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Title: Zika virus infection and differential diagnosis in a cohort of HIV-infected patients

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Running head: Zika virus infection in HIV-infected patients

Abstract

Background

Zika virus (ZIKV) emergence in South America revealed the lack of knowledge regarding clinical manifestations in HIV-infected individuals. Objectives: We described the clinical characteristics, laboratory manifestations, differential diagnosis, and outcome of ZIKV infection in a large, single-center cohort of HIV-infected patients.

Methods

HIV-infected patients aged ≥ 18 years with clinical suspected arboviral disease from an ongoing cohort were followed from February through December 2015. Acute serum samples were tested for ZIKV, DENV, and CHIKV by rRT-PCR, anti-DENV IgM/IgG, and syphilis assays; convalescent samples were tested for anti-DENV IgM/IgG; and urine samples were tested for ZIKV by rRT-PCR. ZIKV disease was defined according to the PAHO guidelines.

Results

Of 101 patients, ZIKV was confirmed in 43 cases and suspected in 34, and another diagnosis was assumed for 24 patients (dengue, secondary/latent syphilis, respiratory infections, human parvovirus B19, adverse drug reaction, musculoskeletal disorders, and acute gastroenteritis). ZIKV-confirmed and suspected patients reported similar signs and symptoms. Pruritic rash was the most common symptom, followed by myalgia, nonpurulent conjunctivitis, arthralgia, prostration, and headache. In the short-term follow-up [median 67.5 days (IQR: 32–104.5)], CD4 cell count ($Z = -.831$, $p = 0.406$) and HIV viral load ($Z = -.447$, $p = 0.655$) did not change significantly post ZIKV infection. There were no hospitalizations, complications, or deaths.

Conclusions

Among HIV-infected patients with suspected arboviral disease, 42.6% were ZIKV-infected. CD4 cell counts and HIV viral load were not different post ZIKV infection. Differential diagnosis with other diseases and adverse drug reaction should be evaluated.

Keywords: Zika; ZIKV; Arboviruses; HIV; Brazil

Introduction

The association between HIV infection and endemic diseases, especially in tropical regions, has been described. [1,2] Some coinfections can modify the clinical course of both HIV infection and the associated disease, worsening their clinical conditions as observed in patients with leishmaniasis, Chagas disease, and malaria. [3-9] HIV and dengue virus (DENV) are often detected together in tropical areas. [10,11] Small series and case reports revealed that coinfected patients are not at an increased risk of severe DENV or accelerated HIV progression. [12-16] However, Pang et al., based on the World Health Organization 2009 classification criteria in a matched case-control study, suggested that patients with DENV/HIV may more likely develop a severe DENV outcome. [17]

Zika virus (ZIKV) is an arbovirus that has emerged as a new public health threat and caused a large outbreak in Brazil and Latin America in 2015–2016 with increased reports of a ZIKV infection-associated congenital syndrome, Guillain-Barré syndrome and other neurological disorders. [18] To date, 48 countries and territories in the Americas have confirmed autochthonous, vector-borne transmission of ZIKV disease. [19]

Information is lacking on whether concurrent HIV with ZIKV infection can affect clinical and laboratory manifestations, the severity of ZIKV disease, and increase in atypical presentations and HIV disease progression. Calvet et al. reported a detailed case with good response to combination antiretroviral therapy (cART) and high CD4+ T-cell count. The patient had mild symptoms, no major laboratory abnormalities, and recovered completely from ZIKV infection. [20] This study aimed to describe the clinical characteristics, laboratory manifestations, differential diagnosis, and outcome of ZIKV infection in a large, single-center cohort of HIV-infected patients in Rio de Janeiro, Brazil.

Methods

Study Design

The study was conducted at the Acute Febrile Illnesses Laboratory (AFIL) of the Evandro Chagas National Institute of Infectious Diseases, Oswaldo Cruz Foundation, from February 1 to December 31, 2015. AFIL is a reference center in Rio de Janeiro, Brazil, for patients with acute febrile diseases. The participants included in this analysis are part of the Institute HIV/AIDS cohort that has been established in 1998 as described elsewhere. [21] HIV-infected patients aged ≥ 18 years, with clinical suspected arboviral disease (i.e., fever, headache, arthralgia, myalgia, prostration, gastrointestinal symptoms, conjunctivitis, or rash) were referred to the AFIL. Patients were interviewed using a standardized case report form to collect demographical information, clinical presentation, and laboratory test results. Blood samples were collected during the acute phase (within 7 days after the onset of symptoms) and during the convalescent phase (14 to 21 days after initial assessment).

