BIOCHEMICAL MARKERS ASSOCIATED WITH MORTALITY IN PATIENTS WITH YELLOW FEVER DURING THE 2017 OUTBREAK IN ESPÍRITO SANTO, BRAZIL

MARCADORES BIOQUÍMICOS ASSOCIADOS À MORTALIDADE EM PACIENTES COM FEBRE AMARELA DURANTE O SURTO DE 2017 NO ESPÍRITO SANTO, BRASIL

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ABSTRACT

Yellow fever (YF) is caused by a virus of the genus *Flavivirus* and family *Flaviviridae*. The severe form of the disease presents a classic triad: jaundice, hemorrhage, and acute renal failure. Brazil experienced one of the largest outbreaks of sylvatic yellow fever transmission in its history in 2017. This retrospective observational cohort study aimed to correlate laboratory test results obtained during the first week of hospitalization with clinical outcome (discharge or death). Medical records of patients with laboratory-confirmed YF (IgM capture) admitted to Hospital Silvio Ávidos (HSA), Colatina, Espírito Santo, during the 2017 outbreak were reviewed. Data were tested for normality (Shapiro-Wilk) and compared between groups using Student's t test, Welch's t test, or the Mann-Whitney U test, as appropriate ($p<0.05$). The majority of patients were male and resided in rural areas of Espírito Santo State. Compared with

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survivors (n=12), non-survivors (n=6) showed significantly higher median ALT levels (3,510.00) vs. (259.50) UI/L, (p=0.015), mean urea (91.83) vs. (26.60) mg/dL, (p=0.045), and mean creatinine (2.40) vs. (0.99) mg/dL, (p=0.027). No statistically significant differences were observed for AST (p=0.310), total bilirubin (p=0.819), or hematological parameters, including hemoglobin, hematocrit, leukocytes, and plate counts (p>0.05) for all. These findings suggest that specific biomarkers of acute hepatic and renal injury assessed during the first week of admission are strongly associated with death in patients with YF, helping in the early identification of patients at higher clinical risk

Keywords: Yellow fever; Biochemistry disorders; Hospitalization; Death.

RESUMO

A febre amarela (FA) é causada por um vírus do gênero Flavivirus e família Flaviviridae. A forma grave da doença apresenta uma tríade clássica: icterícia, hemorragia e insuficiência renal aguda. O Brasil viveu um dos maiores surtos de transmissão de febre amarela silvestre de sua história em 2017. Este estudo de coorte retrospectivo e observacional teve como objetivo correlacionar os resultados de exames laboratoriais obtidos durante a primeira semana de internação com o desfecho clínico (alta ou óbito). Foram revisados os prontuários de pacientes com FA confirmada em laboratório (captura de IgM) internados no Hospital Silvio Ávidos (HSA), Colatina, Espírito Santo, durante o surto de 2017. Os dados foram testados quanto à normalidade (Shapiro-Wilk) e comparados entre os grupos utilizando o teste t de Student, o teste t de Welch ou o teste U de Mann-Whitney, conforme apropriado (p<0,05). A maioria dos pacientes era do sexo masculino e residia em áreas rurais do Estado do Espírito Santo. Em comparação com os sobreviventes (n=12), os não sobreviventes (n=6) apresentaram níveis significativamente maiores de mediana de ALT (3.510,00 vs. 259,50 UI/L, p=0,015), média de ureia (91,83 vs. 26,60 mg/dL, p=0,045) e média de creatinina (2,40 vs. 0,99 mg/dL, p=0,027). Não foram observadas diferenças estatisticamente significativas para AST (p=0,310), bilirrubina total (p=0,819) ou parâmetros hematológicos, incluindo hemoglobina, hematócrito, leucócitos e contagem de plaquetas (p>0,05 para todos). Esses achados sugerem que biomarcadores específicos de lesão hepática e renal aguda avaliados durante a primeira semana de internação estão fortemente associados ao óbito em pacientes com FA, auxiliando na identificação precoce de pacientes com maior risco clínico.

Palavras-chave: *Febre amarela; Alterações bioquímicas; Hospitalização; Óbito.*

1 INTRODUCTION

Yellow fever (YF) is an acute infectious disease extremely relevant regarding morbidity and lethality. Africa and South America are the main endemic regions (Chippaux and Chippaux, 2018). In Brazil, considering the most recent outbreak of the disease, occurred in 2017, there were approximately 780 cases with the majority of the

infections occurring in rural areas of the country (Paules and Fauci, 2017; Chippaux and Chippaux, 2018). The etiologic agent for YF is an arbovirus of the *Flavivirus* genus which is transmitted mainly by mosquitoes of the *Haemagogus* genus (Saraiva *et al.*, 2013). Infection with YF occurs after the virus is inoculated into the human body through the bite of the mosquito, which then enters the lymphatic vessels and especially infects lymphoid cells and macrophages, where the replication process takes place. It then enters the bloodstream and can generate kidney, liver, lung and even brain infections (Vasconcelos, 2003; Saraiva *et al.*, 2013). The disease can originate from cases with low symptoms to episodes with more aggravating aspects and death (Tauil, 2010).

Considering the sources of infection, the wild form is a zoonosis that attacks non-human primates, and accidentally or locally human beings may be infected by entering enzootic regions (Chippaux and Chippaux, 2018). Although the disease is contracted and spread through the bite of the vector mosquito in human population, there are several factors, such as vaccination, that can interfere in the succession of the disease (Possas *et al.*, 2018). Even though most cases of YF show weak symptoms, and may be confused with other febrile diseases, about 20% of the occurrences are severe, generating symptoms such as jaundice, hemorrhages and renal and hepatic failure. In addition, about 20% 50% of patients with YF in its severe form may die (Gardner and Ryman, 2010).

Damage of the YF in the liver result of necrosis and apoptosis of hepatocytes, which ultimately generates an increase in the levels of liver enzymes in the blood, such as bilirubin, aspartate aminotransferase (AST), and alanine transaminase (ALT) (Vasconcelos *et al.*, 2001). In addition, the disease may affect the kidneys and suddenly present a significant loss of blood filtration capacity as well as a higher concentration of residues (Vasconcelos, 2003). From the onset of renal failure, the patient presents alterations metabolic that affect glomerular filtration and elimination of toxins (Chippaux and Chippaux, 2018).

Recent studies have reinforced the importance of laboratory biomarkers in the prediction of clinical severity and mortality in Yellow Fever (YF). Soluble inflammatory mediators have been associated with disease progression, unfavorable outcomes, and relapsing hepatitis, suggesting that the host immune response plays a central role in the pathogenesis of severe cases (Fradico *et al*., 2023). Furthermore, elevated serum

concentrations of viral non-structural protein 1 (NS1) and syndecan-1 have been correlated with endothelial dysfunction and increased disease severity, highlighting the contribution of vascular injury to the progression of YF (Sousa et al., 2024). Recent evidence also demonstrates that coagulation abnormalities are strongly associated with bleeding events and poor prognosis in severe YF, reinforcing the relevance of hemostatic assessment during hospitalization (Jardim et al., 2024). In addition, contemporary reviews emphasize that hepatic dysfunction remains one of the main determinants of mortality in flavivirus infections, including YF, due to extensive hepatocellular injury and systemic inflammatory responses (Pinheiro *et al*., 2023). Finally, recent epidemiological studies continue to demonstrate substantial lethality rates in severe YF outbreaks in South America, reinforcing the need for early identification of prognostic markers capable of supporting clinical decision-making and reducing mortality (Srivastava et al., 2024; Lopes *et al*., 2025). Taken together, these studies indicate that inflammatory mediators, endothelial injury markers, coagulation abnormalities, and hepatic dysfunction are consistently associated with unfavorable outcomes in severe YF (Fradico *et al*., 2023; Pinheiro *et a*l., 2023; Sousa *et al*., 2024; Jardim *et al*., 2024;). However, most of these studies rely on specialized biomarkers (e.g., NS1, syndecan-1, soluble inflammatory mediators) that are not routinely available in the clinical setting of small or resource-limited hospitals during outbreak conditions, and comparative data on which combination of standard, widely accessible biochemical tests best distinguishes survivors from non-survivors remain limited, particularly in Brazilian regional outbreak cohorts. In this context, the present study aimed to evaluate whether routine biochemical markers obtained at hospital admission commonly available even in smaller reference hospitals are associated with in-hospital mortality among patients hospitalized during the 2017 YF outbreak in Espírito Santo, Brazil, contributing regional, real-world evidence to support early risk assessment in similar clinical settings.

