Predictors of mortality in patients with yellow fever: an observational cohort study

Esper G Kallas, Luiz Gonzaga F A B D'Elia Zanella, Carlos Henrique V Moreira, Renata Buccheri, Gabriela B F Diniz, Anna Carla P Castiñeiras, Priscilla R Costa, Juliana Z C Dias, Mariana P Marmorato, Alice T W Song, Alvino Maestri, Igor C Borges, Daniel Joelsons, Natalia B Cerqueira, Nathália C Santiago e Souza, Ingra Morales Claro, Ester C Sabino, José Eduardo Levi, Vivian I Avelino-Silva, Yeh-Li Ho

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Hospital das Clinicas HCFMUSP, Faculdade de Medicina, Universidade de São Paulo, São Paulo, Brazil (Prof E G Kallas PhD, L G F A B D'Elia Zanella MD, C H V Moreira MD, P R Costa PhD, J Z C Dias PhD, M P Marmorato BSc, A T W Song PhD, A Maestri PhD, I C Borges PhD, D Joelsons MD, N B Cerqueira BSc, I Morales Claro BSc, E C Sabino PhD, V I Avelino-Silva PhD, Y-L Ho PhD); **Infectious Diseases Institute "Emilio Ribas", São Paulo, Brazil** (L G F A B D'Elia Zanella, C H V Moreira, R Buccheri MD, G B F Diniz MD, A C P Castiñeiras MD); **Tropical Medicine Institute, University of São Paulo, São Paulo, Brazil** (N C Santiago e Souza BSc, J E Levi PhD); **and DASA Laboratories, São Paulo, Brazil** (J E Levi)

Correspondence to: Prof Esper G Kallas, Hospital das Clínicas da Faculdade de Medicina da Universidade de São Paulo, Faculdade de Medicina, Universidade de São Paulo, São Paulo 01246-903, Brazil
esper.kallas@usp.br

Summary

Background Yellow fever virus infection results in death in around 30% of symptomatic individuals. The aim of this study was to identify predictors of death measured at hospital admission in a cohort of patients admitted to hospital during the 2018 outbreak of yellow fever in the outskirts of São Paulo city, Brazil.

Methods In this observational cohort study, we enrolled patients with yellow fever virus from two hospitals in São Paolo—the Hospital das Clínicas, University of São Paulo and the Infectious Diseases Institute "Emilio Ribas". Patients older than 18 years admitted to hospital with fever or myalgia, headache, arthralgia, oedema, rash, or conjunctivitis were consecutively screened for inclusion in the present study. Consenting patients were included if they had travelled to geographical areas in which yellow fever virus cases had been previously confirmed. Yellow fever infection was confirmed by real-time PCR in blood collected at admission or tissues at autopsy. We sequenced the complete genomes of yellow fever virus from infected individuals and evaluated demographic, clinical, and laboratory findings at admission and investigated whether any of these measurements correlated with patient outcome (death).

Findings Between Jan 11, 2018, and May 10, 2018, 118 patients with suspected yellow fever were admitted to Hospital das Clínicas, and 113 patients with suspected yellow fever were admitted to Infectious Diseases Institute "Emilio Ribas". 95 patients with suspected yellow fever were included in the study, and 136 patients were excluded. Three (3%) of 95 patients with suspected yellow fever who were included in the study were excluded because they received a different diagnosis, and 16 patients with undetectable yellow fever virus RNA were excluded. Therefore, 76 patients with confirmed yellow fever virus infection, based on detectable yellow fever virus RNA in blood (74 patients) or yellow fever virus confirmed only at the autopsy report (two patients), were included in our analysis. 27 (36%) of 76 patients died during the 60 day period after hospital admission. We generated 14 complete yellow fever virus genomes from the first 15 viral load-detectable samples. The genomes belonged to a single monophyletic clade of the South America I genotype, sub-genotype E. Older age, male sex, higher leukocyte and neutrophil counts, higher alanine aminotransferase, aspartate transaminase (AST), bilirubin, and creatinine, prolonged prothrombin time, and higher yellow fever virus RNA plasma viral load were associated with higher mortality. In a multivariate regression model, older age, elevated neutrophil count, increased AST, and higher viral load remained independently associated with death. All 11 (100%) patients with neutrophil counts of 4000 cells per mL or greater and viral loads of 5·1 $\log_{10}$ copies/mL or greater died (95% CI 72–100), compared with only three (11%) of 27 (95% CI 2–29) among patients with neutrophil counts of less than 4000 cells per mL and viral loads of less than 5·1 $\log_{10}$ copies/mL.

