Open Forum Infectious Diseases

BRIEF REPORT

Sofosbuvir Off-label Treatment of Yellow Fever Patients During an Outbreak in Brazil, 2018: A Cohort Study

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We enrolled 21 patients with laboratory-confirmed yellow fever (YF), hospitalized at Eduardo de Menezes Hospital, Brazil, to be treated with sofosbuvir, a drug approved for hepatitis C. Given the absence of specific YF antiviral treatments, the off-label nonrandomized sofosbuvir treatment aimed to address high disease severity and the risk of fatal outcomes. Patients received a daily dose of 400 mg sofosbuvir from 4 to 10 days post–symptom onset. YF viral load (VL) comparisons were made between treated and nontreated patients who either survived or died. The genomic VL for the treated group steadily decreased after day 7 post–symptom onset, suggesting that sofosbuvir might reduce YF VL. This study underscores the urgent need for YF antiviral therapies, advocating for randomized clinical trials to further explore sofosbuvir's role in YF treatment.

Keywords. antiviral; genomic viral load; sofosbuvir; yellow fever; yellow fever virus.

Yellow fever virus (YFV) (Flaviviridae; *Orthoflavivirus*; *Orthoflavivirus flavi*) is the causative agent of yellow fever (YF). YF is endemic in the tropical and subtropical regions of South America and Africa and can manifest with a broad spectrum of symptoms with different degrees of severity, including fatal cases in humans [1, 2]. Despite the existence of a live-attenuated well-tolerated vaccine [3], between December 2016 and June 2019, Brazil faced its largest YF outbreak over the last 80 years, causing 2155 cases and 745 deaths [2].

There is no approved antiviral treatment for YF, but previous studies have shown a potential effect of sofosbuvir, an antiviral widely used against hepatitis C virus (HCV) [4, 5], on YF infection in vitro and in animal models [4, 5]. This broad antiviral activity is in part due to the high conservation of the viral polymerase gene within the members of the Flaviviridae family. Here we present analysis of the impact of sofosbuvir treatment on genomic viral load and biochemical markers during severe YF infection.

MATERIALS AND METHODS

Patient Consent

This study was conducted under a compassionate-model, off-label use and was nonrandomized. A written consent form was obtained from all patients to the use of medication by the team of the Hospital Eduardo de Menezes (HEM), Minas Gerais, Brazil. The design of the work has been approved by the Ethics Committee at Instituto René Rachou/Oswaldo Cruz Foundation (Fiocruz), and Fundação de Hospitais do Estado de Minas Gerais (protocols 72569317.2.0000.5091 and 65910317.0000.5071) and by the institutional review board at Stanford University School of Medicine (eProtocol 53676).

Study Design

This is a cohort study conducted during the YF outbreak in Brazil in 2018 that included hospitalized patients from HEM with YF laboratory-confirmed diagnosis, who received compassionate sofosbuvir off-label treatment, during January and February 2018. YF diagnosis was performed by the reference laboratory in Minas Gerais (Fundação Ezequiel Dias) by detecting immunoglobulin M (IgM) anti-YFV (in addition to negative results for IgM against dengue and Zika viruses)

Received 17 May 2024; editorial decision 28 May 2024; accepted 30 May 2024; published online 6 June 2024

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https://doi.org/10.1093/ofid/ofae312

(in-house protocols), YFV RNA by reverse-transcription quantitative polymerase chain reaction (RT-qPCR) [6], or virus isolation using serum samples. Follow-up was conducted during patient hospitalization.

Sofosbuvir Treatment

Candidates for treatment presented with 1 or more of the signs and symptoms of severity [7], being classified as inclusion criteria <5 days post–symptom onset, aspartate aminotransferase (AST) >1000 U/L, international normalized ratio >1.5, creatinine >2 mg/dL, hepatic encephalopathy (according to West Haven criteria [8]), any bleeding, oliguria (urine output <0.5 mL/kg/hour), breathing disorder (presence of dyspnea, oxygen requirement, or respiratory rate >24 breaths per minute), and poor tissue perfusion (capillary refill >3 seconds). Patients were excluded from treatment if they presented 1 or more of the signs and symptoms classified as exclusion criteria: >5 days of symptoms, use of vasoactive amines, dialysis, and hospitalization at intensive care unit. Due to the limitation of this retrospective study, our control group included patients who were attended at HEM in 2018, with YF laboratory-confirmed diagnosis, who did not satisfy the inclusion criteria for sofosbuvir treatment and with at least 4 serum samples available for YFV RNA detection and quantification. Patients were screened daily, with patients being included in the treatment schedule if they satisfied the inclusion criteria. Sofosbuvir was orally administered at 400 mg once daily (Supplementary Table 1) until clinical improvement or death.