2 MATERIALS AND METHODS

This is a retrospective observational cohort study based on the review of hospital medical records. Given the epidemiological context of the 2017 outbreak, only 18 patients with laboratory confirmed YF met the inclusion criteria at the study site

during the study period, comprising a small sample size (n=18, including 7 deaths) that should be considered when interpreting the results, as detailed in the Limitations section. In 2017, 109 cases of YF were confirmed in Espírito Santo State (Vasconcelos, 2003; Paules and Fauci, 2017). Moreover, eighteen (18) patients diagnosed with YF (IgM Capture) were admitted at the Silvio Ávidos Hospital (HSA), in the Colatina town. Demographic data, laboratory test results at admission, and clinical outcomes (death or discharge) were evaluated. However, information on comorbidities, vaccination status, and the exact time interval between symptom onset and hospital admission was not consistently available in the medical records and could therefore not be systematically analyzed. Laboratory tests included the dosage of hemoglobin, hematocrit, leukocytes, platelets, prothrombin activation time (PAT), total and fractional bilirubin, urea, AST, ALT and creatinine. The data were obtained from medical records, available digitally in the HMSA MV2000 System and authorized by the Espírito Santo State Health Secretariat (SESA) on 05/24/2017). In addition, to complementary exams, patient identification data such as gender, age, origin, resident of urban or rural areas, profession, as well as the main complaint at the time of hospital admission were extracted from the medical record.

The study was approved by the Ethics and Research Committee on Human Beings of the Centro Universitário do Espírito Santo UNESC, number 2,438,317, at 12/14/2017. Patient identification data were kept in absolute secrecy, respecting the regulations established. Laboratory variables (hemoglobin, hematocrit, leukocytes, platelet count, prothrombin activity time [PAT], total bilirubin, indirect bilirubin, direct bilirubin, AST, ALT, urea, and creatinine) were compared between the death and discharge groups and described using mean, standard deviation, median, interquartile range (Q1–Q3), coefficient of variation, skewness, and kurtosis. The normality of the distribution of each variable in each group was assessed using the Shapiro-Wilk test, complemented by inspection of Q-Q plots and histograms, which served as the criterion for the automatic selection of the main statistical test (Shapiro-Wilk for samples with $n < 300$). When normality was confirmed, homogeneity of variances between groups was assessed using Levene's and Bartlett's tests, determining whether Student's t-test (homogeneous variances) or Welch's t-test (heterogeneous variances) was applied. When the normality assumption was not met in at least one of the groups, the nonparametric Mann-Whitney U test was used as an alternative. For

all comparisons, effect size (Cohen's d) was calculated with a corresponding 95% confidence interval and classified as small, medium, or large, allowing evaluation of the practical relevance of the observed differences beyond statistical significance. The significance level adopted for all analyses was 5% ($p < 0.05$). Statistical analyses and comparative graphs between groups were performed using the EasyStatistic platform.

3 RESULTS

YF affected mostly the southeastern states of Brazil (Fig 1, left). Espírito Santo State received the virus through of Minas Gerais (Siqueira *et al.*, 2021) and part of the hospitalizations were carried out at Hospital Silvio Avidos (HAS), in Colatina-ES. The patients admitted in the HAS came from municipalities with rural characteristics of the coffee culture interspersed with Atlantic forest located at the center north of Espírito Santo State (Fig 1, right). This region is close to the habitat of monkeys and the *Haemagogus* mosquito. All eighteen (18) patients reported feeling at least one symptom like fever, nausea or vomiting during the anamnesis at the admission time. Among the admitted patients, eleven (11) were discharged and seven (7) died.

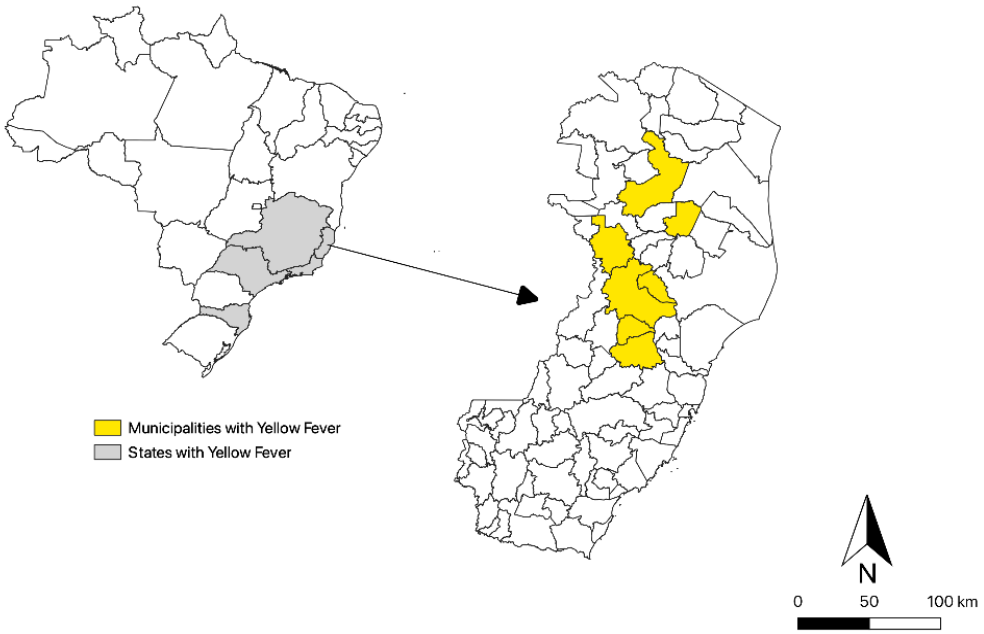


Fig 1: Distribution of autochthonous Yellow Fever (YF) in the states of Brazil among 2017-2018 (left) and in the municipality of Colatina, Marilândia, Nova Venécia, Pancas, Santa Teresa, São Roque do Canaã and Vila Valério located in Espírito Santo State (right) (Siqueira *et al.*, 2021).
The patients followed in this study were grouped according to age, sex and occupation (Table 1). The data show that male patients with rural occupation (56%), with age between 31 to 60 years were the most affected by YF disease.

Table 1: Distribution of patients diagnosed with YF by age group, sex and occupation.