Interpretation We identified clinical and laboratory predictors of mortality at hospital admission that could aid in the care of patients with yellow fever virus. Identification of these prognostic markers in patients could help clinicians prioritise admission to the intensive care unit, as patients often deteriorate rapidly. Moreover, resource allocation could be improved to prioritise key laboratory examinations that might be more useful in determining whether a patient could have a better outcome. Our findings support the important role of the virus in disease pathogenesis, suggesting that an effective antiviral could alter the clinical course for patients with the most severe forms of yellow fever.

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Introduction

There have been several outbreaks and epidemics of arboviruses in recent years, especially in the Americas. In addition to dengue,[1] large epidemics of Zika virus[2] and chikungunya[3] have swept through Central America, the Caribbean, and South America. Despite the high numbers of previously infected individuals who should now be resistant to further infection, these viruses can still cause new outbreaks in susceptible communities. There are four different dengue serotypes that cause disease, therefore subsequent infections can occur.[4] Most recently, a surge in the number of individuals infected

Research in context

Evidence before this study

Yellow fever is a mosquito-borne disease that is endemic in high-risk regions in Africa and South America. Although yellow fever infection has been associated with high mortality, data correlating virological or patient characteristics with death are limited. We searched PubMed using the search terms "risk", "mortality", and "yellow fever", with no language restrictions, for studies published up until Nov 14, 2018. The search returned 73 articles, with most of them dealing with the risk factors of disease after vaccination. Eight articles addressed the epidemiological risk of disease spread following an epidemic. Only four studies analysed the risk factors for mortality: two only with demographic or geographical data, one with clinical symptoms only, and one with clinical and laboratory findings, without virological data. To our knowledge, no previous study has addressed the value of viral load in predicting outcome.

Added value of this study

In this study, we analysed 76 patients infected with yellow fever virus at admission to hospital, and found that older age, elevated neutrophils, elevated aspartate aminotransferase, and higher viral load were independently associated with death. Considering the high mortality after yellow fever virus infection, identification of predictors of poor outcome could aid in the decision making process, as early intensive support care might be lifesaving. Moreover, in point-of-care resource-limited areas, some key laboratory tests might be of greater value than others when allocating resources and referring patients to tertiary care centres.

Implications of all the available evidence

By identifying predictors of death, our findings could substantially assist with allocation of resources in the selection of the most important clinical and laboratory findings to be considered in primary care at the initial clinical evaluation of patients infected with yellow fever virus. Furthermore, our findings could aid in the decision to refer patients who will most benefit from admission to intensive care in tertiary centres. Furthermore, the results of our study give a new perspective on how to advance knowledge on disease pathogenesis and provide the basis for the development of antiviral strategies to treat patients infected with yellow fever virus.

with the yellow fever virus has been observed. Due to the high amount of travel worldwide, there are risks of importation of yellow fever to other regions, as with the previous 2016 dissemination from Africa to Asia.[5]

Large yellow fever virus outbreaks have been documented in Brazil over the period 2016–18,[6] despite the existence of a safe and efficacious vaccine for over 85 years.[7] The 2016 outbreaks were concentrated in the southeast region. In 2017 and 2018, several cases were diagnosed in the outskirts of São Paulo city, ultimately leading to 563 confirmed cases and 214 deaths (data correct as of July 17, 2018[8]). These yellow fever infections were all caused by the modern lineage (sub-lineage 1E) of South American genotype I.[9] All cases to date in this region have been linked to a sylvatic viral cycle. However, concerns exist over a possible urban yellow fever virus resurgence,[10] as observed in the 2015–16 Angola outbreak, in which high population densities and patterns of human mobility and vector suitability were crucial for the exponential risk of sustained transmission of yellow fever.[11]

However, in contrast to previous outbreaks, most patients in the surroundings of São Paulo city were referred to medical care in tertiary hospitals. Hence, a more detailed clinical, laboratory, and virological characterisation of disease presentation could be documented. In this study, we explore the predictors of death at hospital admission in a cohort of patients infected with yellow fever virus who were admitted to one of two reference tertiary hospitals in São Paulo city (either the Hospital das Clínicas, University of São Paulo or the Infectious Diseases Institute "Emilio Ribas").

Methods

Study population

On Jan 10, 2018, a referral system was established where patients with suspected yellow fever were admitted to one of two participating institutions: the Hospital das Clínicas, University of São Paulo and the Infectious Diseases Institute "Emilio Ribas" (both located in São Paulo, Brazil). Patients older than 18 years admitted to hospital with fever or myalgia, headache, arthralgia, oedema, rash, or conjunctivitis were consecutively screened for inclusion in the present study. Consenting patients were included if they had travelled to geographical areas in which yellow fever virus cases had been previously confirmed. Patients were confirmed to be infected with yellow fever virus by detection of the virus in blood collected at admission or tissues at autopsy by real-time PCR. Autopsies were requested for those who died with the disease. Patients with suspected yellow fever who tested negative for yellow fever virus RNA in blood samples collected at admission had their diagnosis confirmed when tissue was positive for yellow fever virus RNA and pathological findings were compatible with the disease. All patients were followed until death or for 60 days after enrolment, whichever occurred first.

