

## Case Report

# Bee sting envenomation severe cases in Manaus, Brazilian Amazon: clinical characteristics and immune markers of case reports

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### Abstract

Bee venom is a natural toxin composed of several peptides. Massive envenoming causes severe local and systemic reactions. We report two cases of severe bee envenomation, of which one was fatal. We also describe clinical characteristics and immune markers. Both victims suffered from respiratory distress, renal failure, rhabdomyolysis, and shock. They required invasive mechanical ventilation, vasoactive drugs, and renal replacement therapy. Moreover, serum levels of chemokines, cytokines, and cell-free circulating nucleic acids demonstrated an intense inflammatory process. Massive envenoming produced systemic injury in the victims, with an uncontrolled inflammatory response, and a more significant chemotactic response in the fatal case.

**Keywords:** Africanized bees. Inflammatory response. Systemic injury.

### INTRODUCTION

In 1956, African bees were imported to Brazil to crossbreed with European bees for commercial purposes. Accidentally, African queens initiated the Africanization of other species of honeybees in the Americas. Africanized bees are aggressive and can attack in large groups. This may result in the death of a patient due to the high volume of venom inoculation<sup>1</sup>. In Brazil, between 2000 and 2018, overall, 159,520 cases of bee stings were reported to the official


surveillance system. These resulted in 466 deaths (0.3%). In the same period, the Brazilian Amazon recorded 6,799 cases and 22 deaths, primarily in urban areas, with similar lethality<sup>2</sup>.

Bee venom (BeV) is a natural toxin comprising peptides, including melittin, adrenaline, dopamine, histamine, hyaluronidase, noradrenaline, phospholipases A<sub>2</sub> and B, and serotonin, apamin, and melittin<sup>3</sup>. After a massive envenomation by Africanized bees, clinical features include intravascular hemolysis, bleeding, acute respiratory distress syndrome, hypertension, myocardial damage, hepatic dysfunction, acute renal failure (ARF), rhabdomyolysis, shock, and coma<sup>3</sup>.

The components of BeV have several local and systemic actions<sup>4</sup>. Immunological interactions between the venom and immune cells show a pattern of response mediated by mast cells, which are

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activated by immunoglobulin E (IgE) antibodies. BeV components may induce faster systemic envenomation through hyaluronidase (spreading factor) and hydrolyzed hyaluronan fragments due to its pro-inflammatory, pro-angiogenic, and immunostimulatory properties, thus leading to rapid spread and massive envenoming<sup>5</sup>.

The inflammatory processes and clinical characteristics involved in massive envenoming that evolve to death remain poorly understood. In this study, we report two cases of severe bee stings that were treated at the *Fundação de Medicina Tropical Doutor Heitor Vieira Dourado* (FMT-HVD), a tertiary hospital in Manaus, Western Brazilian Amazon. This study was approved by the local Ethical Review Board (process #713.140).

### CASE REPORTS

**Case 1:** A 70-year-old man, who resided in a rural area of Careiro da Várzea (approximately 30 km from Manaus by river) presented to the FMT-HVD emergency department (ED), with a sore throat, myalgia, nausea, three vomiting episodes, diarrhea, anuria, and breathlessness. The patient was reported to have been stung by a swarm of bees while working in a rural area. Mild jaundice (+/4+) was noted. Local signs and symptoms included pain, edema, and pruritus in the affected area. The patient was diagnosed with uncontrolled diabetes and hypertension and was treated with enalapril. No allergies were reported.

Physical examination revealed massive bee stings to the head, thorax, arms and legs (**Figure 1A**), hypertension (blood pressure [BP] = 200/100 mmHg), tachycardia (heart rate [HR]=118 bpm), dyspnea, and hyperglycemia (329 mg/dL), although no fever. The time between being stung and hospital admission was ~20 h. Respiratory failure was treated with adrenaline, dexamethasone, methylprednisolone, fenoterol, hydrocortisone, and dexchlorpheniramine. Subsequently, furosemide and captopril were administered. The remaining embedded stings were removed. Despite a slight clinical improvement, the patient developed agitation, dysphonia, apathy, hyperglycemia (313 mg/dL), BP elevation (189/111 mmHg), tachycardia (120 bpm), and tachypnea (respiratory rate [RR] = 35 breaths/min). The patient was transferred to the intensive care unit (ICU). The patient was dehydrated, scored 13 on the Glasgow coma scale, and RR was stabilized with non-invasive oxygen supplementation. However, no electrocardiographic alterations were identified, and upper respiratory tract swelling was absent.