Study definitions

Socio-demographic, lifestyle, health, and HIV/AIDS-related factors

Race/ethnicity, schooling, alcohol consumption, cigarette smoking were self-reported on the day of AFIL assessment. HIV diagnosis, date and types of cART exposure, AIDS-defining illnesses (ADI), CD4+ T-cell counts, and HIV viral load (VL) were extracted from the patient's chart. CD4+ T-cell counts were categorized as < 200, 200–499, and ≥ 500 cells/mm^3. HIV RNA VL was $\log_{10}$-transformed and measured in $\log_{10}$ copies/mL and categorized into < 40, 41–1,000, 1,001–10,000, and > 10,000 copies/mL. Plasma HIV VL and CD4+ T-cell counts were considered in the analysis if determined within 4 months before and up to 4 months after an arboviral infection investigation.

Laboratory analysis and confirmation

Real-time reverse transcriptase polymerase chain reaction (rRT-PCR) assays for ZIKV were performed in the acute serum samples with the QuantiTect Probe RT-PCR kit (QIAGEN), as described previously [22]. Viral RNA was extracted from 140 μL of human serum specimens with the QIAamp Viral RNA Mini Kit (QIAGEN, Valencia, CA, USA) in accordance with the manufacturer's suggested protocol. Acute serum samples were also tested by RT-PCR for DENV [23] and CHIKV [24]. rRT-PCR for ZIKV was performed on acute and convalescent urine specimens according to their availibility. All acute and convalescent serum samples were tested for anti-DENV IgM and IgG as described by the manufacturer *(*PanBio, Brisbane, Australia). Acute serum samples were analyzed for syphilis using VDRL (Labtest Diagnostica, Minas Gerais, Brazil) and treponemal assays (Imuno-ELISA Anti-*Treponema pallidum* from Wama diagnostica, Sao Paulo, Brazil). CD4+ T-cell counts of samples were evaluated on a BD

FACSCalibur cytometer (Becton Dickinson, USA) and Abbott Real Time HIV-1 assay was used for measurement of HIV-1 VL (lower limit of detection, 40 copies/mL). Pregnancy test was not performed during the acute phase of the disease, but a chart review was carried out later to assess whether any woman with reproductive potential became pregnant at follow-up.

Case definition: ZIKV disease was defined according to the latest guidelines of the Pan American Health Organization (PAHO), 2016. [25] Suspected case: the presence of exanthema and at least two of the following signs and symptoms: low-grade fever (temperature < 38.5°C), conjunctivitis, arthralgia, myalgia, or swollen joints. A confirmed case of ZIKV disease was defined as a patient who met the criteria for a suspected case with laboratory confirmation of recent ZIKV infection. This study assessed only detectable RNA of ZIKV in serum and/or urine by rRT-PCR. ZIKV serologies and plaque reduction neutralization test (PRNT) were not performed in this study, thus probable case of Zika virus disease could not be stablished. Another diagnosis was considered if the patient had other clinical or laboratory-confirmed diagnosis.

The investigations were performed as part of the ongoing study on "Detection of unusual clinical presentations of arboviruses", which was reviewed and approved by the local Ethics Committee (CAAE 0026.0.009.000-07). Written informed consent was obtained from all the patients.

Statistical analysis

Median [interquartile range (IQR)] and frequency (%) were used to describe the patient's characteristics for continuous and categorical data, respectively. The median changes in CD4+ T-cell counts and HIV-1 RNA VL measurements were compared using the Wilcoxon test in patients with detectable and undetectable ZIKV before and

up to 4 months after ZIKV infection diagnosis. Categorical variables were compared using a chi-squared test or Fisher's exact test as appropriate. Statistical analyses were performed using the Statistical Packages for Social Sciences, version 17.

Results

A total of 101 HIV-infected dengue-, Zika-, or chikungunya-suspected patients were assessed between February and December 2015. The median age was 41.8 years (IQR: 33.5–49.8). Most of the patients were males (58.4%), white (51.5%), and with more than nine years of schooling (67.3%). Regular alcohol use was reported by 39.6% and only 17.8% had reported current cigarette smoking. The median known HIV infection status was 8.7 years (IQR: 4.9–12.8) and AIDS-defining illnesses occurred in 58.4% patients. CD4+ cell counts and HIV VL data were collected from 71.3% patients with a median time of 54 days (IQR: 36.3–72.8) before the arboviral syndromic investigation. The median CD4+ cell count was 652.5 cells/mm^3 (IQR: 439.8-859.0) and 79.2% patients had undetectable VL. Lifetime cART exposure was reported by 98.5% patients for a median time of 6.1 years (IQR: 3.0–9.0). All but two patients were on cART. The most frequently used cART were lamivudine+tenofovir+efavirenz (31.6%), lamivudine+tenofovir+atazanavir+ritonavir (16.8%), and lamivudine+tenofovir+lopinavir+ritonavir (12.9%). Study population characteristics are given in Table 1.