YFV RNA Detection and Quantitation

Serum samples collected during the hospitalization and sofosbuvir treatment were tested by qualitative RT-qPCR [6]. The number of samples from each patient varied (2–7 samples), and they were collected between days 1 and 10 post–symptom onset (Supplementary Table 1). In brief, total RNA was extracted from serum samples (140 µL) using the QIAmp Viral RNA Mini Kit (Qiagen) and 5 µL of total RNA was used in RT-qPCR [6]. Positive samples were then used for RT-qPCR, using the Bio Gene Research Yellow Fever PCR kit (Bioclin, Brazil), to determine the YFV RNA load. The viral genomic load was expressed as log-transformed genomic copies (GC)/mL.

Clinical Data

Data were maintained in the Stanford University REDCap platform. The following laboratory tests were performed during routine hospital care and analyzed here: alanine aminotransferase (ALT), AST, creatinine (Cr), and indirect bilirubin (Ind Bil), due to previous description of risk factor for severe YF [7].

Statistical Analysis

For analysis of YFV genomic load in sera, we only considered samples collected from days 4 to 10 post–symptom onset due to the low number of samples from other days (n < 4). We analyzed data regarding AST, ALT, Cr, and Ind Bil from days 4 to 10 post–symptom onset. For the control group, we selected patients who were hospitalized with <5 days post–symptom onset at the HEM in 2018. From the control patients, we analyzed YFV genomic load, AST, ALT, Cr, and Ind Bil from >2 time points. Both patients who were discharged or deceased were included. Three groups were analyzed in this study (Supplementary Figure 1): (1) nontreated patients who survived and were discharged from the hospital (NT-S, n = 39); (2) nontreated patients who died during hospitalization (NT-D, n = 7); and (3) treated patients (Treated, n = 21). The data (genomic viral load and laboratory tests) were transformed into log units to improve linearity. Additionally, negative samples in RT-qPCR (where YFV RNA was not detected) were assigned the value of 1 so they could be converted to logarithmic scale. We compared the groups in pairs (NT-S × NT-D; NT-S × Treated; NT-D × Treated) using a *t* test (2-tailed, Welch correction, $P < .05$) either as whole data set or split in timepoints as described in each section of the results.

RESULTS

Among the 230 patients admitted with laboratory-confirmed YF in 2018 at HEM, 21 fit the inclusion criteria and agreed with the off-label treatment with sofosbuvir during the acute phase of YF infection. Given the time the patients were admitted at the hospital, they had different treatment schedules (Supplementary Tables 1 and 2).

Qualitative and quantitative RT-PCR for YFV was performed on samples collected from days 2 to 10 post–symptom onset. Results indicated that the viral genomic load was higher for the NT-D group, followed by the treated group, and finally, the NT-S group in all time points analyzed here (Figure 1). The average viral genomic load was higher at day 4 post–symptom onset with values of 2.04×10^7 GC/mL, 1.25×10^5 GC/mL, and 5.93×10^4 GC/mL for NT-D, Treated, and NT-S, respectively. The viral genomic load values kept decreasing until day 10 post–symptom onset, when the lowest averages were found in each group, with values of 1.93×10^3 GC/mL, 1.03×10^2 GC/mL, and 1.14×10^3 GC/mL, for the NT-D, Treated, and NT-S groups, respectively (Figure 1*A* and Supplementary Table 2).

