Detection of Zika virus in Brazilian patients during the first five days of infection – urine *versus* plasma

R Pessôa [1], JV Patriota [2], MdL de Souza [2], A Abd El Wahed [3], SS Sanabani [1]
1. Department of Pathology, Hospital das Clínicas, School of Medicine, University of São Paulo, São Paulo, Brazil
2. Municipal Hospital of Tuparetama, Pernambuco, Brazil
3. Division of Microbiology and Animal Hygiene, Georg-August-University, Goettingen, Germany

Correspondence: Sabri Saeed Sanabani (sabyem_63@yahoo.com)

Citation style for this article:
Pessôa R, Patriota JV, de Souza MdL, Abd El Wahed A, Sanabani SS. Detection of Zika virus in Brazilian patients during the first five days of infection – urine versus plasma. Euro Surveill. 2016;21(30):pii=30302. DOI: http://dx.doi.org/10.2807/1560-7917.ES.2016.21.30.30302

Article submitted on 08 July 2016 / accepted on 22 July 2016 / published on 28 July 2016

Advantages of testing for Zika virus (ZIKV) in urine have been reported, such as the persistence of ZIKV in this type of specimen for up to 20 days after ZIKV disease onset. We investigate 61 patients in the first 5 days post-symptom onset and find more patients testing positive for ZIKV in plasma samples (n=46), than in corresponding urine samples (n=37). For patients respectively testing positive in both plasma and urine (n=28), respective viral loads appeared similar.

Results of recent studies have suggested that after Zika virus (ZIKV) disease onset, the virus persists at higher levels and for a longer period in urine (up to ca 20 days) than in serum (up to ca 5 days) [1,2]. To provide further data, we tested for the presence of ZIKV in the urine and corresponding plasma specimens of 61 patients presenting symptoms of ZIKV disease in Brazil. The samples were collected between 1 and 5 days after symptom onset. During this phase of infection, the proportion of patients testing positive in plasma samples (46/61) appeared to be higher than those testing positive in urine samples (37/61).

Laboratory investigations

Urine and corresponding plasma samples (i.e. from the same patient at the same date) that had been previously collected during the large outbreak in Tuparetama, Brazil, in 2015, were retrospectively analysed in this study. All samples had been obtained within the first 5 days of onset of symptoms, from a total of 61 individuals, who had been diagnosed as having ZIKV disease on clinical and epidemiological grounds. The main symptoms reported for the patients were rash (n=47), fever ≥38.5°C (n=42), headache (n=40), joint pain (n=39), and conjunctivitis (n=37). The median age of patients was 35 years (range: 1–80 years), with the majority being female (n=41). No patient was co-infected with another flavivirus such as dengue at the time of the sample collection, although previous infection with dengue was not known. None of the women were pregnant.

Specimens were investigated by real-time reverse-transcription polymerase chain reaction (RT-PCR), with a published primer set (FP: 5´-GAAGCCCTTGGATTCTTGAACGAGG-3´ and RP: 5´-CGACTCATCTCTTCTAGGACATATCC-3´) [3] and a fluorescein (FAM)/black hole quencher 1 (BHQ1)-labelled Taqman probe, targeting the non-structural protein (NS)5 genetic region of ZIKV (ZIKAp 5'-FAM-GGGAGAGAGAACTCAGGAGGTGG-BHQ1–3`). A sample was considered positive for ZIKV when the mean cycle threshold (*Ct*) value obtained from three parallel real-time RT-PCRs was ≤ 40 cycles.

Zika virus RNA in urine and plasma

Among the 61 patients, more tested positive for ZIKV RNA in plasma (n=46) than in urine (n=37) within the 5 days post-symptom onset, although the difference was not statistically significant (p=0.12; two-tailed test) (Table).