**Supplementary material: Table 1** presents the laboratory parameters obtained in Case 1. Six hours after admission, the patient presented with swelling in his throat, laryngeal stridor, respiratory distress, and profuse sweating. The patient was not responsive to epinephrine or hydrocortisone nebulization. The patient developed peripheral cyanosis and hypotension, which was treated with norepinephrine. Endotracheal intubation was performed. The tongue, glottis, and epiglottis were swollen. During intubation, the patient developed seizures, which stopped after mechanical ventilation. Peripheral cyanosis and anuria persisted 8 h after intubation. Cardiorespiratory arrest and death occurred after resuscitation attempts. A necropsy was not authorized by the family.

**Case 2:** A 10-year-old boy, residing in the rural area of Urucurituba, approximately 339 km from Manaus by river,



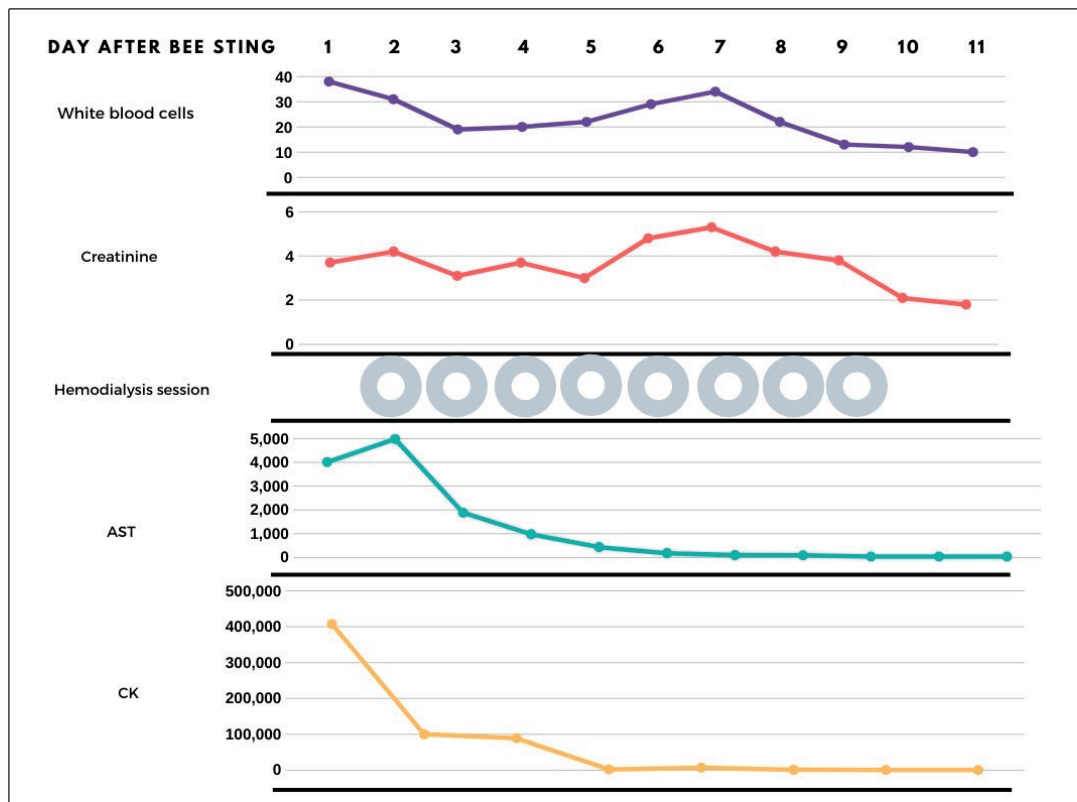
**FIGURE 1:** Clinical presentation of the patients. **1(A):** Case 1 on admission; photo shows bee stings to the head. **1(B):** Case 2 intubated in pediatric ICU; Patient presented tachypnea, swollen face, eyelids and lip edema, myalgia, and hardened edema in his chest.

presented to the FMT-HVD in a somnolent state, but conscious, after suffering a massive bee attack the previous day while playing. Physical examination revealed ~200 bee stings to the head, face, neck, upper limbs, and trunk. No comorbidities were reported. Time between the stinging and hospital admission was 24 h. Intense pain, edema, and pruritus in the affected areas were reported.