	Male	Female	Total
Age Group	n	n	%
15 to 30 years	3	1	22%
31 to 60 years	9	1	56%
≥ 61 years	2	0	11%
Uninformed	1	1	11%
Rural occupation	10	0	56%
Unreported rural occupation	5	3	44%

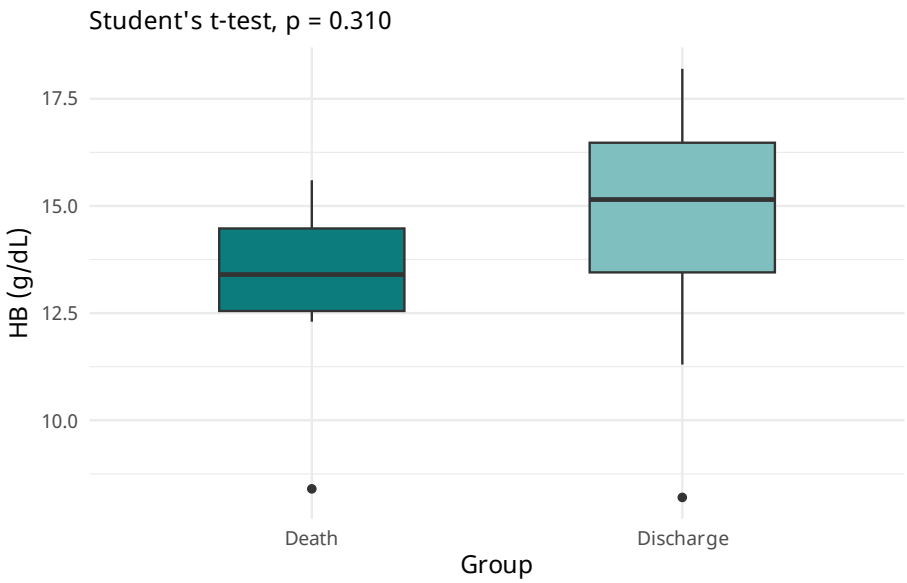
Source: Author, 2025.

On the first day of the admission at the hospital, patients were submitted to several blood tests. In this regard, hemoglobin (HB), hematocrit (HT), leukocyte (LEUKO) (Fig 2), Platelet count and Prothrombin Activation Time (PAT) (Fig 3) levels were measured in all the patients including those who recovered from the infection and also in those who died.

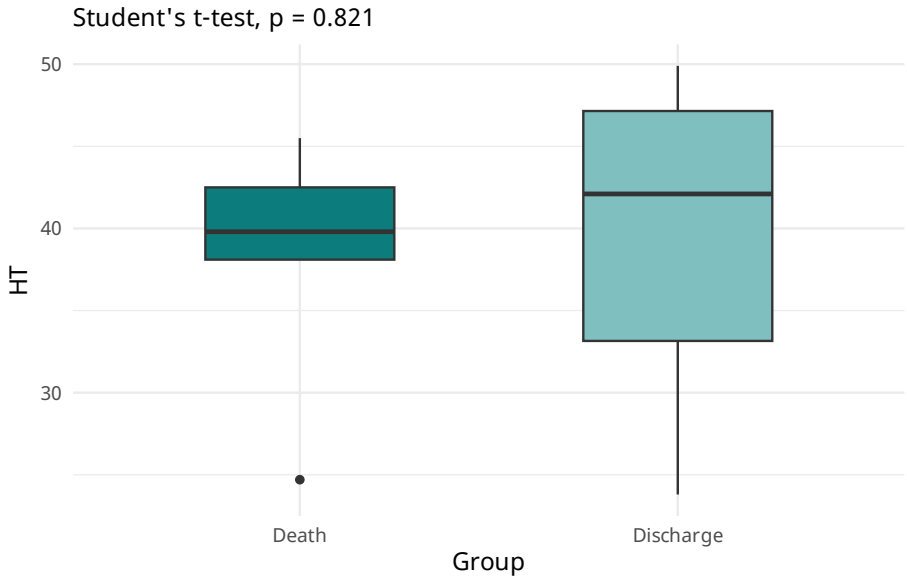
Although there is a tendency of decrease of hemoglobin (Fig 2A) and hematocrit (Fig 2B) values in those patients that progressed to death, no statistical significance was observed between the groups.

Then, the leukocytes levels were analyzed and data did not show important differences in relation to its respective reference values (4,000 - 10,000 m^3) and also between the two groups of patients studied (Fig 2C).

A


Student's t-test, p = 0.310
17.5
15.0
12.5
10.0
HB (g/dL)
Death
Discharge
Group

B


Student's t-test, p = 0.821
50
40
30
HT
Death
Discharge
Group

C

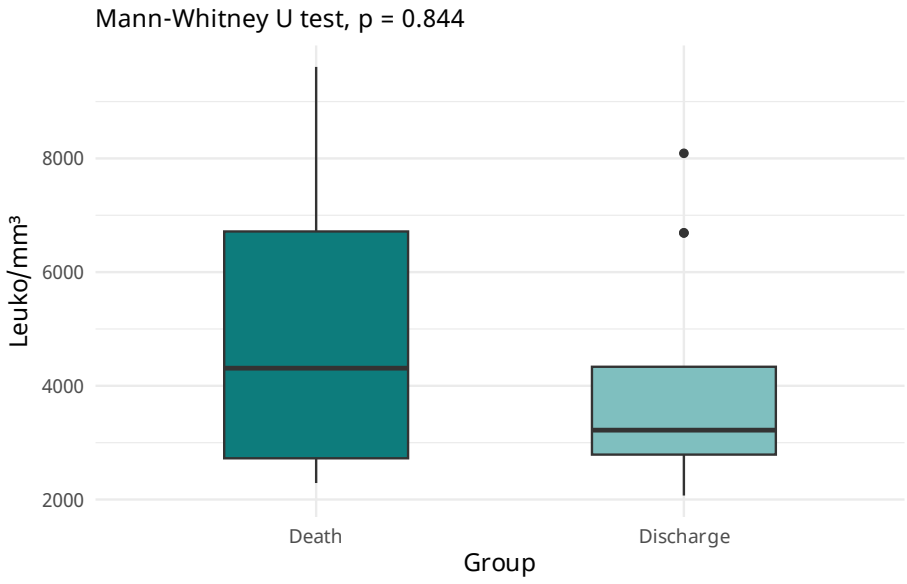
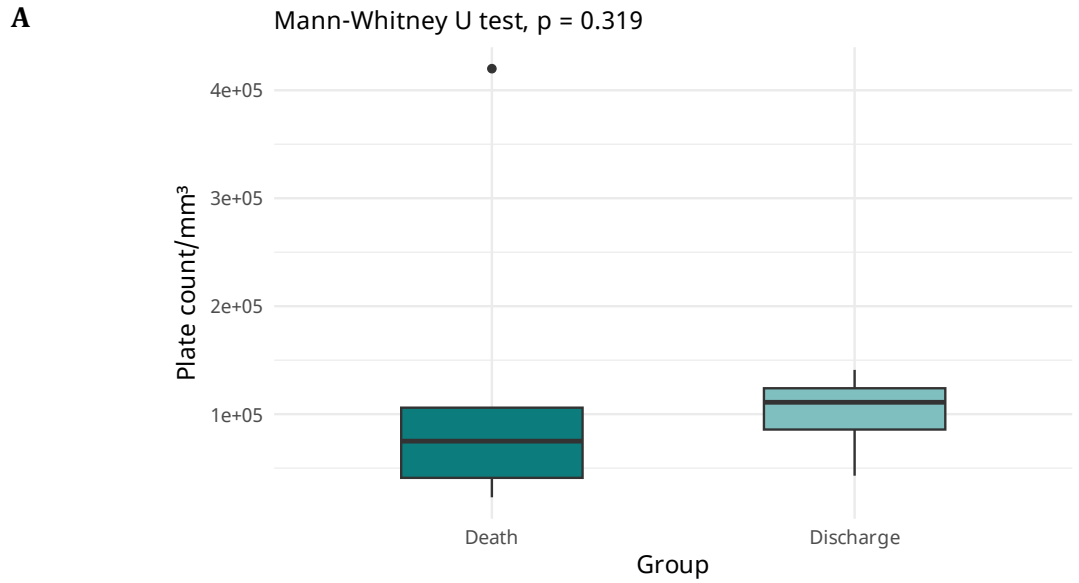