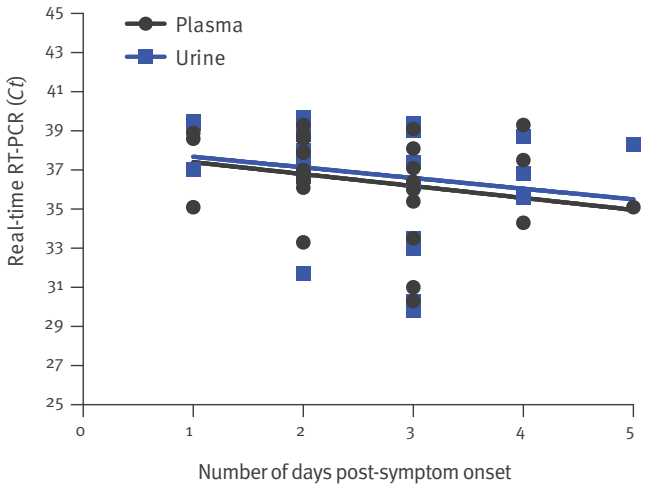
In 28 cases, ZIKV RNA was detected in both types of samples. *Ct* values from plasma and urine samples collected at the same time post-symptom onset did not seem to differ (Figure).

Background and discussion

ZIKV belongs to the genus Flavivirus, a group of RNA viruses transmitted by arthropods. The virus name originates from the Zika Forest of Uganda, where it was first isolated in 1947 from an infected rhesus monkey [4]. In 2007, ZIKV caused an outbreak in Yap State, Micronesia [5]. The outbreak was characterised by relatively mild disease including symptoms such as rash, arthralgia, and conjunctivitis, which are commonly observed upon ZIKV infection [5,6]. Seven years later, the virus appeared in French Polynesia and larger

Figure

Comparison between the cycle threshold (*Ct*) values of plasma and corresponding urine samples from Zika virus infected patients testing positive for both types of samples in the first five days post-symptom onset, Brazil, 2015 (n=28 patients)



RT-PCR: reverse transcription-polymerase chain reaction; ZIKV: Zika virus.

A linear regression analysis was performed by plotting the Ct values of plasma and urine samples versus the time post onset of symptoms using PRISM (Graphpad SoJware Inc., San Diego, California). The Ct values of ZIKV RNA in plasma and urine samples appear similar.

outbreaks were reported in New Caledonia, the Cook Islands, and Easter Island, whereby coincident with the French Polynesia ZIKV outbreak, an increased incidence of neurological complications was observed [7]. The first cases of ZIKV infection in the Americas were detected in Brazil in May 2015 [3,6,8] and, since, the virus spread to other countries in North and South America [9]. In Brazil, concurrent with ZIKV infections, neurological complications were also reported, as well as congenital malformations including microcephaly [10].

Due to cross-reactivity between members of the Flavivirus genus, the serological diagnostic of ZIKV is difficult [11]. Although there are molecular tests based on virus isolation and/or detection of ZIKV RNA during the acute phase of infection, the low-level and short period of viraemia remains a challenge [3]. Recently, Gourinat et al [1] observed that ZIKV RNA in six patients from French Polynesia was detectable at higher levels and for longer periods after symptom onset in urine samples (up to ca 20 days) than in corresponding serum samples (up to ca 5 days). The larger time window post-disease onset of possible ZIKV detection in urine was confirmed by others who investigated returning travellers to the United States (US) [2]. It was further noted that among 55 persons with urine and serum samples obtained within the first 5 days of symptom onset, a higher proportion tested positive for ZIKV RNA in urine

Table 1a

Real-time reverse transcription-polymerase chain reaction results of screening plasma and corresponding urine samples from patients in Brazil, 2015 (n=61 patients)