Study oversight

The study protocol was approved by the institutional review boards at the Hospital das Clínicas, School of Medicine, University of São Paulo, and the Infectious Diseases Institute "Emilio Ribas". All study participants or their legal representatives provided signed informed

For the **REDCap platform** see https://www.project-redcap.org/

consent to participate in this study. All individual identifiable information was maintained in secured cabinets and electronic files using the REDCap platform in a secured server at the School of Medicine, University of São Paulo. We vouch for the accuracy and completeness of the data, the analyses, and for the fidelity of the study to the established protocol.

Clinical data and laboratory testing

Tests were done at clinical laboratories located at the Hospital das Clínicas, School of Medicine, University of São Paulo, and the Infectious Diseases Institute "Emilio Ribas". Demographic and clinical data were age, sex, race, duration of symptoms upon admission, fever, myalgia, rash, headache, arthralgia, abdominal pain, and bleeding. The following laboratory tests were done at admission: aspartate transaminase (AST) and alanine aminotransferase (ALT) concentrations, prothrombin time (measured by the international normalised ratio [INR]), total, direct, and indirect bilirubin, creatinine, haemoglobin, and the number of leukocytes, neutrophils, and platelets.

Clinical management

All patients were admitted to the intensive care unit. Laboratory assessments were repeated every 8 h, but we considered only data at hospital admission in this study. Briefly, patients with any neurological impairment underwent electroencephalogram and computed tomography, as well as optic nerve sheath measurement and transcranial Doppler ultrasound if there were signs of intracranial hypertension; early initiation of renal replacement therapy; systematic collection of blood and urine cultures and prophylactic administration of antibiotics in cases of severe hepatic insufficiency (cefotaxime and fluconazole at Hospital das Clínicas and piperacillin plus tazobactam and fluconazole at Infectious Diseases Institute "Emilio Ribas"); blood, fresh frozen plasma, or cryoprecipitate transfusions depending on the grade of haemorrhage and coagulation disorders; thrombo-elastogram if indicated (available only at Hospital das Clínicas); monitoring by the liver transplantation team in case of factor V less than 50%, presence of any grade of hepatic encephalopathy, INR greater than 2·5, or ammonia above 70 µL/L. Our discharge criteria mainly relied on the absence of clinical symptoms and normal, or progressive improvement in, laboratory assessment results.

Yellow fever virus RNA detection and quantitation

We extracted viral RNA from 500 µL of plasma on the automated platform NucliSENS easyMag (Biomérieux; São Paulo, Brazil). 50 µL RNA was eluted and 14 µL was used for rtPCR with primers and probes allowing codetection and differentiation between the 17DD yellow fever vaccine strain and the wild-type virus circulating in the Brazilian outbreak of 2016–17, as previously described.[12] We constructed a calibration curve with serial dilutions of the yellow fever vaccine (live attenuated virus yellow fever vaccine; Fiocruz/Bio-manguinhos; Rio de Janeiro, Brazil; 4·81 $\log_{10}$ plaque forming units [PFU] per 0·5 mL) in yellow fever virus RNA-negative human plasma spanning 9 to 9x10³ PFUs per ml. We obtained viral loads from the interpolating curve, which had an R^2 value of 0·99 and a limit of detection of 0·1 PFUs per mL (95% detection rate). We initially calculated viral loads in equivalence to PFUs per mL, since the calibration curve was built with the 17DD vaccine diluted in human plasma. Conversion to yellow fever virus RNA copies per mL was done using the following formula: $\log_{10}$ PFU per mL=[0·974×$\log_{10}$ copies per mL]−2·807. This conversion was based on a linear correlation described by Fernandes-Monteiro and colleagues,[13] corroborating a previous estimate of a ratio of yellow fever virus genomes to infectious particles of between 1000 to one and 5000 to one.[14]

Whole-genome sequencing

Yellow fever virus RNA-positive rtPCR samples underwent whole-genome sequencing with the yellow fever virus primers scheme.[15] Briefly, we produced cDNA from RNA-positive samples using random hexamers (Invitrogen; Carlsbad CA, USA) and ProtoScript II Reverse Transcriptase (New England BioLabs; Ipswich, MA, USA) according to the manufacturers' instructions. We then amplified cDNA with a multiplex PCR assay that produced overlapping 500 base pair amplicons across the whole coding genome of the recent South American genotype I outbreak clade. PCR products were purified, quantified, and pooled in an equimolar fraction for normalisation. We used the Native Barcoding kit (Oxford Nanopore Technologies; Oxford, UK) and Ligation Sequencing kit (Oxford Nanopore Technologies) for library preparation, which included an end repair, dA-tailing, barcode ligation (1–12), and adapter ligation process. The sequencing library was loaded into a R9.4 flow cell and run for up to 48 h. Raw files were basecalled and demultiplexed using Albacore software version 2.2.7 and trimmed using Porechop software version 0.2.3_seqan2.1.1. We mapped sequences to the reference genome (GenBank accession no JF912190) and obtained the consensus sequence for each sample through Geneious version 11.0.5. We uploaded consensus sequences with the YFV Typing Tool 2016 to reconstruct maximum likelihood trees.[16]