Among the 101 enrolled patients, 43 (42.6%) had confirmed ZIKV infection, assessed by rRT-PCR assay as detectable RNA in the serum (n = 28), urine (n = 12), or both (n = 3). The median threshold cycles (Ct) for serum and urine specimens was 33.4 (IQR: 29.4–35.4) and 32.7 (IQR: 30.0–34.6), respectively.

The median time of ZIKV detection by rRT-PCR was two days (IQR: 1–3 days) in serum and three days (IQR: 2–5 days) in urine in these confirmed cases. Suspected

ZIKV infection according to PAHO guidelines was observed in 34 patients with negative rRT-PCR. The median time of serum collection in these patients was three days (IQR: 2–5 days).

The rRT-PCR for DENV was positive for two patients (DENV-1 and DENV-4); both were negative for ZIKV RNA. All samples from the 101 patients were negative for CHIKV RNA. Seven patients had secondary/latent syphilis—one patient had confirmed ZIKV infection, two had suspected ZIKV infection, and four had only this diagnosis. Past syphilis infection was detected in 25.7% of the cohort. The other diagnosed diseases were upper respiratory tract infections (n = 8), pneumonia (n = 3), human parvovirus B19 (n = 2), adverse drug reaction (n = 2), musculoskeletal disorders (n = 2), and acute gastroenteritis (n = 1). None of the 101 patients were hospitalized, developed complications, or died. None of the women reported pregnancy at the first interview or during the follow-up.

In the 43 patients with positive rRT-PCR for ZIKV, the most commonly reported signs and symptoms were rash (macular or maculopapular) (93.0%), pruritis (83.7%), myalgia (69.8%), nonpurulent conjunctivitis (65.1%), arthralgia (62.8%) associated with joint swelling in 12 of 27 patients (44.4%), prostration (58.1%), and headache (55.8%). Reported fever or measured body temperature ≥ 37.5°C were recorded in 51.2% patients. Similar frequencies of these signs and symptoms were observed in the negative rRT-PCR for ZIKV suspected group. (Table 2).

In the short-term follow-up [median 67.5 days (IQR: 32–104.5)], no significant change in CD4+ cell count ($Z = -.831$, $p = 0.406$), which was 670.5 cells/mm^3 (IQR: 423.0–941.5 cells/mm^3) and 644.5 cells/mm^3 (IQR: 506.3–889.5 cells/mm^3) pre and post ZIKV infection, respectively, was noted as per the Wilcoxon signed-rank test. No statistical difference was observed in HIV VL at baseline and post ZIKV infection (both

undetectable) ($Z = -.447$, $p = 0.655$). Furthermore, when the 34 patients with negative rRT-PCR for ZIKV were included in this analysis, there were no statistical difference observed in the CD4+ cell count and HIV VL pre and post ZIKV infection.

Patients showing negative rRT-PCR for ZIKV with other diagnosis had less rash ($p = 0.001$), nonpurulent conjunctivitis ($p = 0.005$), pruritis ($p < 0.001$), periarticular edema ($p = 0.023$), more chills ($p = 0.025$), and more cough ($p = 0.001$) when compared to rRT-PCR confirmed ZIKV patients. Laboratory characteristics of the patients are given in Table 3. Hematological test results were similar among the groups. No abnormal liver enzyme levels were observed.

Reactive acute phase anti-DENV IgM was detected in six patients (14.0%) with positive rRT-PCR for ZIKV, which increased to nine (20.9%) in the paired convalescent samples. In contrast, reactive anti-DENV IgM was detected in 11 (35.5%) of the acute serum samples among negative rRT-PCR for ZIKV suspected patients. Only two (9.1%) acute samples were detected with reactive anti-DENV IgM in the other diagnosis group—one patient with acute DENV diagnosis and one patient with pneumonia. During the course of both suspected ZIKV and other diagnosis group disease, serum anti-DENV IgM percentage reactiveness remained similar to that in the acute phase. High prevalence of reactive anti-DENV IgG was detected in the acute serum samples in all the groups, suggesting previous DENV infection (Table 3).

Discussion

The study describes the clinical characteristics, laboratory features, differential diagnosis, and outcomes within a cohort of HIV-infected patients with concurrent ZIKV infection in a single reference center. The study demonstrated that most coinfected patients had mild clinical symptoms and no major laboratory abnormalities. There were no hospitalizations, complications, or deaths. All the patients recovered completely

from ZIKV infection. Additionally, differential diagnosis with other diseases and antiretroviral therapy cutaneous adverse reactions should be evaluated. No patients had chikungunya possibly because the first autochthonous transmission in Rio de Janeiro was detected in October 2015.