To better understand the impact of sofosbuvir on the kinetics of YFV genomic load, we performed statistical analysis comparing the median values of viral genomic load among the groups in pairs (NT-S × NT-D; NT-S × Treated; NT-D × Treated) using a *t* test (2-tailed, Welch correction, $P < .05$), considering each day post–symptom onset. All the mean values of viral genomic load were different for the groups compared: NT-S × Treated ($P = .002$), NT-S × NT-D ($P = .002$), and NT-D × Treated ($P < .0001$). YFV genomic loads from the Treated group had intermediary values between the 2

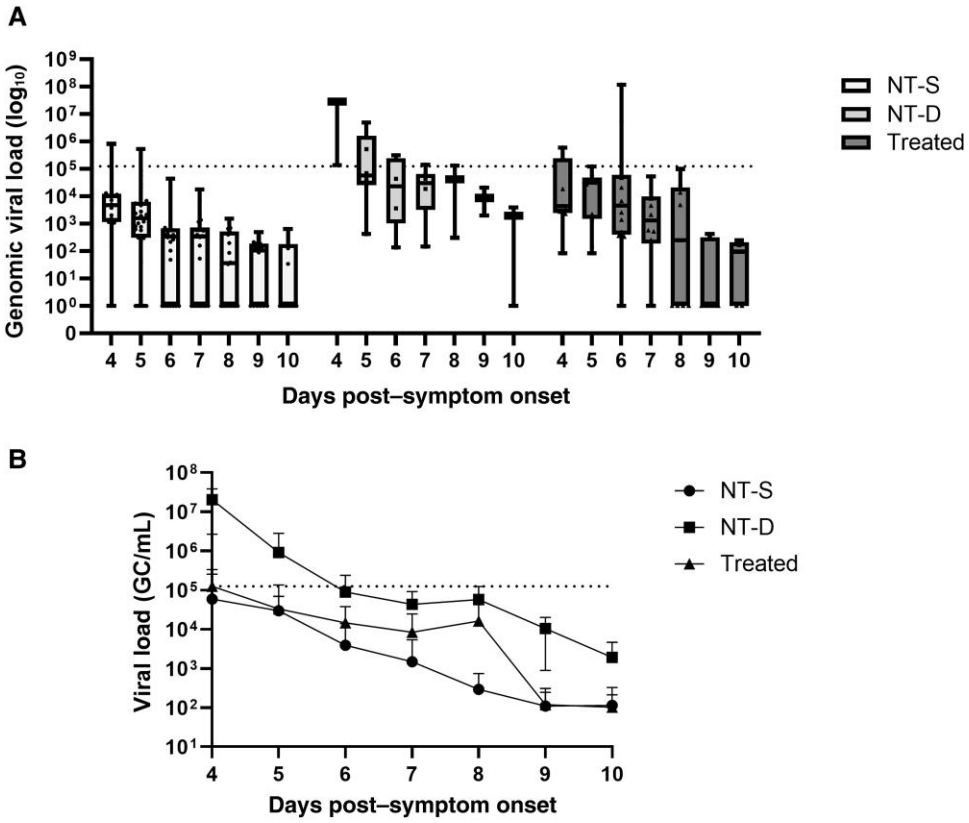

Figure 1. Yellow fever viral load. *A*, Genomic viral load values for group and day postsymptoms analyzed. Bars indicate maximum and minimum values. The dashed line represents the threshold value as described by Kalllas et al [7] as a risk factor for severe YF. *B*, Average genomic viral load for each group on each day post–symptom onset analyzed. Bars indicate standard deviation. Abbreviations: GC, genomic copies; NT-D, nontreated death; NT-S, nontreated survived.

untreated groups, suggesting that the antiviral could be able to reduce the viral genomic load of the patients to levels near those of the healthier patients (NT-S group) (Figure 1*B*).

We also compared the groups by day of symptom onset (days 4, 5, 6, 7, 8, 9, and 10) (Table 1) to investigate a possible impact of sofosbuvir on the YFV genomic load (2-tailed, Welch correction, $P < .05$). We observed significant differences between the 2 untreated groups (NT-S and NT-D) from days 4 to 9 post–symptom onset ($P < .038$), showing a clear separation in viral genomic load between those who survived and those who evolved to death from YF infection. The difference between the untreated and treated groups depended on the time point of YF infection (Table 1).

At days 5 and 6 post–symptom onset, the viral genomic load values of the Treated group were different ($P < .0295$) compared to the NT-S group, but not different compared to NT-D ($P > .097$). On day 4 post–symptom onset and days 7 to 9 post–symptom onset, differences in the viral genomic load values were observed when the Treated group was compared to NT-D ($P < .0443$), but not observed when compared to NT-S ($P > .069$). These results indicate a possible effect of the antiviral sofosbuvir in the YFV genomic viral load in the Treated group, starting on day 7 post–symptom onset (Table 1).