Statistical analyses

For our analysis of predictors of mortality among patients with yellow fever admitted to hospital, we initially compared demographic and clinical characteristics and laboratory findings at admission of survivors and deceased patients by use of χ^2 or Fisher's exact test for categorical variables, and Wilcoxon rank-sum test for numerical variables. Baseline for survival analysis was considered as days since symptoms onset, as reported at admission. Follow-up data were censored

	Overall (n=76)	Survivors (n=49)	Deceased (n=27)	p value*
Age, years	42 (32–54)	40 (27–46)	50 (36–63)	0·012
Sex				
Male	68 (89%)	41 (84%)	27 (100%)	0·045
Female	8 (11%)	8 (16%)	0	..
Race†				
White	49 (65%)	29 (59%)	20 (77%)	0·124
Non-white	26 (35%)	20 (41%)	6 (23%)	..
Time with symptoms at presentation, days	8 (5–10)	8 (6–10)	7 (5–8)	0·163
Any comorbidity‡	28 (42%)	15 (35%)	13 (54%)	0·125
Fever	69 (91%)	44 (90%)	27 (100%)	1·000
Headache	48 (63%)	31 (63%)	17 (63%)	0·979
Rash	2 (3%)	2 (4%)	0	0·398
Myalgia§	56 (74%)	36 (73%)	20 (74%)	0·954
Arthralgia	25 (33%)	19 (39%)	6 (22%)	0·142
Abdominal pain	35 (46%)	20 (41%)	15 (56%)	0·217
Bleeding	28 (37%)	17 (35%)	11 (41%)	0·601
Leucocytes, per µL¶	3900 (3100–5800)	3500 (2700–4200)	5500 (4200–10 000)	0·0001
Lymphocytes	820 (540–1300)	800 (600–1200)	830 (500–1530)	0·628
Neutrophils	2410 (1470–4360)	1840 (1250–2650)	4290 (3000–8220)	<0·0001
Platelets, per µL	77 000 (52 000–116 000)	79 000 (53 000–129 000)	67 000 (52 000–99 000)	0·3539
Aspartate transaminase, U/L	2528 (865–5793)	1626 (694–3550)	6103 (2775–15500)	<0·0001
Alanine aminotransferase, U/L	1846 (938–2797)	1473 (847–2762)	2370 (1404–5194)	0·044
International normalised ratio	1·26 (1·12–1·66)	1·18 (1·06–1·40)	1·69 (1·39–2·06)	<0·0001
Total bilirubin, mg/dL	3·80 (1·15–6·28)	2·70 (0·89–5·37)	5·62 (4·60–6·79)	0·0012
Direct	2·97 (0·79–5·29)	1·76 (0·56–4·63)	4·68 (2·69–5·60)	0·0040
Indirect	0·50 (0·20–1·12)	0·37 (0·13–0·61)	1·13 (0·70–1·73)	<0·0001
Creatinine, mg/dL	1·25 (0·82–3·76)	1·02 (0·80–1·35)	4·06 (2·99–5·28)	<0·0001
Yellow fever virus RNA, $\log_{10}$ copies/mL‖	7·71 (4·31–9·59)	6·01 (2·17–8·32)	9·01 (7·71–11·07)	0·00020

Data are n (%) or median (IQR). Groups were divided according to survival. *p values describe the comparison between survivors and deceased. †Data missing for one patient. ‡Data missing for nine patients. §Data are missing for 20 patients. ¶Data missing for seven patients. ‖For patients with negative yellow fever virus RNA in quantitative assay but a positive result in qualitative assay in any sample at admission or in autopsy findings, an arbitrary value of 0·5 plaque forming units per ml was applied for calculation of yellow fever viral load.