Mann-Whitney U test, p = 0.844
8000
6000
4000
2000
Leuko/mm³
Death
Discharge
Group

Figure 2: Evaluation of blood count in patients with Yellow Fever. Hemoglobin measurement – HB (A), hematocrit – HT (B), and leukocytes – Leuko (C), compared between death (n = 6, 5, and 4, respectively) and discharge (n = 10, 11, and 12, respectively) groups (HB: 12.98 ± 2.53 vs. 14.52 ± 2.98 g/dL; HT: 38.12 ± 8.01 vs. 39.27 ± 9.70%; Leuko: 5130 ± 3348.03 vs. 4010 ± 1967.38/mm³), with no statistically significant differences observed ($p < 0.05$). Reference values (RV): Hemoglobin 13–17 g/dL (men) and 12–18 g/dL (women); Hematocrit 35–47%; Leukocytes 4000–10,000/mm³.
Source: Author, 2025.

After, the blood clotting ability from the patients were assessed through analysis of platelets levels and prothrombin activation time (PAT) (Figure 3A and 3B). The plate count test showed an evident thrombocytopenia when compared to the reference values (150,000 to 450,000 m^3) in both groups of patients analyzed (Fig 3A). Interestingly, some patients who died showed platelet levels very low (around to 50,000 m^3).

The PAT results (Fig 3B) showed a increase in patient mortality. These results indicate that bleeding and clotting time would be prolonged, thus suggesting a clotting disorder since the virus in the bloodstream reduces blood flow in the organs and causes hemorrhages (Duarte-Neto *et al.*, 2019).



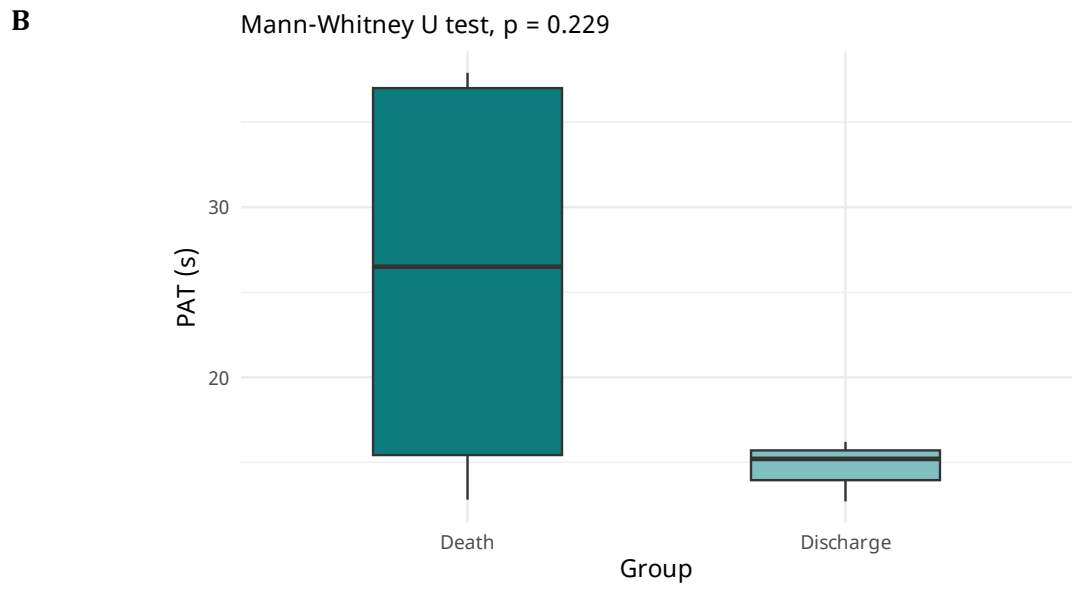
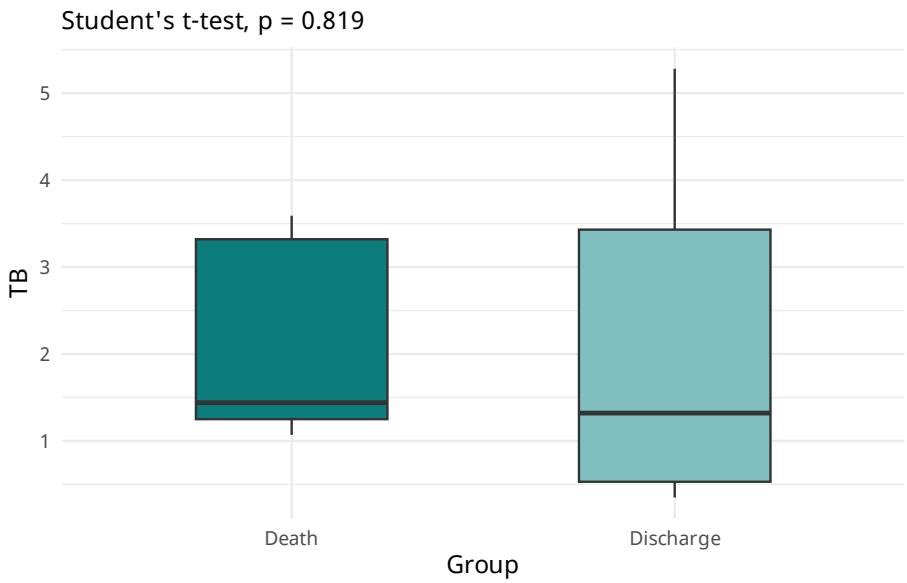


Figure 3. Evaluation of the coagulative ability in patients with Yellow Fever. (A) Platelet count; (B) Prothrombin Activation Time – PAT. Platelet count and PAT were compared between death (n = 5 and 4, respectively) and discharge (n = 12 and 3, respectively) groups (Platelets: 133,000 ± 163,574.14 vs. 100,083.33 ± 33,407.83/mm³; PAT: 25.93 ± 13.22 vs. 14.70 ± 1.80 s), with no statistically significant differences observed ($p < 0.05$). Reference values (RV): Platelets 150,000–450,000/mm³; PAT 1–1.2 INR.
Source: Author, 2025.