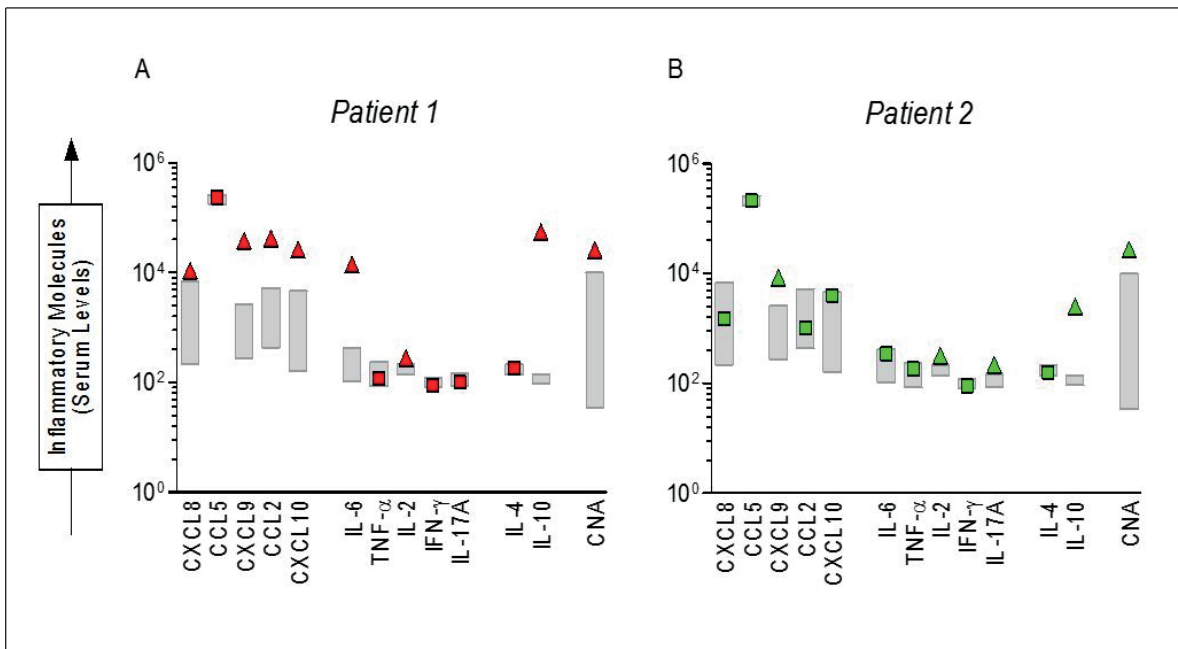
On physical examination, the patient presented with tachypnea, a swollen face, eyelids and lip edema, mild jaundice (+/4+), myalgia, sialorrhea, moaning, hardened chest edema, and a flat rigid abdomen painful to palpation, diarrhea, and elevated serum creatinine (**Figures 1B and 2**). The remaining stingers were removed. The patient was transferred to the pediatric ICU and intubated. Adrenaline, methylprednisolone, fenoterol, hydrocortisone, and dexchlorpheniramine were administered. Two days after admission, elevated muscle enzymes, aminotransferases, and lactate dehydrogenase revealed severe rhabdomyolysis (**Figure 2**). Oliguric ARF required eight hemodialysis sessions.

**Supplementary material: Table 2** presents the detailed laboratory follow-up of Case 2. The patient developed a hypertensive crisis, which was treated with captopril, amlodipine, and losartan. Abdominal ultrasonography revealed mild ascites, alithiasic cholecystitis, splenomegaly, and bilateral acute glomerulopathy. Otorrhea, with neutrophilia and fever, was treated with ceftriaxone, metronidazole, and clindamycin. The patient improved, and was discharged after 15 days. No pain or discomfort was reported during follow-up.