Sample ID	Sex	Age	*Ct* plasma	*Ct* urine
1 day post-symptom onset				
64	F	5	38.6	37.0
60	M	15	Neg	39.3
77	M	46	38.9	39.4
30	F	26	35.1	39.5
2 days post-symptom onset				
74	F	50	Neg	31.5
18	F	16	36.6	31.7
70	F	50	Neg	32.0
46	F	31	Neg	34.7
61	F	44	36.1	36.7
51	F	45	37.9	36.9
20	F	10	33.3	37.0
40	F	4	38.6	37.1
53	F	19	39.3	37.1
67	F	37	38.8	37.6
31	F	20	36.8	38.0
58	M	46	Neg	38.5
32	F	33	37.0	38.8
11	F	44	37.9	38.8
22	M	8	36.4	39.1
16	F	39	36.7	39.7
57	F	1	26.2	Neg
2	M	37	36.6	Neg
62	F	45	39.2	Neg
65	M	45	Neg	Neg
75	F	10	Neg	Neg
73	F	36	Neg	Neg
3 days post-symptom onset				
39	F	8	39.1	29.8
23	F	80	37.1	30.3
19	M	28	36.0	33.0
43	F	55	38.1	33.5
44	F	35	Neg	35.2
34	F	40	Neg	36.1
69	F	38	31.0	36.3
21	M	42	36.4	37.4
28	F	39	35.4	39.0
54	M	48	30.3	39.2
29	M	28	33.5	39.4
9	F	56	34.1	Neg
13	M	5	35.1	Neg
55	F	10	37.4	Neg
63	F	34	37.5	Neg
76	M	9	38.8	Neg
71	F	62	38.9	Neg

Ct: cycle threshold; F: female; ID: identity; M: male; Neg: negative.

The *Ct* value is the mean of the three *Ct*s of each sample.

Table 1B

Real-time reverse transcription-polymerase chain reaction results of screening plasma and corresponding urine samples from patients in Brazil, 2015 (n=61 patients)

Sample ID	Sex	Age	*Ct* plasma	*Ct* urine
4 days post-symptom onset				
35	M	65	Neg	35.0
7	F	31	37.5	35.6
50	F	70	39.3	36.8
27	F	46	34.3	38.7
47	F	18	Neg	39.3
15	F	43	34.4	Neg
5	F	28	36.5	Neg
17	F	73	36.6	Neg
10	M	34	37.3	Neg
24	M	11	37.4	Neg
37	F	15	Neg	Neg
56	M	13	Neg	Neg
5 days post-symptom onset				
33	F	27	35.1	38.3
1	M	42	33.6	Neg
4	M	3	37.0	Neg
14	M	49	37.2	Neg
6	M	8	37.6	Neg
8	F	36	Neg	Neg

Ct: cycle threshold; F: female; ID: identity; M: male; Neg: negative.

The *Ct* value is the mean of the three *Ct*s of each sample.

than in serum [2]. The findings opened the door for the use of urine for the diagnosis of ZIKV infection [1].

In our study, we focused on the detection of ZIKV in samples from 61 patients collected within the first 5 days of symptom onset. In contrast to the two previous studies mentioned above [1,2], patients in our study had an overall a higher ZIKV detection rate in plasma than in urine during active infection. Moreover, our data do not support the recent finding of higher level of ZIKV RNA in urine than in serum during the acute phase of the disease [1]. The reasons for this discrepancy remain unclear, although this might have been due to the small number of patients investigated in all three studies. Another hypothesis could be that host immune and genetic factors might affect the viral load of ZIKV in distinct body fluids, whereby this may vary among individuals. A similar suggestion has been reported in cases of infection with dengue virus, another flavivirus [12]. In this respect, patients in our study were Brazilian citizens, while the two other studies investigated patients from French Polynesia and travellers returning to the US. Previous infection with other flaviviruses might also alter and/or enhance the replication of ZIKV in specific organs [13]. Information about past dengue infection was however not available for our patients. Finally, beside the small sample size, one limitation of our study was the cross-sectional design that only permitted obtaining data at single time points.

In conclusion, we recommend the simultaneous testing of blood and urine samples in ZIKV infected individuals with focus on plasma samples in the first 5 days of infection. Further investigations will be required to more fully determine factors influencing ZIKV pathogenesis.

Acknowledgements

This work was partially supported by grants 2014/24596-2 from the Fundação de Amparo à Pesquisa do Estado de São Paulo. The funders had no role in design of the study, data collection and analysis, decision to publish, or preparation of the manuscript. We would like to acknowledge our colleague Shereen Petersen, DPZ, for English proofreading of the manuscript.

Conflict of interest

None declared.