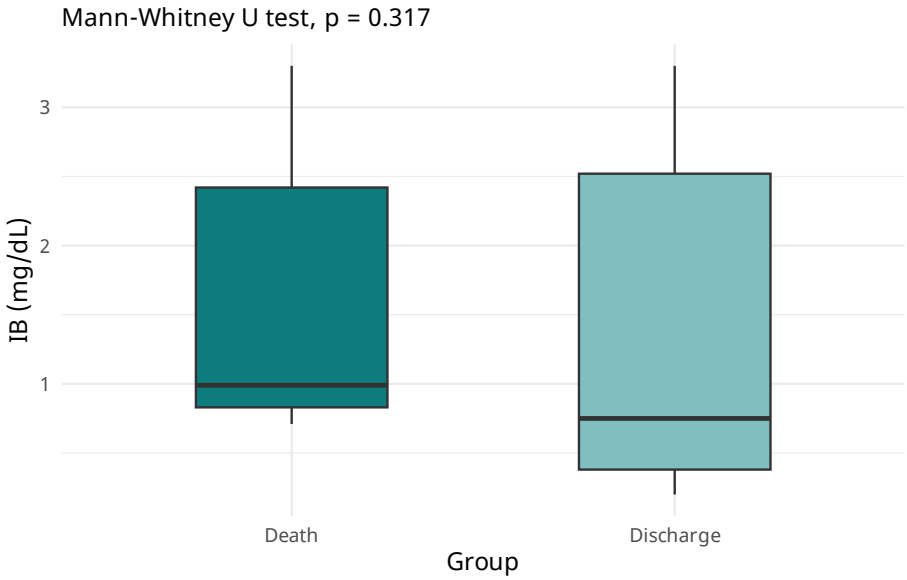
Besides affecting blood coagulation, the YF virus attacks the lymph nodes, bone marrow, brain, heart, kidney and liver. Especially, the liver damage caused by hepatocytes cell death increases the values of bilirubin, aspartate aminotransferase (AST), and alanine transaminase (ALT) (Kallas *et al.*, 2019). Then, the patients studied had liver function assessed by the quantification of total bilirubin (Fig 4A), indirect bilirubin (Fig 4B), direct bilirubin (Fig 4C), AST (Fig 5A) and ALT (Fig 5B).

The results showed elevated levels of bilirubin and its fractions in both groups of patients analyzed when the results were compared with the reference value (Fig 4A, 4B e 4C).

A


Student's t-test, p = 0.819
TB
5
4
3
2
1
Death
Discharge
Group

B


Mann-Whitney U test, p = 0.317
IB (mg/dL)
3
2
1
Death
Discharge
Group

C

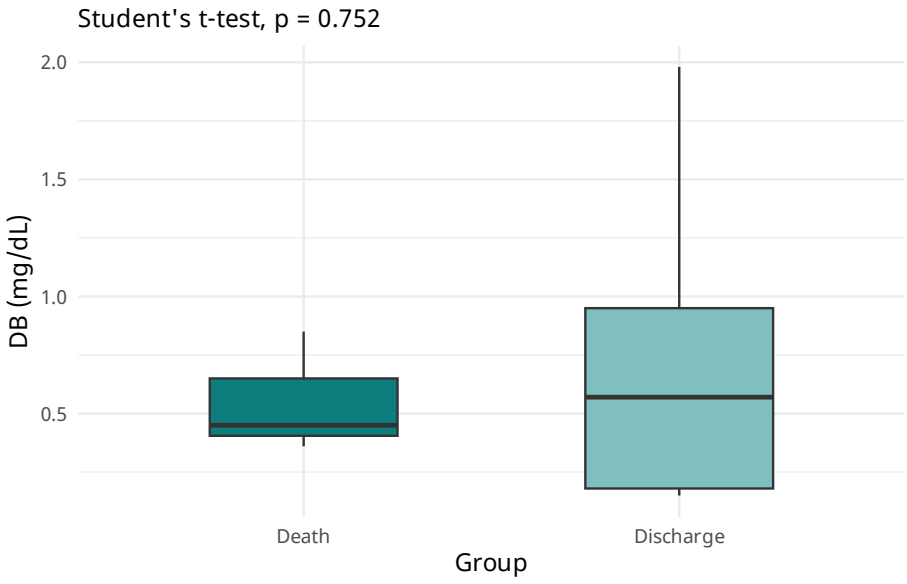
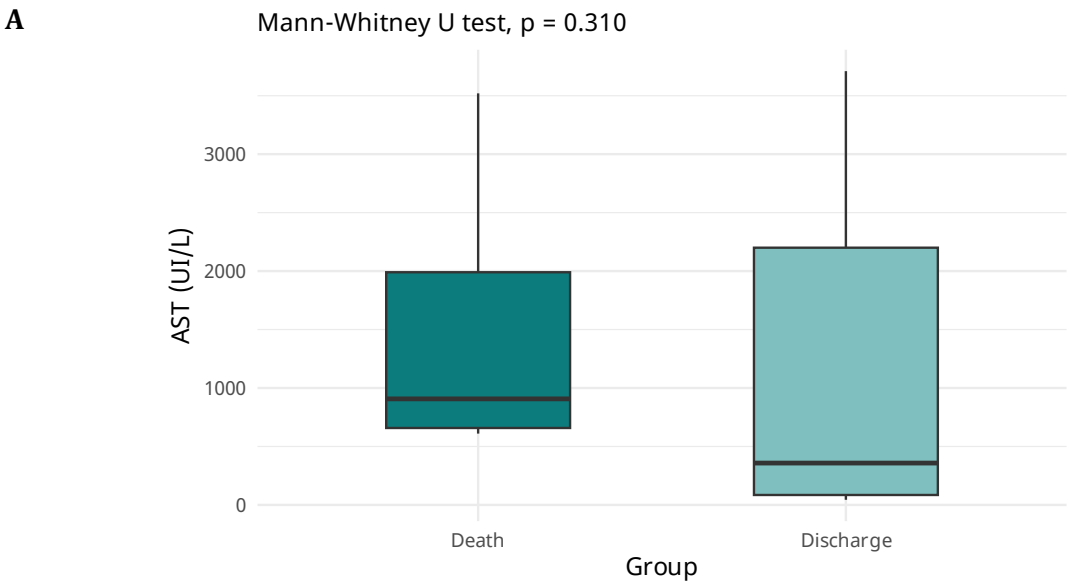

Student's t-test, p = 0.752
DB (mg/dL)
2.0
1.5
1.0
0.5
Death
Discharge
Group

Figure 4. Measurement of total bilirubin – TB (A), indirect bilirubin – IB (B), and direct bilirubin – DB (C) concentrations, in mg/dL, from blood samples from patients on the first day of hospitalization, who were discharged and those who died. TB, IB, and DB were compared between death (n = 5, 5, and 3, respectively) and discharge (n = 9, 9, and 9, respectively) groups (TB: 2.13 ± 1.22 vs. 1.92 ± 1.76 mg/dL; IB: 1.65 ± 1.15 vs. 1.25 ± 1.20 mg/dL; DB: 0.55 ± 0.26 vs. 0.67 ± 0.61 mg/dL), with no statistically significant differences observed ($p < 0.05$). Reference values (RV): DB < 0.30 mg/dL; IB 0.20–0.80 mg/dL; TB < 1.2 mg/dL.
Source: Author, 2025.

When the liver enzymes were assessed, the AST (Fig 5A) and ALT (Fig 5B) levels displayed an increase around 20 times more in both groups of patients analyzed when the results were compared with the reference value. Interestingly, the increase of ALT levels in the group that progressed to death was much higher (60 times) than the group that was discharged, with statistical significance (Fig 5B). These data indicate that this remarkable increase in ALT levels was associated with mortality and may represent a potential prognostic marker requiring validation in larger cohorts, consistent with data already described in the literature (Kallas *et al.*, 2019). The dosage of glutamic-pyruvic transaminase (ALT) in the group that progressed to death was higher than the group that was discharged, with statistical significance, several $p<0.05$ (Fig 5B) which may have contributed to the metabolic acidosis (Scheiner *et al.*, 2017). Taken together, these findings reveal severe liver damage in patients infected with the YF virus.



