharyngeal cultures and rectal cultures for gonorrhea and chlamydia, as well as serologies for syphilis and viral hepatitides ruled out concomitant sexually transmitted infections. The patient did not have classic symptoms of acute HIV seroconversion, but did report severe epigastric discomfort in the month prior to day 0. On day 15, the patient again experienced an episode of severe epigastric pain and had a temperature of 38.2°C. Computed tomography revealed thickening of the sigmoid colon, ascending colon and rectum. Bi-directional endoscopy revealed erythematous patches of the sigmoid colon but was otherwise normal, and biopsies were noncontributory. All symptoms resolved spontaneously.

When informed of the day 0 HIV serology results on day 4, the patient refused to discontinue FTC/TDF while further investigations were pending, suspecting a false positive test and citing his uninterrupted adherence. Therefore, darunavir 600mg twice daily, ritonavir 100mg twice daily and raltegravir 400mg twice daily were added to his daily FTC/TDF due to concern about emergent HIV drug resistance.

On day 7, the CD4 count was 710 cells/mm3 (31.2%) and HIV viral load was 28,000 copies/mL (4.45 $\log_{10}$ copies/mL). After 21 days of combination therapy, the viral load became undetectable (target not detected; Figure S1). One week later when genotyping data became available, the drug regimen was simplified to once daily darunavir/cobicistat 800/150mg, rilpivirine 25mg and dolutegravir 50mg. HIV-1 RNA has

since remained undetectable.

Figure S1. Clinical course in days. *DRV/r darunavir/ritonavir. RAL raltegravir. DTG dolutegravir. DRV/COBI darunavir/cobicistat. RPV rilpivirine. VL HIV-1 viral load. U undetectable viral load.*



2. Estimated infection date

We estimated the HIV infection date, time to the most recent common ancestors (TMRCAs) of HIV envelope lineages present within this individual, in a coalescent framework under a relaxed molecular clock as implemented in BEAST v1.8.3[1]. This approach incorporates uncertainty in estimates of the phylogeny, lineage specific variance in substitution rates and the possibility of alternate demographic histories[2].

The mean and highest probability density (HPD confidence limits) of infection date based on the best-fit model suggests that this patient acquired HIV on approximately April 21, 2015, but with a broad confidence interval (95% HPD: March 6, 2015 – May 3, 2015). The mean estimate of the infection date (April 21, 2015), estimated with BEAST while blinded to the infection status of the patient, falls directly within the period the patient disclosed as the exposure period.

The broad confidence limits around this mean estimate are expected when estimating times on the order of days with short sequence lengths, following recent infection[3].

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