**Circulating immune marker level measurements in both cases:** Levels of circulating chemokines, cytokines, and nucleic acids (CNAs) are shown in **Figure 3** for both cases. **Supplementary material: Material and Methods** shows how the measurements of circulating immune markers were performed. There was an increase in the levels of chemokines (CXCL-8, CXCL-9, CCL-2, and CXCL10) in Case 1, considering the 95% CI of the mean values



**FIGURE 2:** Follow-up of Case 2. Rhabdomyolysis is a well-known cause of acute kidney injury and renal failure. Details of laboratory tests are presented in Supplementary material: Table 2.



**FIGURE 3:** Serum levels of chemokines, cytokines and cell-free nucleic acids in severe bee sting cases at hospital admission. 3(A): Case 1. 3(B): Case 2. The gray boxes represent serum levels of a control group, that consisted of 20 healthy subjects, ages ranging from 22 to 36 years (median = 27 years).

observed for a control group of age-matched healthy individuals. However, only the CXCL-9 chemokine was increased in Case 2. No difference was observed in CCL-5. The pro-inflammatory cytokine IL-6 level increased in Case 1. The regulatory cytokine IL-10, cell proliferation cytokine IL-2, and CNAs increased in both patients.

## DISCUSSION

Bee stings cause pain, edema, nausea, pruritus, erythema, vomiting, diarrhea, myalgia, respiratory distress, tachycardia, and vomiting, as previously reported<sup>3</sup>. Moreover, both cases presented exuberant clinical manifestations due to massive bee stings and the volume of venom injected. In fact, both cases showed signs of respiratory distress, intense rhabdomyolysis, and increased levels of inflammatory markers. Furthermore, the dynamic river topography in the Amazon greatly delays access to proper health care.

Multiple bee stings always constitute a medical emergency, and patients who have suffered a mass attack by Africanized bees (>50 stings) should be monitored, as the venom may persist in circulation for days and cause delayed reactions<sup>2</sup>. Inflammation results from an unbalanced immune response associated with the elevated production of pro-inflammatory cytokines, chemokines, and histamine. These are secreted through the degranulation of mast cells triggered by allergen stimuli, which are central in acute hypersensitivity reactions. This hypersensitivity inflammation has been associated with mitogen-activated protein kinase and nuclear factor- $\kappa$ B signaling cascade of mast cells and leukocytes with an inhibitory effect. This effect has been shown to be dependent on BeV concentrations<sup>6</sup>.

The immune profile of Case 1 showed a remarkable increase in chemokines, which was probably associated with severe envenomation owing to the massive envenoming, as the patient did not mention any bee allergy, excluding anaphylaxis. The consequences of an increase in immune-mediated reactions secondary to massive exposure to venom might have produced acute envenoming syndrome and immune regulation<sup>1,3</sup>. Moreover, BeV action is dependent on the time, dose, and type of cell affected, producing a significant suppression of the immune response, with less leukocyte migration and concentrations of inflammatory mediators<sup>5</sup>. Hyperglycemia can also be considered a risk factor during envenoming. This has been shown to be associated with cytokine-mediated disruption of insulin secretion in severe scorpion envenoming.

The presence of comorbidities in Case 1 may have influenced the higher levels of pro-inflammatory biomarkers and, consequently the patient's outcome. The exact extent to which both comorbidities contribute to immune regulatory exhaustion and clinical outcomes in such cases is currently unknown. Unfortunately, venom-specific IgE level analysis to confirm hypersensitivity to BeV allergens and plasma mast cell tryptase activity were not available for either patient. However, it is possible that the wide inflammatory activation with extensive release of anaphylactogenic mediators may have contributed to anaphylactic symptoms and clinical outcomes (bronchoconstriction, hypotension, angioedema, abdominal pain, etc.).

Envenoming, such as those generated by BeV, induces an intense inflammatory process, in which neutrophils influence the

concentrations of other inflammatory molecules. Although we did not perform absolute counts of neutrophils, eosinophils, basophils, lymphocytes, and monocytes, Case 1 presented a marked increase in chemokines (CXCL-8, CXCL-9, CCL-2, and CXCL10). The high levels of these chemokines released by endothelial cells in the skin and mucosal bite locations, promoted intense cell recruitment and inflammatory response<sup>7</sup>. In many cases, chemokines activate target cells, increasing the production of inflammatory molecules, oxygen radicals, and nitric oxide, which induce the degranulation of neutrophils and other cell types. Furthermore, this response contributes to host immune defense locally. However, the exacerbated production of these inflammatory molecules promotes tissue destruction and impaired function of the affected organs<sup>7</sup>.