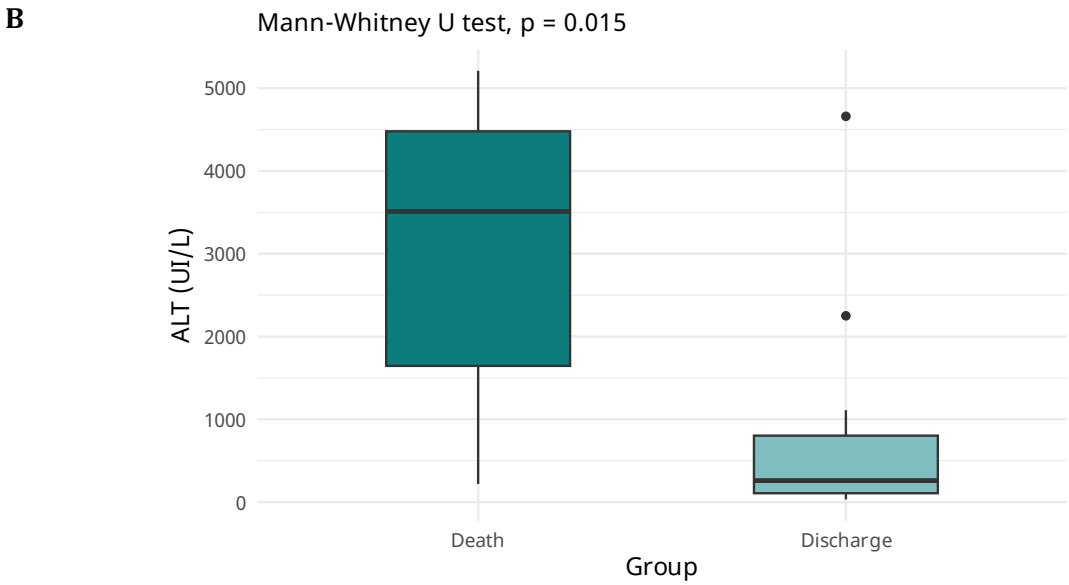


Figure 5. Measurement of aspartate aminotransferase (AST) (A) and alanine transaminase (ALT) (B) concentrations, in U/L, from blood samples from patients on the first day of hospitalization, who were discharged and those who died. AST and ALT were compared between death (n = 5 and 6, respectively) and discharge (n = 11 and 12, respectively) groups (AST: 1537.00 ± 1241.61 vs. 1167.64 ± 1369.82 U/L, p = 0.310; ALT: 3045.50 ± 1988.97 vs. 835.42 ± 1364.39 U/L, p = 0.015*). The asterisk (*) indicates a statistically significant difference between groups ($p < 0.05$). Reference values (RV): AST/TGO < 40 U/L; ALT/TGP < 56 U/L.
Source: Author, 2025.

Studies have shown that the systemic lesions caused by the virus have induced damage to the liver and other organs and may have led to the death of patients Duarte-Neto *et al.*, 2019; Duarte-Neto *et al.*, 2019; Ho *et al.*, 2019; Kallas *et al.*, 2019; Ribeiro *et al.*, 2019).

These findings are in line with Kallas *et al*. (2019), who identified elevated AST, creatinine, and neutrophil count as independent predictors of death in a larger observational cohort of YF patients, reinforcing the biological plausibility of the biochemical alterations observed in our sample despite its smaller size (Table 2). Similarly, Ribeiro et al. (2019) reported that elevated transaminase levels were associated with fatal outcomes in a reference hospital cohort in São Paulo, corroborating the pattern of hepatic injury observed in the present study. In addition, Fradico *et al*. (2023) demonstrated that soluble inflammatory mediators were associated with disease severity and late-relapsing hepatitis in YF patients, suggesting that the biochemical alterations described here may reflect a broader systemic inflammatory process rather than isolated hepatic or renal dysfunction.

The kidneys from patients with YF have difficulty in blood filtration, because the pathogenesis is based on acute tubular necrosis and fatty tubular degeneration, in addition to glomerular alterations perceived to a lesser extent, associated with altered permeability to proteins and albuminuria. The renal function of the observed patients was assessed through the levels of Urea (Fig 6A) and Creatinine (Fig 6B). Both parameters are noticeably altered and above the reference values in the group that evolves to death, with statistical significance at $p<0.05$.

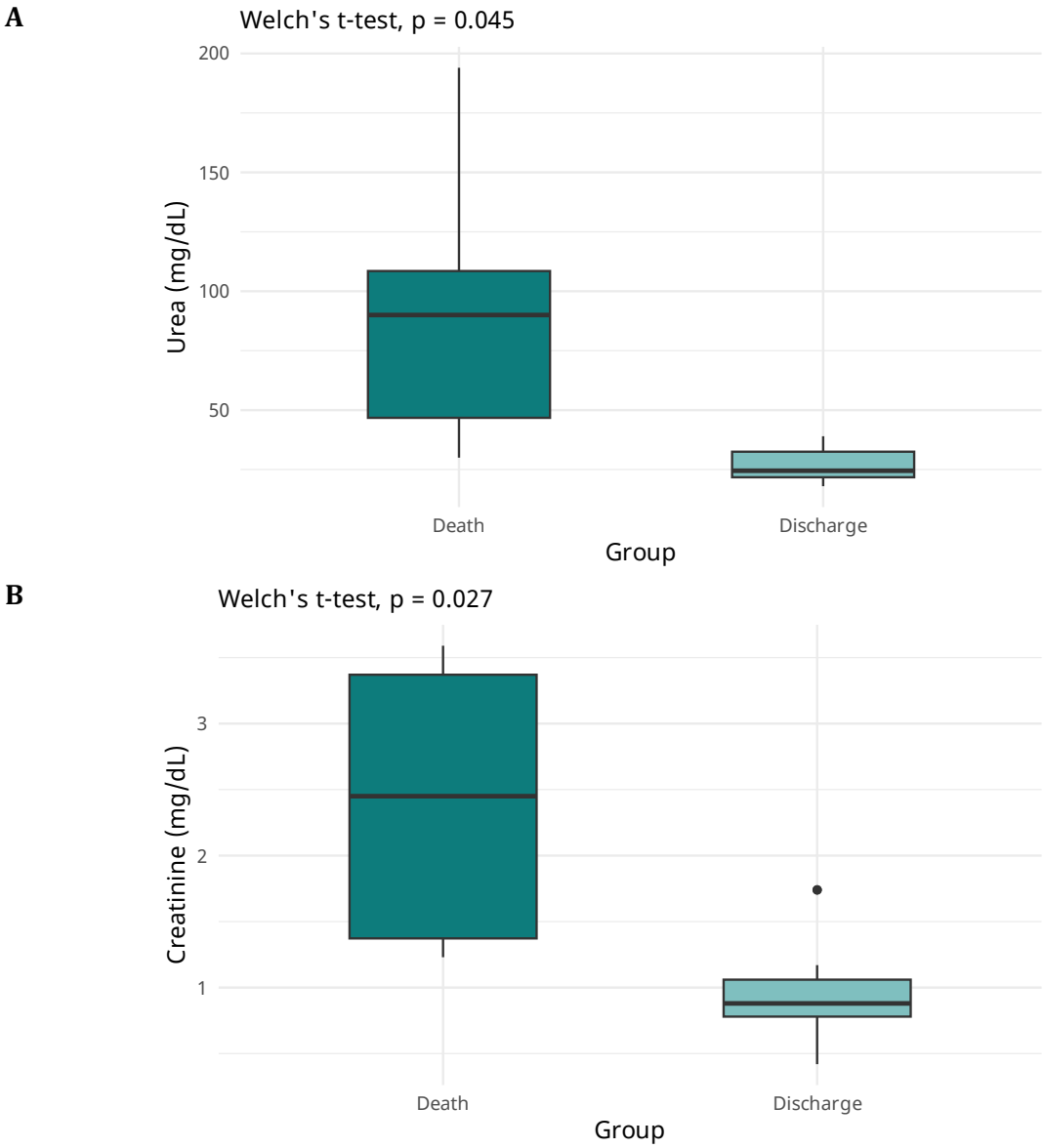


Figure 6. Urea (A) and creatinine (B) concentrations, in mg/dL, of blood samples from patients on the first day of hospitalization who were discharged and those who died. Urea and creatinine were compared between death (n = 6 and 6, respectively) and discharge (n = 10 and 11, respectively) groups (Urea: 91.83 ± 60.05 vs. 26.60 ± 7.41 mg/dL, p = 0.045*; Creatinine: 2.40 ± 1.13 vs. 0.99 ± 0.42 mg/dL, p =