In this study, ZIKV-confirmed and suspected patients had similar signs and symptoms of HIV-uninfected patients at the same setting. [26] Rash was the most common symptom, followed by pruritis, and myalgia, nonpurulent conjunctivitis, arthralgia associated with joint swelling in some patients, prostration, and headache at similar frequencies. Fever, when present, was generally of a low-grade and short term, similar to that reported in other studies. [26-28]

Two patients developed rash just a few days after introduction of fosamprenavir and efavirenz, which was considered a cutaneous adverse drug reaction, typically manifesting as a maculopapular rash with or without systemic symptoms. [29] Seven (7.5%) patients had secondary/latent syphilis, including one ZIKV-confirmed patient and two ZIKV-suspected patients. Our findings are consistent with syphilis prevalence in HIV-infected patients reported in other settings. [30,31] Past syphilis history was reported in 25.7% of the cohort. Syphilis is a disease that still represents a persistent public health problem both in the HIV-infected and uninfected population. [32]

Another infectious disease that should be investigated in the differential diagnosis of rash in HIV-infected patients is human parvovirus B19 infection, [33,34] which was confirmed in two patients. Despite adherence to cART, one patient developed severe anemia requiring blood transfusion and immunoglobulin, but finally recovered. The second patient had mild symptoms without anemia. Both had exanthema.

Gonzales et al. reported two cases of DENV/HIV coinfection during a DENV-3 epidemic in Havana, 2001–2002. CD4+ cell counts remained within reference ranges, and HIV disease progression was not observed .[12] Mendes et al. reported a case of DENV hemorrhagic fever in an HIV-infected patient on cART. Both plasma HIV VL and CD4+ cell counts did not change significantly within 30 days before the diagnosis of DENV or 10 days later. [14] In the present study, in the short-term follow-up (two months) post ZIKV infection, significant change in CD4+ cell count and HIV VL was not noted. ZIKV infection complications or new AIDS-defining conditions were not observed.

The similarity and frequency of clinical manifestations between confirmed and suspected cases suggested that a high proportion of suspected patients probably were ZIKV cases because, in an epidemic setting, a negative RT-PCR does not exclude Zika as a diagnosis. [35] Some studies suggested that ZIKV RNA detection in the plasma/serum by RT-PCR is only possible for a few days after the onset of symptoms. [36,37] The median time of ZIKV detection by rRT-PCR was two days (IQR: 1–3 days) in serum of confirmed cases. Although median serum collection was slightly different in suspected cases [three days (IQR: 2–5 days)], it may have decreased the assay sensitivity for virus detection in the serum. ZIKV is shed in urine for a longer duration after onset of symptoms and usually has a higher titer than that in serum. [38-40] Unfortunately, in this cohort, urine samples were not collected from most of the patients.

In the present study, reactive anti-DENV IgM was detected in 20.9% and 35.5% during the course of disease in the rRT-PCR ZIKV-confirmed and suspected groups, respectively. Conversely, only one patient with pneumonia in the other diagnosis group had reactive anti-DENV IgM. This reactivity of anti-DENV IgM could

be explained in at least two different ways. The first, but less probable, could correspond to patients with DENV showing non-detectable DENV rRT-PCR. The second, and more plausible, is antibody cross-reactivity amongst previously DENV-infected individuals. [22,28,41-43] In fact, highly reactive serum anti-DENV IgG was detected in acute serum samples from all patients, suggesting a high prevalence rate of past DENV infection. More specific serologic testing comparing neutralizing antibody titers to ZIKV and other flavivruses would be useful for more definitive results. [37]

Our study has some limitations. As most patients in the cohort responded well to cART and had high CD4+ cell counts, a clear insight on whether ZIKV affects (negatively or positively) the disease outcomes in severely immunocompromised AIDS patients was not obtained. More ZIKV-suspected cases could have been confirmed by rRT-PCR if urine or body fluids such as saliva had been collected from all the patients. [44,45] ZIKV serologies and PRNT were not performed in this study. Probable cases of Zika virus disease according to PAHO guidelines could not be stablished.To the best of our knowledge, this is the first study to evaluate HIV/ZIKV coinfection based on a larger cohort of HIV-infected patients from a single center during a ZIKV outbreak.

In conclusion, in a cohort of immunologically and virologically stable HIV-infected patients with suspected arboviral disease, 42.6% were ZIKV infected. CD4 cell counts and HIV viral load were not different post ZIKV infection. Differential diagnosis with other diseases and adverse drug reactions should be evaluated. Whether patients with more severe immunodeficiency have differential manifestations and/or viral shedding is yet to be investigated.

References

1. Karp CL, Auwaerter PG. Coinfection with HIV and tropical infectious diseases. I. Protozoal pathogens. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America.* Nov 1 2007;45(9):1208-1213.

2. Karp CL, Auwaerter PG. Coinfection with HIV and tropical infectious diseases. II. Helminthic, fungal, bacterial, and viral pathogens. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America.* Nov 1 2007;45(9):1214-1220.