Table 1: **Baseline characteristics of patients with yellow fever**

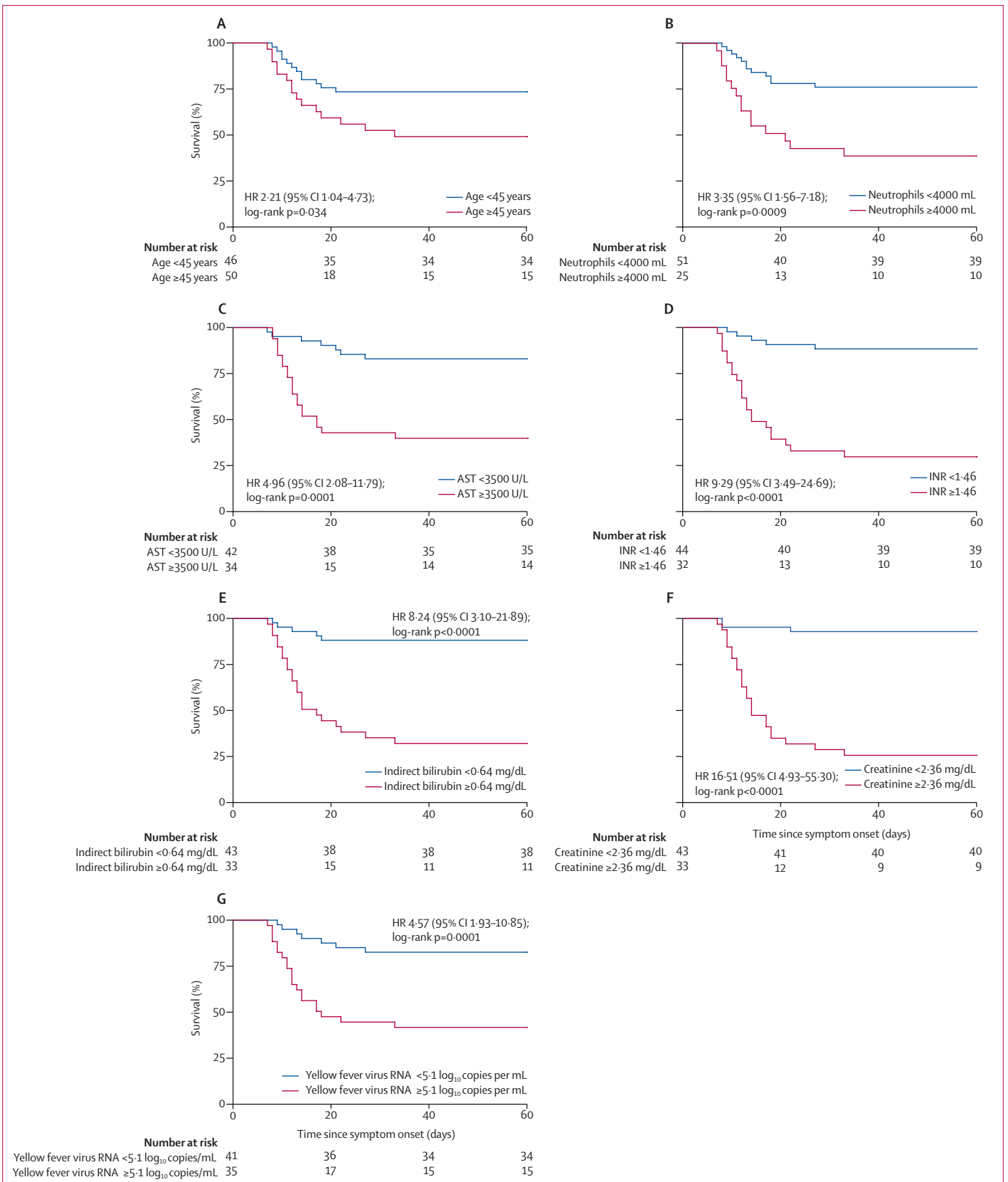
at 60 days after enrolment or death, whichever occurred first. We log-transformed yellow fever viral load values to improve linearity. We used receiver operating characteristic (ROC) curves to select cutoff points for each numerical predictor according to visual assessment of the highest sensitivity or specificity, and we constructed a Kaplan-Meier curve to illustrate the association between each binary predictor and patient survival. We used a multivariate Cox proportional hazards model with robust standard errors to estimate the independent effect of potential predictors on mortality. For the multivariate model, we used numerical variables in their original scale (not dichotomised). Next, we created event-based algorithms using two variables with statistically significant association with mortality in the multivariate model. We selected the algorithm with the highest discriminatory capacity to depict a simple predictive tool. We used Stata version 15.1 with a two-tailed α error of 0·05 in all analyses.

Role of the funding source

The funder 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

Between Jan 11, 2018, and May 10, 2018, 118 patients with suspected yellow fever were admitted to Hospital das Clínicas, and 113 patients with suspected yellow fever were admitted to Infectious Diseases Institute "Emilio Ribas". 95 patients with suspected yellow fever were included in the study. 136 patients were excluded for the following reasons: 57 had a diagnosis of yellow fever ruled out before inclusion, and 79 for other reasons, including refusal to participate, death shortly after admission (up to 1 day), or admission to hospital on a weekend or holiday. Three (3%) of 95 patients with suspected yellow fever who were included in the study were excluded with a different