0.027*). The asterisk (*) indicates a statistically significant difference between groups ($p < 0.05$). Reference values (RV): Urea 10–45 mg/dL; Creatinine 0.50–1.20 mg/dL.
Source: Author, 2025.

This excess of urea in the body and the malfunction of the kidneys, evidenced by the high values of creatinine in the bloodstream associated with (Ho *et al.*, 2019) that proved the need to perform renal replacement therapy in 73% of patients and a rate of 84% of renal failure among those hospitalized for YF are aggravating factors for blood acidosis (Leal, Leite Júnior and Mafra, 2008; Ho *et al.*, 2019). Among the consequences of acidosis are the worsening of renal failure, systemic inflammation, and protein degradation, factors that worsen the health of patients (Leal, Leite Júnior and Mafra, 2008; Ho *et al.*, 2019).

Table 2. Consolidated biochemical results — official EasyStatistic output: D = Death; A = Discharge (Alta). All statistics, exact p-values and effect sizes (with 95% CI) below are taken directly from the EasyStatistic report (Silva, B. S. EasyStatistic, v.1.0, 2026), not recalculated. Rows shaded blue reached statistical significance ($p < 0.05$).

Variable	n (D/A)	Test used	Statistic	p-value	Effect size (95% CI)	Type	Magnitude
Hemoglobin (g/dL)	6/10	Student's t-test	t=-1.05, df=14	0.310	d = 0.54 [-0.50, 1.57]	Cohen's d	Medium
Hematocrit (%)	5/11	Student's t-test	t=-0.23, df=14	0.821	d = 0.12 [-0.94, 1.18]	Cohen's d	Negligible
Leukocytes (/mm³)	4/12	Mann-Whitney U	U=22.00	0.844	r = -0.08 [-0.64, 0.53]	Rank-biserial	Negligible
Platelets (/mm³)	5/12	Mann-Whitney U	U=40.00	0.319	r = 0.33 [-0.27, 0.75]	Rank-biserial	Medium
PAT (s)	4/3	Mann-Whitney U	U=2.00	0.229	r = -0.67 [-0.94, 0.12]	Rank-biserial	Large*
Total bilirubin (mg/dL)	5/9	Student's t-test	t=0.23, df=12	0.819	d = -0.13 [-1.22, 0.97]	Cohen's d	Negligible
Indirect bilirubin (mg/dL)	5/9	Mann-Whitney U	U=14.50	0.317	r = -0.36 [-0.77, 0.27]	Rank-biserial	Medium
Direct bilirubin (mg/dL)	3/9	Student's t-test	t=-0.33, df=10	0.752	d = 0.22 [-1.10, 1.52]	Cohen's d	Small
AST (U/L)	5/11	Mann-Whitney U	U=18.00	0.310	r = -0.35 [-0.76, 0.26]	Rank-biserial	Medium
ALT (U/L)	6/12	Mann-Whitney U	U=10.50	**0.015**	r = -0.71 [-0.90, -0.29]	Rank-biserial	**Large**

Variable	n (D/A)	Test used	Statistic	p-value	Effect size (95% CI)	Type	Magnitude
Urea (mg/dL)	6/10	Welch's t-test	t=2.65, df=5.09	**0.045**	d = -1.79 [-2.98, -0.56]	Cohen's d	**Large**
Creatinine (mg/dL)	6/11	Welch's t-test	t=2.96, df=5.75	**0.027**	d = -1.92 [-3.11, -0.69]	Cohen's d	**Large**

* PAT effect size is large but based on very small groups (n = 4 vs. 3) and a wide, boundary-crossing 95% CI [-0.94, 0.12]; interpret with caution, as also noted for platelets, leukocytes, and bilirubin fractions.

4 DISCUSSION

Of the total number of hospitalized patients, 40% died during the 2017 outbreak. Great characteristic of the injuries of the yellowish infection in the liver is the inflammatory mid-zonal necrosis. As it is a type of death that expels intracellular contents, hepatic necrosis caused by YF results in the spillage of hepatic enzymes into the blood (Vasconcelos, 2003).

Interestingly, the severity of bleeding is not always associated with the number of platelets, but with problems linked to complement activation and consumption of clotting factors, especially prothrombin, factor VIII, and thromboplastin. This indicates that in YF there is consumption coagulopathy and disseminated intravascular coagulation (Chippaux and Chippaux, 2018).

Aspartate aminotransferase (AST), which is found in the cytoplasm and mitochondria of different cell types, undergoes impaired energy production in severe cases, potentially causing ATP depletion and, above all, necrosis (Avaria *et al*., 2012). However, considering that AST is located across various cell types, it may be indicative not only of hepatic complications but also of damage to other tissues (Kim *et al*., 2008). Although AST did not show statistical significance regarding the clinical outcome in this study, the enzyme alanine aminotransferase (ALT) demonstrated statistical significance and is markedly increased in the blood (Tuboi et al., 2007), reinforcing that the increase in the concentration of this specific enzyme is directly related to the severity of liver conditions (Kim *et al*., 2008).

Simultaneously with the increase in transaminases, an increase in the concentration of total bilirubin is also observed, mainly due to the greater increase in

indirect bilirubin (Tuboi *et al.*, 2007) (Figure 4) which is a product resulting from the lysis of red blood cells and is found circulating in the blood associated with albumin. Bilirubin tests are extremely important to diagnose a liver problem, which can also cause a decrease in the liver's metabolism rate (Vítek and Ostrow, 2009). In summary, direct or conjugated bilirubin, which is formed after the action of glucuronyl transferase on indirect bilirubin, may also be elevated. Hepatic and biliary complications also associated with increased AST and ALT may be an indication of biliary obstruction, dysfunctions in the regulation of protein, carbohydrate and lipid as wel storage and release of substances (Bertolami, 2005; Vítek and Ostrow, 2009).