3. Guerra JA, Coelho LI, Pereira FR, et al. American tegumentary leishmaniasis and HIV-AIDS association in a tertiary care center in the Brazilian Amazon. *The American journal of tropical medicine and hygiene.* Sep 2011;85(3):524-527.

4. Martins-Melo FR, Ramos AN, Jr., Alencar CH, Heukelbach J. Mortality Related to Chagas Disease and HIV/AIDS Coinfection in Brazil. *Journal of tropical medicine.* 2012;2012:534649.

5. Berg A, Patel S, Aukrust P, et al. Increased severity and mortality in adults co-infected with malaria and HIV in Maputo, Mozambique: a prospective cross-sectional study. *PloS one.* 2014;9(2):e88257.

6. van Griensven J, Carrillo E, Lopez-Velez R, Lynen L, Moreno J. Leishmaniasis in immunosuppressed individuals. *Clinical microbiology and infection : the official publication of the European Society of Clinical Microbiology and Infectious Diseases.* Apr 2014;20(4):286-299.

7. Anema A, Ritmeijer K. Treating HIV/AIDS and leishmaniasis coinfection in Ethiopia. *CMAJ : Canadian Medical Association journal = journal de l'Association medicale canadienne.* May 24 2005;172(11):1434-1435.

8. Diazgranados CA, Saavedra-Trujillo CH, Mantilla M, Valderrama SL, Alquichire C, Franco-Paredes C. Chagasic encephalitis in HIV patients: common presentation of an evolving epidemiological and clinical association. *The Lancet infectious diseases.* May 2009;9(5):324-330.

9. Chen X, Xiao B, Shi W, et al. Impact of acute vivax malaria on the immune system and viral load of HIV-positive subjects. *Chinese medical journal.* Dec 2003;116(12):1810-1820.

10. UNAIDS. Global AIDS update 2017. Available at: http://www.unaids.org/sites/default/files/media_asset/Global_AIDS_update_2017_en.pdf.

11. Guzman MG, Harris E. Dengue. *Lancet.* Jan 31 2015;385(9966):453-465.

12. Gonzalez D, Limonta D, Bandera JF, Perez J, Kouri G, Guzman MG. Dual infection with dengue virus 3 and human immunodeficiency virus 1 in Havana, Cuba. *Journal of infection in developing countries.* May 1 2009;3(4):318-320.

13. Siong WC, Ching TH, Jong GC, Pang CS, Vernon LJ, Sin LY. Dengue infections in HIV patients. *The Southeast Asian journal of tropical medicine and public health.* Mar 2008;39(2):260-265.

14. Mendes Wda S, Branco Mdos R, Medeiros MN. Clinical case report: Dengue hemorrhagic fever in a patient with acquired immunodeficiency syndrome. *The American journal of tropical medicine and hygiene.* May 2006;74(5):905-907.

15. Watt G, Kantipong P, Jongsakul K. Decrease in human immunodeficiency virus type 1 load during acute dengue fever. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America.* Apr 15 2003;36(8):1067-1069.

16. Joob B, Wiwanitkit V. Dengue in HIV infected patients: clinical profiles. *Asian Pacific journal of tropical biomedicine.* 2014;4:S568-S569.

17. Pang J, Thein TL, Lye DC, Leo YS. Differential clinical outcome of dengue infection among patients with and without HIV infection: a matched case-control study. *The American journal of tropical medicine and hygiene.* Jun 2015;92(6):1156-1162.

18. World Health Organization. Situation Report. Zika virus microcephaly, Guillain-barré syndrome, January 05, 2017. Available at: http://apps.who.int/iris/bitstream/10665/252762/1/zikasitrep5Jan17-eng.pdf?ua=1.

19. Pan American Health Organization/World Health Organization Regional Office for the Americas. Regional Zika epidemiological update (Americas), August 25, 2017. Washington, D.C.: Pan American Health Organization, World Health Organization Regional Office for the Americas; 2017. Available at: https://www.paho.org/hq/index.php?option=com_content&view=article&id=11599&Itemid=41691&lang=en.

20. Calvet GA, Filippis AM, Mendonca MC, et al. First detection of autochthonous Zika virus transmission in a HIV-infected patient in Rio de Janeiro, Brazil. *Journal of clinical virology : the official publication of the Pan American Society for Clinical Virology.* Jan 2016;74:1-3.

21. Moreira RI, Luz PM, Struchiner CJ, et al. Immune status at presentation for HIV clinical care in Rio de Janeiro and Baltimore. *Journal of acquired immune deficiency syndromes.* Aug 2011;57 Suppl 3:S171-178.