Authors' contributions

Conceived and designed the experiments: RP, AAEW, SSS. Performed the experiments: RP, JVP, MLS, AAEW, SSS. Data analysis: RP, JVP, MLS, AAEW, SSS. Drafted the manuscript: AAEW, SSS. Critical revision: RP, JVP, MLS. All authors read and approved the final manuscript.

References

1. Gourinat AC, O'Connor O, Calvez E, Goarant C, Dupont-Rouzeyrol M. Detection of Zika virus in urine.Emerg Infect Dis. 2015;21(1):84-6. DOI: 10.3201/eid2101.140894 PMID: 25530324
2. Bingham AM, Cone M, Mock V, Heberlein-Larson L, Stanek D, Blackmore C, 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 Morb Mortal Wkly Rep. 2016;65(18):475-8. DOI: 10.15585/mmwr.mm6518e2 PMID: 27171533
3. Pessôa R, Patriota JV, Lourdes de Souza M, Felix AC, Mamede N, Sanabani SS. Investigation Into an Outbreak of Dengue-like Illness in Pernambuco, Brazil, Revealed a Cocirculation of Zika, Chikungunya, and Dengue Virus Type 1.Medicine (Baltimore). 2016;95(12):e3201. DOI: 10.1097/MD.0000000000003201 PMID: 27015222
4. Kirya BG. A yellow fever epizootic in Zika forest, Uganda, during 1972: Part 1: Virus isolation and sentinel monkeys.Trans R Soc Trop Med Hyg. 1977;71(3):254-60. DOI: 10.1016/0035-9203(77)90020-7 PMID: 407675
5. Duffy MR, Chen TH, Hancock WT, Powers AM, Kool JL, Lanciotti RS, et al. Zika virus outbreak on Yap Island, Federated States of Micronesia. N Engl J Med. 2009;360(24):2536-43. DOI: 10.1056/NEJMoa0805715 PMID: 19516034
6. Campos GS, Bandeira AC, Sardi SI. Zika Virus Outbreak, Bahia, Brazil.Emerg Infect Dis. 2015;21(10):1885-6. DOI: 10.3201/eid2110.150847 PMID: 26401719
7. Roth A, Mercier A, Lepers C, Hoy D, Duituturaga S, Benyon E, et al. Concurrent outbreaks of dengue, chikungunya and Zika virus infections - an unprecedented epidemic wave of mosquito-borne viruses in the Pacific 2012-2014. Euro Surveill. 2014;19(41):20929. DOI: 10.2807/1560-7917.ES2014.19.41.20929 PMID: 25345518
8. Cardoso CW, Paploski IA, Kikuti M, Rodrigues MS, Silva MM, Campos GS, et al. Outbreak of Exanthematous Illness Associated with Zika, Chikungunya, and Dengue Viruses, Salvador, Brazil. Emerg Infect Dis. 2015;21(12):2274-6. DOI: 10.3201/eid2112.151167 PMID: 26584464

9. Centers for Disease Control and Prevention (CDC). Travel notices. Zika Virus. Atlanta: CDC. [Accessed Jan 26 2016]. Available from: http://wwwnc.cdc.gov/travel/notices
10. Paixão ES, Barreto F, da Glória Teixeira MG, da Conceição N. Costa M, Rodrigues LC. History, Epidemiology, and Clinical Manifestations of Zika: A Systematic Review.Am J Public Health. 2016;106(4):606-12. DOI: 10.2105/AJPH.2016.303112 PMID: 26959260
11. Musso D, Gubler DJ. Zika Virus.Clin Microbiol Rev. 2016;29(3):487-524. DOI: 10.1128/CMR.00072-15 PMID: 27029595
12. Lan NT, Hirayama K. Host genetic susceptibility to severe dengue infection.Trop Med Health. 2011;39(4Suppl);73-81. DOI: 10.2149/tmh.2011-S08 PMID: 22500139
13. Dejnirattisai W, Supasa P, Wongwiwat W, Rouvinski A, Barba-Spaeth G, Duangchinda T, et al. Dengue virus sero-cross-reactivity drives antibody-dependent enhancement of infection with zika virus. Nat Immunol. 2016: Epub ahead of print. DOI: 10.1038/ni.3515 PMID: 27339099

License and copyright