The inflammatory process caused by viruses and bacteria potentiates the expression of pro-inflammatory cytokines which in turn increase the generation of Reactive Oxygen Species (ROS) as well as cell injury and reduction of the population of white cells (Deakin *et al*., 1995; Steffensen *et al*., 2014; Hirano *et al.*, 2017; Li Li *et al*., 2018; McGarry *et al*., 2018). Ammonia, one of the residues of bacterial metabolism, is no longer eliminated by the liver and can attack neurons and other cells of the central nervous system (Ho *et al*., 2019). Thus, high rates of direct and indirect Bilirubin, AST, ALT, Urea, and Creatinine represented in Figure 5 and 6, seem to be correlated with the death of hospitalized patients (Duarte-Neto *et al*., 2019; Kallas *et al*., 2019; Ho *et al*., 2019). The schematic representation (Fig 7) shows that in the healthy liver, the GOT and GPT enzymes are mostly contained within the liver cells, with insignificant doses in the bloodstream, and the indirect bilirubin is converted into direct bilirubin in the hepatocytes, to be excreted in the liver and intestine. In the kidney, urea and creatinine are filtered from the blood and excreted in the urine (Figure 7A). On the other hand, the pathophysiology of YF involves direct damage to hepatocytes, resulting in the release of liver enzymes into the bloodstream. Furthermore, bilirubin conversion is reduced which elevates indirect bilirubin while subsequent biliary complications may explain the increase in direct bilirubin. In the renal system, in turn, the virus compromises the glomerular filtration rate of urea and creatinine, promoting a consequent increase in their serum levels (Figure 7B).

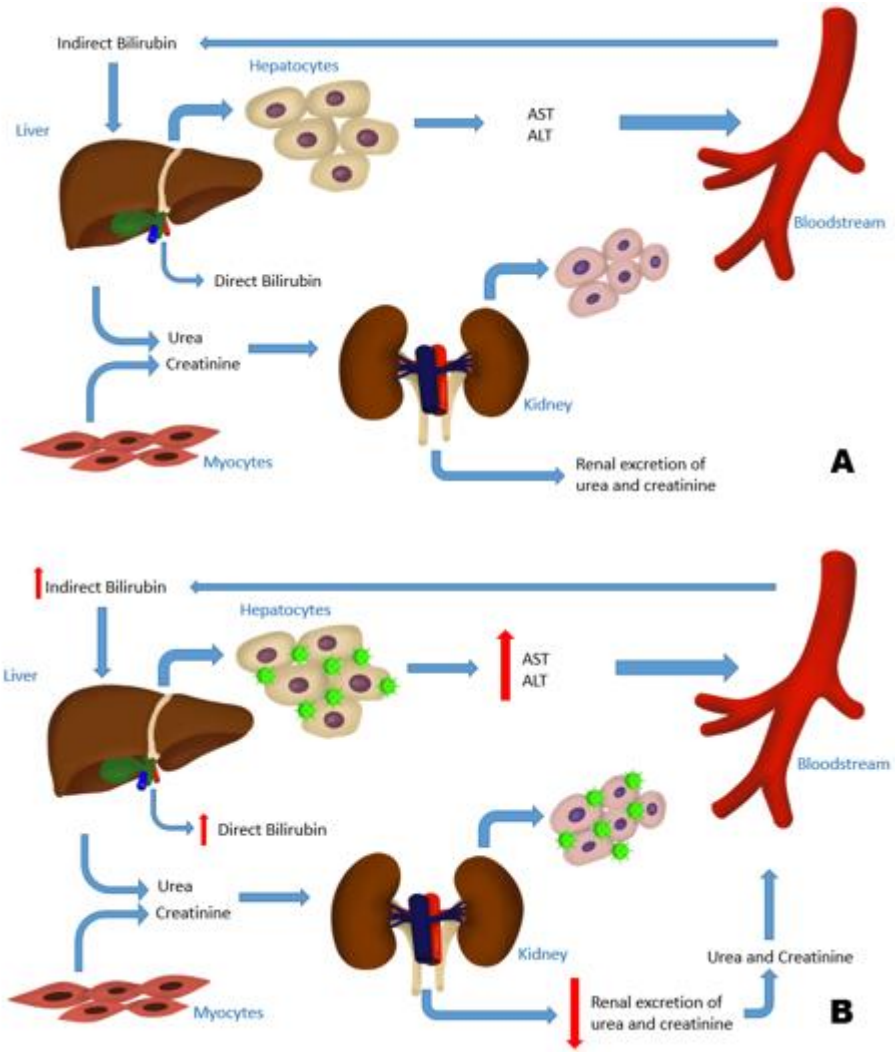


Figure 7. Schematic representation of the biochemical/pathological effects caused by the YF virus. **A** represents liver and kidney physiology. **B** represents the pathophysiology of YF in the liver and kidney. **Source:** Author, 2025.

Effects of these renal disorders is the increase in urea and creatinine levels, which may generate the occurrence of proteinuria in the urine, due to damage to the renal tubules (Vasconcelos, 2003; Arogundade *et al.*, 2020). In addition, renal failure in YF is characterized by a decrease in urine volume, which may progress to anuria due to necrosis hosted in the renal tubules (Vasconcelos, 2003; Arogundade *et al.*, 2020).

By diagnosis, the severe form of the disease must present at least one of the classic symptoms, which are, hematemesis, jaundice or oliguria. The combination of these symptoms characterizes hepatorenal failure, which is the malignant form of the disease (Vasconcelos, 2003; Bernuau, 2006; Arogundade *et al.*, 2020). In severe

cases of YF, disseminated intravascular coagulation (Chippaux and Chippaux, 2018), a decrease in fibrinogen and Factor VIII, essential for blood clotting, can occur, in addition to thrombocytopenia, which is the reduction of platelet level. The association of these conditions is an increased risk of bleeding (Vinholt, 2019; Hadid, Kafri and Al-Katib, 2021). Researchers showed that blood transfusion has been favorable in the recovery of patients with YF (Ho *et al.*, 2019). In addition to the direct damage to the liver, spleen, lymph nodes, bone marrow, brain, and heart caused by the virus, bacterial action is also associated with the worsening of the pathology as well as the release of toxins that cause damage to the lung, intestine, and pancreas. Although not investigated in this study, the high number of deaths may be involved in diabetic patients (Ho *et al.*, 2019), due to the predisposition that diabetics have to present blood acidosis, and release of AST/ALT, urea, and creatinine can potentiate the death of patients with YF. Some studies have shown that serum transaminase levels are associated with disease severity and clinical course in patients with YF who died, reinforcing their relevance as candidate markers warranting further investigation in larger cohorts (Ribeiro *et al*., 2019).

This study has some limitations that should be considered when interpreting these findings. The relatively small sample size (n=18) reflects the epidemiological reality of a single-center cohort during the 2017 YF outbreak in Espírito Santo, and limits the precision of the estimates as well as the generalizability of the results to other populations or outbreaks. Therefore, the associations observed between biochemical markers and mortality should be interpreted as preliminary and hypothesis-generating, consistent with the retrospective, single-center design of this study, rather than as validated predictors of clinical outcome. Additionally, clinically relevant variables such as comorbidities, vaccination status, time from symptom onset to hospitalization, and a standardized measure of clinical severity at admission were not systematically available in this retrospective cohort. The absence of these variables limits the ability to adjust for potential confounding factors and should be addressed in future prospective studies with more comprehensive clinical data collection.

5 CONCLUSION

Elevated levels of ALT, urea, and creatinine on admission were significantly associated with in hospital death among patients hospitalized during the 2017 yellow fever outbreak in Espírito Santo (Mann-Whitney U, p = 0.015, r = -0.71; Welch's t-test, p = 0.045, d = -1.79; and p = 0.027, d = -1.92, respectively), whereas hematological parameters, bilirubin fractions, AST, and coagulation markers showed no statistically significant differences between groups. Given the small, single-center retrospective cohort (n = 18, 7 deaths) and the absence of multivariable adjustment, these findings should be interpreted as preliminary evidence of association rather than validated prognostic capacity, warranting confirmation in larger, prospective, multicenter studies.

Conflict of Interest: The authors declare no conflict of interest.

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