A
Survival (%)
HR 2·21 (95% CI 1·04–4·73); log-rank p=0·034
Age <45 years
Age ≥45 years
Number at risk
Age <45 years 46 35 34 34
Age ≥45 years 50 18 15 15
B
HR 3·35 (95% CI 1·56–7·18); log-rank p=0·0009
Neutrophils <4000 mL
Neutrophils ≥4000 mL
Number at risk
Neutrophils <4000 mL 51 40 39 39
Neutrophils ≥4000 mL 25 13 10 10
C
HR 4·96 (95% CI 2·08–11·79); log-rank p=0·0001
AST <3500 U/L
AST ≥3500 U/L
Number at risk
AST <3500 U/L 42 38 35 35
AST ≥3500 U/L 34 15 14 14
D
HR 9·29 (95% CI 3·49–24·69); log-rank p<0·0001
INR <1·46
INR ≥1·46
Number at risk
INR <1·46 44 40 39 39
INR ≥1·46 32 13 10 10
E
HR 8·24 (95% CI 3·10–21·89); log-rank p<0·0001
Indirect bilirubin <0·64 mg/dL
Indirect bilirubin ≥0·64 mg/dL
Number at risk
Indirect bilirubin <0·64 mg/dL 43 38 38 38
Indirect bilirubin ≥0·64 mg/dL 33 15 11 11
F
HR 16·51 (95% CI 4·93–55·30); log-rank p<0·0001
Creatinine <2·36 mg/dL
Creatinine ≥2·36 mg/dL
Time since symptom onset (days)
Number at risk
Creatinine <2·36 mg/dL 43 41 40 40
Creatinine ≥2·36 mg/dL 33 12 9 9
G
HR 4·57 (95% CI 1·93–10·85); log-rank p=0·0001
Yellow fever virus RNA <5·1 log10 copies per mL
Yellow fever virus RNA ≥5·1 log10 copies per mL
Time since symptom onset (days)
Number at risk
Yellow fever virus RNA <5·1 log10 copies/mL 41 36 34 34
Yellow fever virus RNA ≥5·1 log10 copies/mL 35 17 15 15

diagnosis (one with acute hepatitis A, one with hepatitis C flare, and one with leptospirosis). 16 patients with undetectable yellow fever virus RNA were excluded. 76 patients with confirmed yellow fever virus infection, based on detectable yellow fever virus RNA in blood (74 patients) or yellow fever virus confirmed only at the autopsy report (two patients), were included in this analysis. We recorded the cohort demographics, clinical presentation, and laboratory findings (table 1).

Patients with yellow fever virus were mostly young and middle-aged men (median age 42 years, IQR 32–54). 27 (36%) of 76 patients died during the 60 day period after hospital admission. Patients who died were generally older than survivors and more likely to be male (table 1). Upon admission, patients reported a median of 8 days of symptoms (IQR 5–10), with no statistically significant difference observed between those who survived and those who died (p=0·163). Comorbidities were more common in the deceased group, but were not significantly different compared with the surviving group (p=0·125; table 1). In all patients, fever was the most common clinical finding, followed by myalgia, headache, abdominal pain, bleeding, and arthralgia. We did not observe any significant differences in the frequency of these findings between patients who survived and patients who died (table 1). Overall, length of hospital stay was median 7 days (range 1–60; IQR 6–11) in patients who survived and 7 days (range 2–27; IQR 5–10) in patients who died.

We generated 14 complete yellow fever virus genomes from the first 15 viral load-detectable samples. The genomes belonged to a single monophyletic clade of the South America I genotype, sub-genotype E. All samples were clustered together, suggesting that there was a single yellow fever virus entry causing the present outbreak (appendix).

Upon hospital admission, several variables were significantly associated with mortality, including higher leukocyte count, higher neutrophil count, higher AST and ALT concentrations, greater prothrombin time (measured by INR), higher bilirubin concentration, elevated creatinine, and higher viral load (table 1).

We selected variables associated with mortality in univariate analyses and plotted ROC curves to select cutoff points for survival curves (data not shown). These cutoff points were also selected on the basis of biological and clinical parameters.

To assess variables independently associated with death, we carried out a multivariate Cox proportional hazards analysis including selected potential predictors. Given the limited sample size and frequency of outcomes, we selected six variables to construct the model, based on the univariate analysis. We selected neutrophil count rather than total lymphocyte count as we observed stronger association with death and this measure represents a more specific cell subset. We excluded INR and ALT because of collinearity with AST,[17] and we observed higher AST concentration to be more strongly associated with death. We selected indirect bilirubin as this measure had a stronger association with death and better represents liver damage compared with direct bilirubin. We also included age, creatinine concentration, and viral load in the final model. We constructed Kaplan-Meier survival curves for the seven selected variables using death as the outcome (figure 1). In this analysis with original numerical variables categorised in two levels, all variables maintained a statistically significant association with death.