22. Lanciotti RS, Kosoy OL, Laven JJ, et al. Genetic and serologic properties of Zika virus associated with an epidemic, Yap State, Micronesia, 2007. *Emerging infectious diseases.* Aug 2008;14(8):1232-1239.

23. Lanciotti RS, Calisher CH, Gubler DJ, Chang GJ, Vorndam AV. Rapid detection and typing of dengue viruses from clinical samples by using reverse transcriptase-polymerase chain reaction. *Journal of clinical microbiology.* Mar 1992;30(3):545-551.

24. Lanciotti RS, Kosoy OL, Laven JJ, et al. Chikungunya virus in US travelers returning from India, 2006. *Emerging infectious diseases.* May 2007;13(5):764-767.

25. Pan American Health Organization / World Health Organization. Case Definitions. Available at: http://www.paho.org/hq/index.php?option=com_content&view=article&id=11117&Itemid=41532⟨=en.

26. Brasil P, Calvet GA, Siqueira AM, et al. Zika Virus Outbreak in Rio de Janeiro, Brazil: Clinical Characterization, Epidemiological and Virological Aspects. *PLoS neglected tropical diseases.* Apr 2016;10(4):e0004636.

27. Cerbino-Neto J, Mesquita EC, Souza TM, et al. Clinical Manifestations of Zika Virus Infection, Rio de Janeiro, Brazil, 2015. *Emerging infectious diseases.* Jul 2016;22(7):1318-1320.

28. Duffy MR, Chen TH, Hancock WT, et al. Zika virus outbreak on Yap Island, Federated States of Micronesia. *The New England journal of medicine.* Jun 11 2009;360(24):2536-2543.

29. Yunihastuti E, Widhani A, Karjadi TH. Drug hypersensitivity in human immunodeficiency virus-infected patient: challenging diagnosis and management. *Asia Pacific allergy.* Jan 2014;4(1):54-67.

30. Shimelis T, Lemma K, Ambachew H, Tadesse E. Syphilis among people with HIV infection in southern Ethiopia: sero-prevalence and risk factors. *BMC infectious diseases.* Apr 17 2015;15:189.

31. Heiligenberg M, Rijnders B, Schim van der Loeff MF, et al. High prevalence of sexually transmitted infections in HIV-infected men during routine outpatient visits in the Netherlands. *Sexually transmitted diseases.* Jan 2012;39(1):8-15.

32. Hook EWR. Syphilis. *Lancet.* Apr 15 2017;389(10078):1550-1557.

33. Azevedo KM, Setubal S, Camacho LA, et al. Parvovirus B19 seroconversion in a cohort of human immunodeficiency virus-infected patients. *Memorias do Instituto Oswaldo Cruz.* May 2012;107(3):356-361.

34. Pereira RF, Garcia Rde C, Azevedo KM, Setubal S, Siqueira MA, Oliveira SA. Clinical features and laboratory findings of human parvovirus B19 in human immunodeficiency virus-infected patients. *Memorias do Instituto Oswaldo Cruz.* Apr 2014;109(2):168-173.

35. Rabe IB, Staples JE, Villanueva J, et al. Interim Guidance for Interpretation of Zika Virus Antibody Test Results. *MMWR. Morbidity and mortality weekly report.* Jun 3 2016;65(21):543-546.

36. Gabaglia CR. Zika virus and diagnostics. *Current opinion in pediatrics.* Feb 2017;29(1):107-113.

37. Landry ML, St George K. Laboratory Diagnosis of Zika Virus Infection. *Archives of pathology & laboratory medicine.* Jan 2017;141(1):60-67.

38. Bingham AM, Cone M, Mock V, et al. Comparison of Test Results for Zika Virus RNA in Urine, Serum, and Saliva Specimens from Persons with Travel-Associated Zika Virus Disease - Florida, 2016. *MMWR. Morbidity and mortality weekly report.* May 13 2016;65(18):475-478.

39. Campos Rde M, Cirne-Santos C, Meira GL, et al. Prolonged detection of Zika virus RNA in urine samples during the ongoing Zika virus epidemic in Brazil. *Journal of clinical virology : the official publication of the Pan American Society for Clinical Virology.* Apr 2016;77:69-70.

40. Gourinat AC, O'Connor O, Calvez E, Goarant C, Dupont-Rouzeyrol M. Detection of Zika virus in urine. *Emerging infectious diseases.* Jan 2015;21(1):84-86.

41. Stettler K, Beltramello M, Espinosa DA, et al. Specificity, cross-reactivity, and function of antibodies elicited by Zika virus infection. *Science.* Aug 19 2016;353(6301):823-826.

42. Steinhagen K, Probst C, Radzimski C, et al. Serodiagnosis of Zika virus (ZIKV) infections by a novel NS1-based ELISA devoid of cross-reactivity with dengue virus antibodies: a multicohort study of assay performance, 2015 to 2016. *Euro surveillance : bulletin Europeen sur les maladies transmissibles = European communicable disease bulletin.* Dec 15 2016;21(50).