Regarding toxic reactions, rhabdomyolysis was the hallmark of both cases, and both evolved to renal failure. One possible mechanism for muscle damage involves the direct action of venom toxic components, such as melittin and phospholipase A<sub>2</sub>, on skeletal muscle tissue<sup>8</sup>. Generally, rhabdomyolysis causes problems that range from asymptomatic elevations of creatine kinase (CK) to life-threatening electrolyte changes and severe acute kidney injury. An in-hospital mortality risk score for patients with rhabdomyolysis may be obtained using demographic, clinical, and laboratory admission data. According to this score, both patients presented a 60% risk of death and a need for continuous renal replacement therapy<sup>9</sup>.

In this study, an elevation of CK-MB levels was recorded in both patients. Enzymatic changes and morphological lesions of the acute myocardial infarction type were found in rats, showing a possible direct toxic action of the venom on cardiac muscle<sup>1</sup>. The severe toxic effects of BeV would ideally be treated with antivenom. Although there have been several successful attempts to produce bee antivenoms, there are no antivenoms currently available in Brazil<sup>10</sup>.

Increased levels of CNAs have been previously reported in sepsis, trauma, and cancer. Furthermore, they are speculated to be useful biomarkers for clinical outcomes. Their origin is debatable. CNAs have been associated with neutrophil extracellular traps<sup>11</sup>. Nonetheless, an experimental sepsis model showed otherwise, which led us to hypothesize an association with necrotic cells<sup>11</sup>. Besides, the association between BeV and DNA damage is controversial<sup>12</sup>. In this report, both cases presented higher CNAs than controls. However, neutrophils were not quantified. The relationship between CNAs and neutrophils was not established, and therefore deserves further investigation in massive bee envenoming.

According to the two cases presented here, the patient that evolved to death presented a clinical case of severe respiratory distress and more significant inflammatory/chemotactic response when compared to the patient with predominant rhabdomyolysis and milder respiratory distress, despite being under intensive clinical support. Thromboembolic and hemorrhagic markers were not available for these patients. Nonetheless, such markers would help to further understand the pathogenesis and clinical outcomes. Immune markers, such as chemokines (CXCL-8, CXCL-9, CCL-2, and CXCL10), also seem to be distinct between the two clinical presentations, which could be used as predictors of severity. This study depicts the outcome-impacting aspects of bee sting

envenomation in the Amazon: delayed access to proper health care, the complex management of such cases, and how intricate the immune response is to massive loads of BeV, besides how this appears to influence the clinical outcome. Prospective investigation of the BeV immune response is warranted to further comprehend such aspects.

### ETHICS APPROVAL

This study was approved by the local Ethics Review Board (Approval #713.140). The family gave consent in both cases.

### ACKNOWLEDGMENTS

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### AUTHORS' CONTRIBUTIONS

IMS, WMM, JAGS, AGC and FFAV designed study and wrote the manuscript. MCS, AGC and FFAV analysed and interpreted data. ESB, GCM and AGC performed the experiments. WMM, JAGS, MCS and MVGL revised the manuscript. All authors read and approved the final manuscript.

### CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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**Supplementary material: TABLE 1:** Laboratory parameters obtained from Case 1.

Parameters	April 25 <sup>th</sup> , 2019	April 26 <sup>th</sup> , 2019
Hemoglobin (g/dL)	15.5	15.7
White blood cells ( $10^3/\text{mm}^3$ )	27.9	38.4
Platelets ( $10^3/\text{mm}^3$ )	438.9	328.6
Creatinine (mg/dL)	4.1	7.1
Alkaline phosphatase (IU/L)	251	340
Gamma-glutamyl transferase (IU/L)	26	36
Total bilirubin (mg/dL)	1.33	1.23
Direct bilirubin (mg/dL)	0.02	0.02
Indirect bilirubin (mg/dL)	1.31	1.21
Glucose (mg/dL)	344	149
Potassium (mmol/L)	6.9	7.9
Sodium (mmol/L)	135	137
Aspartate aminotransferase (IU/L)	513	1998
Alanine aminotransferase (IU/L)	62	384
Urea (mg/dL)	99	174
Albumin (g/dL)	4.2	3.5
Creatine phosphokinase (IU/L)	19,995	40,801
Creatine phosphokinase-MB (IU/L)	381	606
Lactate dehydrogenase (IU/L)	1036	8604
pH	7.41	7.07
pCO <sub>2</sub>	22.1	42.9
pO <sub>2</sub>	69.3	50.3
sO <sub>2</sub>	93.1	74.6
Lactate (mg/dL)	44	69
Base excess (mmol/L)	-9.8	-16.3
HCO <sub>3</sub> <sup>-</sup> (mmol/L)	13.9	11.9