Age, neutrophil count, AST, and viral load were independently associated with death in the adjusted analysis (table 2). According to this model, the hazard ratio (HR) for each 5-year increase in age was 1·28 (95% CI 1·07–1·55; p=0·0080), for each 1000 cells per µL increase in neutrophil count was 1·21 (1·09–1·34;

	HR (95% CI)	p value
Age (per 5-year increase)	1·28 (1·07–1·55)	0·0080
Neutrophils (per 1000 cells per µL increase)	1·21 (1·09–1·34)	0·00044
Aspartate transaminase (per 100 U/L increase)	1·01 (1·00–1·02)	0·0030
Indirect bilirubin (per 1 mg/dL increase)	1·41 (0·98–2·06)	0·065
Creatinine (per 1 mg/dL increase)	1·07 (0·88–1·32)	0·444
Yellow fever RNA viral load (per 1 $\log_{10}$ copies/mL increase)	1·27 (1·42–2·07)	<0·0001

HR=hazard ratio.

***Table 2*: HRs for death for variables included in a multivariate Cox proportional regression model**

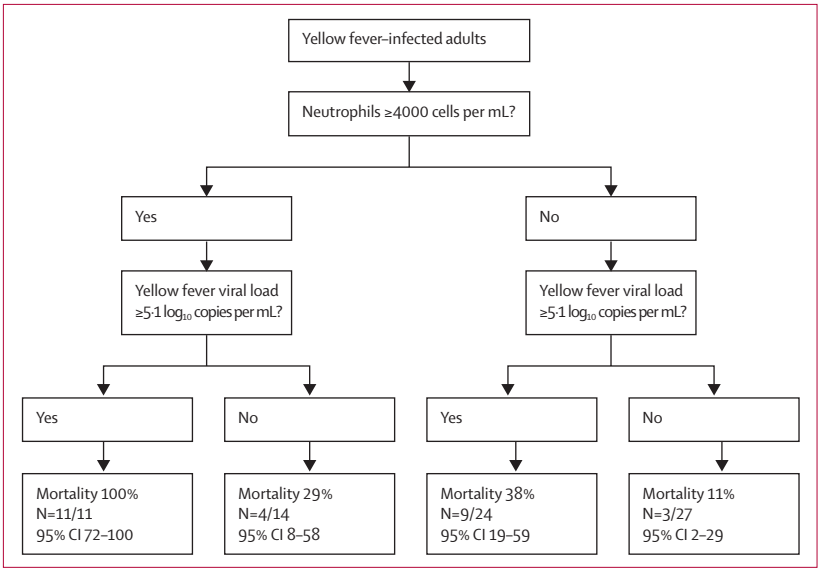


***Figure 2*: Event-based algorithm**
We used the two variables independently and most significantly associated with death identified in the Cox proportional regression model—yellow fever virus load and neutrophil count—to identify subgroup mortality.

See **Online** for appendix

***Figure 1*: Kaplan-Meier curves showing the effect of binary converted predictors on survival**
Analyses were done for age (A), neutrophil count (B), AST (C), INR (D), indirect bilirubin (E), creatinine (F) and yellow fever virus load (G). AST=aspartate transaminase. INR=international normalised ratio.

p<0·00044), for each 100 U/L increase in AST was 1·01 (1·00–1·02; p=0·0030), and for each 1 $\log_{10}$ copies/mL increase in yellow fever virus RNA was 1·27 (1·42–2·07; p<0·0001; table 2).

We then constructed an event associated algorithm that included neutrophil counts and viral load—the two variables independently and most significantly associated with death—with the same cutoff points as identified for the Kaplan-Meier survival curves (neutrophils ≥4000 cells per mL and viral load ≥5·1 $\log_{10}$ copies/mL; figure 2). All 11 (100%) patients with neutrophil counts of 4000 cells per mL or greater and viral loads of 5·1 $\log_{10}$ copies/mL or greater died (95% CI 72–100), compared with only three (11%) of 27 patient deaths (95% CI 2–29) with neutrophil counts less than 4000 cells per mL and viral loads less than 5·1 $\log_{10}$ copies/mL (figure 2).

Discussion

Although only 10–50% of patients infected with yellow fever virus develop symptoms, yellow fever virus infection is recognised as a very severe disease, with associated mortality as high as 50% in symptomatic patients.[18] However, which variables could predict poor patient outcome after yellow fever virus infection remained to be elucidated. In this study, we determined which demographic, clinical, and laboratory findings upon admission were associated with death in a prospective cohort of 76 patients with yellow fever virus.

The outbreak investigated in this study was caused by a monophyletic South American 1 genotype virus. This fact simplified our analyses, as differences in patient outcomes during this outbreak were not caused by viral diversity. Therefore, differential outcomes could be associated with the inoculum burden, immune response,[19,20] individual genetic susceptibility,[21] or other predisposing factors. Another strength of our study is that all patients were evaluated at only two referral institutions in the São Paulo (Hospital das Clínicas, School of Medicine, University of São Paulo, and the Infectious Diseases Institute "Emilio Ribas").