Table 1. Comparison of Yellow Fever Virus Genomic Loads Between Groups of Patients at Different Days Post–Symptom Onset

	P Values/Day Post–Symptom Onset						
Group	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10
NT-S × NT-D	**.0202**	**.0103**	**.0384**	**.0002**	**.0309**	**.0002**	.3644
NT-S × T	.1534	**.0249**	**.0295**	.0696	.1614	.2570	.3126
NT-D × T	**.0270**	.0970	.2044	**.0241**	**.0443**	**.0009**	.4189

Values in bold indicate significant differences between each pair (2-tailed *t* test, Welch correction, $P < .05$).

Abbreviations: NT-D, nontreated death; NT-S, nontreated survival; T, treated.

Given that certain laboratory values are associated with higher mortality and severity of YFV [7], we analyzed AST, ALT, Cr, and Ind Bil in our cohort. Almost all comparisons had a significant difference, with higher absolute values being observed in the NT-D group, followed by Treated and NT-S, respectively (Supplementary Figure 2, Supplementary Tables 3 and 4).

DISCUSSION

To date, there is no available treatment for YF. However, previous studies have demonstrated that sofosbuvir may be

effective for human YF: an in vitro study, which demonstrated that the drug inhibited the replication of both vaccine and wild-type strains of YFV on human hepatoma cells [4, 5], and in vivo studies showing that sofosbuvir had antiviral activity against the YFV [4, 5]. In another off-label study with sofosbuvir, the drug was used in 2 patients diagnosed with YF for 7 days [5]. Both had a prominent reduction in the genomic viral load and improvement in the biochemical markers used to evaluate clinical status. Our larger study confirms these findings and lends evidence to show that sofosbuvir has a positive impact on lowering the YFV genomic load around the seventh day post–symptom onset.

It is important to note that this study has several limitations. Given that this study was conducted during the largest YF outbreak in Minas Gerais in 80 years, we were unable to implement a standardized protocol and conduct a randomized controlled trial. Even though our study indicates an antiviral activity of sofosbuvir against YFV in infected patients, due to these issues we cannot be sure of sofosbuvir efficacy as the sole reason that treated patients survived.

Considering the rarity of YF outbreaks and therefore the issues of having an ideal sample population that could fulfill the requirements for the use of sofosbuvir, our study can be used as a reference for further development of effective therapy for use against this important human health threat during a future outbreak.

In summary, our results show the potential benefit of the antiviral sofosbuvir in reducing the YFV genomic load in patients with severe YF.

Supplementary Data

Supplementary materials are available at *Open Forum Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

Acknowledgments. The authors thank their colleagues from Eduardo de Menezes Hospital, Laboratory of Virus, Federal University of Minas Gerais, and the teams of Ezequiel Dias Foundation and Secretary of Health of Minas Gerais, Brazil. This work was developed in the context of the research group "Grupo de Estudos de Pesquisa e Resposta em Febre Amarela do Estado de Minas Gerais," and the authors thank all members of the group.

Financial support. I. M. R., P. A. A., B. P. D., A. T.-C., O. A. M.-F., and A. D. L. were supported by Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq)/National Institutes of Health (NIH) (grant number 404192-2019-0/R01 AI149614; principal investigators A. D. L. and O. A. M.-F.). B. P. D., A. T.-C., and O. A. M.-F. were supported by Secretaria de Estado de Saúde de Minas Gerais, Secretaria de Estado de Planejamento de Minas Gerais, Instituto René Rachou, Fundação Oswaldo Cruz. This study was financed in part by Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) (grant numbers 88882.348380/2010-1 and 0001). This study was supported by Rede Mineira de Antivirais, Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG) (grant number RED-00170-23). B. P. D., A. T.-C., and O. A. M.-F. are CNPq Research Fellows. A. T.-C. reports support for this work from the US Collaborative Biomedical Research Program, CNPq/Ministério da Saúde/NIH. B. P. D. and I. M. R. report support for this work from Hospital Eduardo de Menezes (provision of study materials).

Potential conflicts of interest. All authors: No reported conflicts.

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