43. Priyamvada L, Quicke KM, Hudson WH, et al. Human antibody responses after dengue virus infection are highly cross-reactive to Zika virus. *Proceedings of the National Academy of Sciences of the United States of America.* Jul 12 2016;113(28):7852-7857.

44. Tauro LB, Bandeira AC, Ribeiro GS, et al. Potential use of saliva samples to diagnose Zika virus infection. *Journal of medical virology.* Jan 2017;89(1):1-2.

45. Bonaldo MC, Ribeiro IP, Lima NS, et al. Isolation of Infective Zika Virus from Urine and Saliva of Patients in Brazil. *PLoS neglected tropical diseases.* Jun 2016;10(6):e0004816.

Table 1. Baseline characteristics of 101 HIV-infected patients from Rio de Janeiro between February and December 2015, stratified by rRT-PCR Zika virus detection and diagnosis (N=101)

Characteristics	Positive rRT-PCR for Zika virus Confirmed case (n = 43)	Negative rRT-PCR for Zika virus Suspected case (n = 34)	Negative rRT-PCR for Zika virus Other diagnosis (n = 24)
Median age, years (IQR)	41.8 (36.4–49.7)	40.0 (30.3–51.1)	42.7 (33.6–49.3)
Gender			
Male	24 (55.8)	16 (47.1)	19 (72.2)
Female	19 (44.2)	18 (52.9)	5 (20.8)
Race/ethnicity			
Non-white	22 (51.2)	15 (44.1)	12 (50.0)
White	21 (48.8)	19 (55.9)	12 (50.0)
Schooling, years			
> 11	9 (20.9)	8 (23.5)	9 (37.5)
> 9-11	20 (46.5)	13 (38.2)	9 (37.5)
≤ 9	14 (32.6)	13 (38.2)	6 (25.0)
Regular alcohol use (yes)	19 (44.2)	10 (29.4)	11 (45.8)
Current cigarette smoking	6 (14.0)	3 (8.8)	9 (37.5)
Median known HIV infection status, years (IQR)	8.4 (4.6–11.9)	9.4 (5.2–15.4)	7.2 (5.3–11.2)
AIDS-defining illness (yes)	30 (69.8)	15 (44.1)	14 (58.3)
Current cART exposure	44 (100.0)	33 (97.1)	23 (95.8)

PI-based	22 (51.2)	18 (54.5)	13 (56.5)
NNRTI	20 (46.5)	11 (33.3)	9 (39.1)
Other	1 (2.3)	4 (12.1)	1 (4.3)
Median CD4+ count (cells/mm^3) (IQR)	670.5 (423.0–941.5)*	709.0 (468.0–855.0)†	584.0 (302.0–838.0)‡
≥ 500	20 (66.7)	19 (70.4)	10 (66.7)
200–499	8 (26.7)	8 (29.6)	3 (20.0)
< 200	2 (6.7)	-	2 (13.3)
Median HIV viral load (log_{10} copies/mL) (IQR)	1.59 (1.59–1.59)	1.59 (1.59–2.57)	1.59 (1.59–1.59)
≤ 40	29 (96.7)	16 (59.3)	12 (80.0)
41–1,000	-	7 (25.9)	2 (13.3)
1,001–10,000	-	3 (11.1)	-
> 10,000	1 (3.3)	1 (3.7)	1 (6.7)

Data are presented as n (%) and median (interquartile interval). IQR: Interquartile interval, cART: Combination antiretroviral therapy, PI: Protease inhibitor, NNRTI: Non-nucleoside reverse transcriptase inhibitors. Missing CD4+ cell count and HIV viral load collected 120 days prior to onset of Zika virus infection symptoms: *13 patients, †7 patients, ‡9 patients.

Table 2. Clinical characteristics of 101 HIV-infected patients from Rio de Janeiro between February and December 2015, stratified by rRT-PCR Zika virus detection and diagnosis (N=101)