Several variables were associated with death in our univariate analysis, including age, sex, leukocyte and neutrophil counts, liver transaminase concentration, INR, bilirubin concentration, creatinine concentration, and yellow fever viral load. However, in a multivariate model, age, neutrophil count, AST, and viral load remained as independent predictors of death.

Our findings suggest that four different factors can affect patient outcome after yellow fever virus infection. The first factor is increasing age, possibly reflecting immune system senescence or diminished functional reserve, supporting the findings of a previous study in patients with yellow fever in Ghana and Nigeria,[22] as well as in patients with dengue in Singapore.[23] The second factor, higher numbers of circulating neutrophils, might reflect increased inflammation due to a cytokine storm, sepsis, or bacterial product translocation—the latter has been previously described in severe dengue.[24] The third factor, elevated AST, is a proxy for liver damage and multiorgan failure. These results support the findings of Tuboi and colleagues,[25] who retrospectively analysed 251 yellow fever virus cases and showed that elevated AST and jaundice were independently associated with increased mortality. The fourth possible factor is the pathogen itself. Although viral load has not been previously identified as a predictor of death in human beings, we were able to document this association, supporting the idea that there is a direct viral effect on disease pathogenesis. In other arboviruses, the association between viral load and disease severity has been previously shown in patients with dengue,[26] but not with Zika virus.[27] In yellow fever, the association between viral replication detected in the blood and outcome has been observed in a rhesus macaque model. Higher peak viraemia after challenge was associated with fulminant disease resulting in euthanasia, whereas all animals that controlled viral replication during the first week of infection survived.[28] The association between viral load and disease severity could have been stronger in our cohort if we had viral load data from earlier time points after the onset of symptoms. However, as the median time since onset of symptoms at admission in the present cohort was 8 days (IQR 5–10), and the earliest a patient was admitted was 4 days after the onset of symptoms, the study did not have sufficient power to further explore this issue. Nonetheless, this finding suggests that antiviral drugs or neutralising antibodies[29] should be used early in the treatment of yellow fever virus to decrease disease-related mortality. Additionally, our findings support the development of point-of-care quantitative viral load tests, which should be made available in areas at risk of yellow fever virus outbreaks. This strategy would provide a useful diagnostic tool and help in the assessment of risk of death.

Potential limitations of this study are the restricted area in which the study took place and the single yellow fever virus genotype responsible for this outbreak (the modern lineage genotype I, responsible for outbreaks in South America since 2000[9]). Therefore, caution should be used when applying our findings to yellow fever virus cases caused by other genotypes, such as those documented in the 2016 Uganda outbreak,[30] or the 2015–16 Angola outbreak,[31] which subsequently spread to the neighbouring Democratic Republic of the Congo.[32] We also had a relatively small sample size, which restricted the number of predictors included in the multivariate model. Moreover, only clinical and laboratory data at admission were considered, therefore some variables that might have changed during the course of hospital treatment were not analysed.

Our findings have several implications for the care of patients with yellow fever virus. Identification of poor outcome markers could help guide resource allocation and strategies to provide intensive care for patients with potentially severe disease, supporting a rational approach

during disease outbreaks. Medical services and intensive care units can be overwhelmed during the peak of epidemics and decision making referral algorithms might take advantage of simple clinical and laboratory evaluation. Notably, liver transplantation has been investigated as a last resort intervention in severe, life-threatening cases.[33]

Moreover, despite the availability of an efficacious yellow fever vaccine, the occurrence of such epidemics highlights the need for improved vaccine coverage. Although increased vaccine coverage has been achieved, coverage remains insufficient considering the yellow fever risk zones, both in tropical and subtropical areas in the Americas and Africa.[34] Long-lasting protection has been shown in the immunocompetent population with a single vaccine dose.[35] However, a booster dose might be recommended in specific populations, such as children younger than 2 years, people living with HIV, immunocompromised patients, and those in areas at high risk of yellow fever.[36]

In conclusion, our findings identified predictors of mortality in patients with yellow fever, providing useful information to improve understanding of disease pathogenesis and supporting the decision making process in the care of these patients.

Contributors

EGK, CHVM, AM, and NBC designed the study. LGFABD'EZ, CHVM, RB, GBFD, ACPC, ATWS, ICB, DJ, and Y-LH participated in data collection. PRC, JZCD, MPM, NCSeS, IMC, ECS, and JEL did the laboratory procedures. EGK and VIA-S participated in the data analysis. EGK, ATWS, JEL, VIA-S, and Y-LH wrote the manuscript. All authors revised and approved the final version of the manuscript.

Declaration of interests

We declare no competing interests.

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