**Reference values:** Hemoglobin: 13.0-16.0 g/dL; White blood cells: 4.000-10.000/mm<sup>3</sup>; Platelets: 130,000-400.000/mm<sup>3</sup>; Creatinine: 0.5-1.2 mg/dL for adults; Alkaline phosphatase: male  $\leq$ 390 IU/L; Gamma-glutamyl transferase: 7-60 IU/L for males; Total bilirubin: 0.01-1.3 mg/dL; Direct bilirubin: 0.01-0.3 mg/dL; Indirect bilirubin: 0.01-0.7 mg/dL; Glucose: <99 mg/dL; Potassium: 3.6-5.2 mmol/L; Sodium: 135-145 mmol/L; Aspartate aminotransferase: 2-38 IU/L; Alanine aminotransferase: 2-44 IU/L; Urea: 10-45 mg/dL; Albumin: 3.5-5 g/dL; Creatine phosphokinase: 24-190 IU/L; Creatine phosphokinase-MB: 2-25 IU/L; Lactate dehydrogenase: 211-423 IU/L; pH: 7.35-7.45; pCO<sub>2</sub>: 35-45; pO<sub>2</sub>: 80-100; sO<sub>2</sub>: 94-97%; Lactate  $\geq$ 45 mg/dL; Base excess -2 to +2 (mmol/L); HCO<sub>3</sub><sup>-</sup>: 22-26 mmol/L.

**Supplementary material: TABLE 2:** Laboratory parameters obtained from Case 2.

Parameters	July 18 <sup>th</sup> , 2019	July 19 <sup>th</sup> , 2019	July 20 <sup>th</sup> , 2019	July 22 <sup>nd</sup> , 2019	July 23 <sup>rd</sup> , 2019	July 25 <sup>th</sup> , 2019	July 26 <sup>th</sup> , 2019	July 28 <sup>th</sup> , 2019	July 30 <sup>th</sup> , 2019	August 1 <sup>st</sup> , 2019	August 2 <sup>nd</sup> , 2019
Hemoglobin (g/dL)	10.94	10.99	8.6	7.9	8.0	10.1	10.0	11.39	8.11	8.31	7.90
White blood cells (10 <sup>3</sup> /mm <sup>3</sup> )	37.930	31.520	18.790	20.000	22.350	29.150	34.620	21.920	13.080	12.310	9.856
Platelets (10 <sup>3</sup> /mm <sup>3</sup> )	188.100	196.700	128.400	146.300	120.700	218.300	321.000	537.000	367.400	235.100	238.400
Creatinine (mg/dL)	3.7	4.2	3.1	3.7	3.0	4.8	5.3	4.2	3.8	2.1	1.8
Alkaline phosphatase (IU/L)	378	...	...	221	...	...	...	339	...	...	...
Amylase (IU/L)	499	...	...	...	...	...	...	...	...	...	...
Gamma-glutamyl transferase (IU/L)	...	...	...	...	...	...	...	...	...	...	...
Glucose (mg/dL)	128	...	...	119	...	...	86	86	...	...	...
Potassium (mmol/L)	5.0	5.3	3.9	3.7	3.5	3.6	3.9	4.0	3.5	4.2	3.8
Sodium (mmol/L)	135	132	140	142	140	137	135	137	139	138	139
Calcium	...	...	8.4	8.2	8.7	9.4	10.3	10.0	8.9	9.0	8.4
Magnesium	...	2.2	2.8	2.5	2.2	2.6	2.4	2.3	1.7	...	...
Aspartate aminotransferase (IU/L)	4009	4979	1884	976	435	181	97	91	41	36	36
Alanine aminotransferase (IU/L)	590	818	856	644	520	297	233	187	124	92	92
Urea (mg/dL)	162	188	137	131	130	176	135	86	104	97	96
Albumin (g/dL)	3.0	3.2	3.5	3.4	3.4	3.1	3.3	3.8	3.4	...	...
Creatine phosphokinase (IU/L)	...	407.708	99.986	88.874	21.006	6.655	...	882	306	...	208
Creatine phosphokinase-MB (IU/L)	...	3.888	1.385	691	288	133	...	48	26	...	30
Lactate dehydrogenase (IU/L)	12.753	...	9.803	...	5.658	4.296	...	3.275	1.726	...	...