Characteristics	Positive rRT-PCR for Zika virus Confirmed case (n = 43)	Negative rRT-PCR for Zika virus Suspected case (n = 34)	Negative rRT-PCR for Zika virus Other diagnosis (n = 24)
Macular/maculopapular rash	40 (93.0)	34 (100.0)	14 (58.3)
Pruritis	36 (83.7)	28 (82.4)	9 (37.5)
Myalgia	30 (69.8)	17 (50.0)	16 (66.7)
Nonpurulent conjunctivitis	28 (65.1)	19 (55.9)	7 (29.2)
Arthralgia	27 (62.8)	27 (79.4)	18 (75.0)
Prostration	25 (58.1)	15 (44.1)	18 (75.0)
Headache	24 (55.8)	17 (50.0)	16 (66.7)
Fever*	22 (51.2)	21 (61.8)	17 (70.8)
Low back pain	19 (44.2)	10 (29.4)	9 (37.5)
Retro-orbital pain	19 (44.2)	14 (41.2)	12 (50.0)
Lymphadenopathy	14 (32.6)	5 (14.7)	6 (25.0)
Anorexia	14 (32.6)	11 (32.4)	13 (54.2)
Periarticular edema	12 (27.9)	13 (38.2)	1 (4.2)
Diarrhea	12 (27.9)	7 (20.6)	5 (20.8)
Oropharyngeal pain	11 (25.6)	5 (14.7)	7 (29.2)
Sweating	11 (25.8)	5 (14.7)	9 (37.5)

Photophobia	11 (25.6)	8 (23.5)	5 (20.8)
Chills	10 (23.3)	10 (29.4)	12 (50.0)
Nausea	10 (23.3)	11 (32.4)	8 (33.3)
Taste alteration	9 (20.9)	9 (26.5)	7 (29.2)
Abdominal pain	6 (14.0)	6 (17.6)	5 (20.8)
Hoarseness	5 (11.6)	2 (5.9)	3 (12.5)
Nasal congestion	4 (9.3)	3 (8.8)	6 (25.0)
Coryza	4 (9.3)	3 (8.8)	6 (25.0)
Dyspnea	2 (4.7)	3 (8.8)	4 (16.7)
Dysuria	2 (4.7)	1 (2.9)	1 (4.2)
Cough	1 (2.3)	5 (14.7)	8 (33.3)
Bleedings			
Petechiae	1 (2.3)	3 (8.8)	-
Bleeding gums	-	1 (2.9)	-
Epistaxis	-	2 (5.9)	1 (4.2)
Enanthema	1 (2.3)	-	1 (4.2)
Earache	1 (2.3)	3 (8.8)	2 (8.3)
Vomiting	1 (2.3)	5 (14.7)	2 (8.3)
Hepatomegaly	-	3 (5.2)	1 (4.2)
Splenomegaly	-	1 (1.7)	1 (4.2)

Data are presented as n (%). *Fever: reported or body temperature ≥ 37.5°C.

Table 3. Laboratory characteristics of 101 HIV-infected patients from Rio de Janeiro between February and December 2015, stratified by rRT-PCR Zika virus detection (N=101)

Characteristics	Positive rRT-PCR for Zika virus* Confirmed case (n = 43)	Negative rRT-PCR for Zika virus† Suspected case (n = 34)	Negative rRT-PCR for Zika virus‡ Other diagnosis (n = 24)
Hematocrit (%)	41.1 (39.2–44.5)	40.7 (37.5–42.5)	41.7 (39.3–43.1)
Hemoglobin (g/dL)	13.8 (13.1–14.8)	13.7 (12.6–14.7)	13.9 (13.3–14.8)
Platelet (× 10^3/mm^3)	200 (176–223)	221.5 (183.5–266.3)	209 (158–249)
Leukocytes count (/mm^3)	4,170 (3,750–5,020)	5,100 (3,985–5,745)	5,630 (4,580–6,960)
Neutrophils (%)	57.0 (49.0–65.0)	60.0 (50.0–66.3)	62.0 (52.0–75.0)
Lymphocytes (%)	33.0 (25.0–38.0)	33.0 (29.0–40.5)	27.0 (16.0–36.0)
Serum AST (U/L)	32.0 (24.5–47.8)	27.0 (20.5–44.5)	26.5 (22.0–29.3)
Serum ALT (U/L)	40.0 (27.0–54.0)	29.0 (23.5–48.5)	33.5 (25.3–44.8)
Reactive acute serum anti-DENV IgM	6 (14.0%)	11 (35.5%)	2 (9.1%)§
Reactive acute serum anti-DENV IgG	39 (90.7%)	24 (77.4%)	20 (90.9%)

AST: Aspartate aminotransferase, ALT: Alanine aminotransferase

Data are presented as n (%) and median (interquartile interval). Missing: *AST: n = 11; ALT: n = 12; n = 7; †Hematocrit, hemoglobin, platelet, leukocytes count, neutrophils, and lymphocytes: n = 4; AST: n = 13; ALT: n = 13, acute serum anti-DENV-IgM and IgG; n = 3; ‡Hematocrit, hemoglobin, platelet, leukocytes count,

neutrophils and lymphocytes: n = 1; AST: n = 6; ALT: n = 8, acute serum anti-DENV-IgM and IgG; n = 2. [§] Among the two patients with positive rRT-PCR for DENV, only one patient showed reactive anti-DENV IgM response during the course of the infection. The second reactive anti-DENV IgM in this group was detected in one patient with pneumonia.