**Reference values:** Hemoglobin: 13.0-16.0 g/dL for males; White blood cells: 4.000-10.000/mm<sup>3</sup>; Platelets: 130.000-400.000/mm<sup>3</sup>; Creatinine: 0.3-1.0 mg/dL for children; Alkaline phosphatase: male ≤390 IU/L; Gamma-glutamyl transferase: 7-60 IU/L for males; Glucose: <99 mg/dL; Potassium: 3.6-5.2 mmol/L; Sodium: 135-145 mmol/L; Aspartate aminotransferase: 2-38 IU/L; Alanine aminotransferase: 2-44 IU/L; Urea: 10-45 mg/dL; Albumin: 3.5-5 g/dL; Creatine phosphokinase: 24-190 IU/L; Creatine phosphokinase-MB: 2-25 IU/L; Lactate dehydrogenase: 211-423 IU/L; pH: 7.35-7.45; pCO<sub>2</sub>: 35-45; pO<sub>2</sub>: 80-100; Lactate ≥45 mg/dL; Base excess -2 to +2 (mmol/L); HCO<sub>3</sub><sup>-</sup>: 22-26 mmol/L.

## Supplementary material: Material and Methods

### Biological sample collection

Approximately 4 mL of peripheral blood was collected at admission from each patient and from 20 age-matched healthy controls by venous puncture in tubes containing EDTA (BD Vacutainer® EDTA K2). Samples were centrifuged (3.000 rpm, 10 min) and plasma aliquots were stored in a freezer at -80°C for measurement of inflammatory molecules.

### Immune markers measurements

The plasma soluble immune markers (CXCL-8, CCL-5, CXCL-9, CCL-2, CXCL-10, IL-6, TNF, IL-2, IFN- $\gamma$ , IL-17A, IL-4 and IL-10,) were quantified using the Cytometric Bead Array (CBA). BD™ Human Chemokine Kit (BD Biosciences, San Diego, CA, USA) and BD™ Human Th1, Th2, Th17 Cytokine Kit (BD Biosciences, San Diego, CA, USA) were used following the manufacturer's technical guidelines and protocols. A FACS Canto II flow cytometer (BD Biosciences, San Jose, CA, USA) at Fundação Hospitalar de Hematologia e Hemoterapia do Amazonas (HEMOAM) was used for sample acquisition. FCAP-Array software v3 (Soft Flow Inc., USA) was used to calculate the cytokine levels (MFI). The analyses were performed in triplicate.

### CNAs measurements

CNAs were isolated from plasma from patients or healthy donors using the QIAamp DNA Blood Mini Kit (QIAGEN, Chatsworth, CA, USA) according to the manufacturer's instructions. To quantify CNAs levels in plasma, amplification of the genomic sequence of the human telomerase reverse transcriptase (hTERT), a ubiquitous single copy gene mapped on 5p 15.33, was used as a marker of the total amount of DNA present in the samples. We used the following specific primers: **F:** 5GGC ACA CGT GGC TTT TCG 3; **R:** 5 GGT GAA CCT GCT AAG TTT ATG CAA 3; **Probe VIC5:** TCA GGA CGT CGA GTG GAC ACG GTG-3 TAMRA, as previously described (1). The DNA was amplified using an Applied Biosystems 7500 Fast System®. To normalize the amount of DNA in plasma samples, we used the dilution of plasmids containing the target region. The analysis was performed in triplicate.



**Data analysis**

Data analyses were performed using the software GraphPad Prism (v5.0). The cases' results were compared to the control group, which consisted of 20 healthy subjects, with ages ranging from 22 to 36 years (median = 27 years), all living in Manaus-AM. The values for each inflammatory molecule were compared with the interquartile range (IQR) values of the reference group.

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