Cytokine 152 (2022) 155825

Contents lists available at ScienceDirect

Cytokine

journal homepage: www.elsevier.com/locate/cytokine

Lower levels of CXCL-8 and IL-2 on admission as predictors of early adverse reactions to *Bothrops* antivenom in the Brazilian Amazon

Frandison G S Soares [a,b], Hiochelson N. Ibiapina [a,b], Marco A. Sartim [a,c], Iran Mendonça-da-Silva [a,b], Elizandra F. Nascimento [a,b], Luiz C.L. Ferreira [a,b], Felipe A. Cerni [d], Adriana Malheiro [e,h], Manuela B. Pucca [d,f], Fan H. Wen [g], Ana Maria Moura-da-Silva [b,g], Allyson G. Costa [a,e,h], Wuelton M. Monteiro [a,b,1], Jacqueline A.G. Sachett [a,i,1,*]

[a] *School of Health Sciences, Amazonas State University, Manaus, Brazil*
[b] *Department of Research, Dr. Heitor Vieira Dourado Tropical Medicine Foundation, Manaus, Brazil*
[c] *Postgraduate Department, Nilton Lins University, Manaus, Brazil*
[d] *Health Sciences Postgraduate Program, Federal University of Roraima, Boa Vista, Brazil*
[e] *Immunology Postgraduate Program, Amazonas Federal University, Manaus, Brazil*
[f] *Medical School, Federal University of Roraima, Boa Vista, Brazil*
[g] *Butantan Institute, São Paulo, Brazil*
[h] *Department of Research, Amazonas Hematology and Hemotherapy Hospital Foundation, Manaus, Brazil*
[i] *Department of Research, Alfredo da Matta Foundation, Manaus, Brazil*

ARTICLE INFO

Keywords:
Snakebite
Bothrops
Antivenom
Adverse reaction
Hypersensitivity
Cytokine
Chemokine
CXCL-8
IL-2

ABSTRACT

Snakebite envenomings are considered a global health problem. The specific therapy for these envenomings consists of administering animal-derived antivenoms aiming to neutralize the venom toxins. Antivenoms have been used effectively to treat snakebites for more than a century; however, their administration may result in early and/or late adverse reactions. The present study presents the prevalence of early adverse reactions (EARs) towards *Bothrops* antivenom therapy in a health tertiary unit in the Brazilian Amazon and explores if specific plasma cytokines and chemokines from envenomed patients could be used as predictors of EARs. A cohort of patients bitten by *Bothrops atrox* was followed-up at the *Fundação de Medicina Tropical Dr. Heitor Vieira Dourado* (FMT-HVD), from 2014 to 2016. Patients were treated with the Brazilian *Bothrops* antivenom and CXCL-8, CCL-5, CXCL-9, CCL-2, CXCL-10, IL-6, TNF, IL-2, IL-10, IFN-y, IL-4, and IL-17A were evaluated in patients' plasma samples before and after antivenom administration. From the total of patients (n = 186), mostly were male (82.3%), inhabiting rural areas (87.1%), with an average age of 35 years. Most of the patients (83.8%) were admitted to the hospital within 6 h after the accident, 26 (14%) reported having suffered a previous snakebite, and 97 (52.1%) received between 7 and 9 antivenom vials. The frequency of antivenom-induced EARs was 11.8% (22), resulting mostly of mild reactions. Urticaria was the major EAR manifestation (46.4%). Interestingly, CXCL-8 and IL-2 showed significantly lower levels in patients who progressed to EARs, although IL-2 levels might not represent biological relevance due the small magnitude difference between groups. This study reveals that CXCL-8 and IL-2 could play a role in the onset of EARs in pit viper envenomings.

1. Introduction

Snakebites cause high rates of morbidity and mortality in tropical and subtropical regions [1]. Globally, it is estimated that occur 5,500,000 cases of snakebites per year, with 1,841,000 envenomings, 400,000 permanent disabilities, and 138,000 deaths [2]. In 2019, 30,482 snakebites were reported in Brazil [14.5 cases/100,000 inhabitants), 13,601 of which were in the Amazon Region of the country (52.7 cases/100,000 inhabitants) [3]. In the Brazilian Amazon, snakes of the genus *Bothrops*, especially *B. atrox*, the Amazonian pit viper, are involved in ~ 90% of snakebites [4,5].

Venom from *Bothrops* snakes consists of a cocktail of toxins that

* Corresponding author at: Av. Carvalho Leal, 1777 – Cachoeirinha, Manaus/AM, CEP 69065-001 Brazil.
E-mail address: jac.sachett@gmail.com (J.A.G. Sachett).
[1] These authors equally contributed to the manuscript.

https://doi.org/10.1016/j.cyto.2022.155825
Received 22 November 2021; Received in revised form 25 January 2022; Accepted 6 February 2022
Available online 12 February 2022
1043-4666/© 2022 Elsevier Ltd. All rights reserved.

induce a variety of local and systemic manifestations in patients [6,7]. Among the most important components of the venom are metalloproteases (MPs: P-I, P-II, and P-III classes), phospholipases A_2 (PLA_2), serine proteases (SPs), type C lectin (CLEC), and L-amino acid oxidases (LAAO) [8–12]. Local and systemic manifestations occur due to the direct action of toxins and the release of inflammatory mediators [13–17]. Envenomings caused by *B. atrox* usually trigger an intense acute inflammatory response with pain, edema, ecchymosis, bleeding, and blistering at the bite site [18,19]. Systemic manifestations include bleeding, acute renal failure, and rarely disseminated intravascular coagulation [20–22]. In animal models, the inflammatory process caused by *Bothrops* venom leads to an increase in the total number of leukocytes, mainly polymorphonuclear and/or mononuclear cells [23], and an increase of local cytokines TNF-α, IL-1β, IL-6, IL-12p70, and IL-10 [24–26]. Furthermore, specific components of *Bothrops* venom such as *batroxase* also demonstrated to cleave directly a few components of the complement system (C3, C4, and C5), leading to uncontrolled production of anaphylatoxins, which play an important role in the progression of signs and symptoms during snake envenomings [27]. In humans, a similar effect occurs with an increase of circulating CXCL-8, CCL-5, CXCL-9, CCL-2 CXCL-10, and IL-6, resulting in activation of the immune response, and cell migration [28,29]. The levels of CXCL-8, CCL-2, and TNF-alpha are significantly elevated in patients with severe local manifestations [28].

Currently, antivenoms are the only specific treatment available for snakebite envenomings, and the World Health Organization (WHO) list of essential medicines includes them in the basic package of health care in affected countries [30]. So far, the antivenoms currently available are produced via hyperimmunization of large animals, such as horses in Brazil [13], being heterologous and polyclonal immunobiologicals, resulting in bottlenecks regarding safety [31]. Thus, early adverse reactions (EAR) to antivenom therapy of anaphylactic origin (IgE or non-IgE-mediated reactions) are expected events of varied frequency according to the type of antivenom and individual hypersensitivity to heterologous proteins [32]. Clinically, patients may present urticaria, itching, tachycardia, nausea, vomiting, abdominal colic, bronchospasm, hypotension, and angioedema [33–35]. Although the frequency and severity of EARs have decreased due to the improvement of the antivenom purification process, the incidence of EARs is still around 20% in Brazil [33,35]. This frequency is similar to other Latin American countries [34], and lower than in some Asian countries, where incidence is higher than 50% and antivenom-related anaphylaxis is a common event [36,37]. Premedication with antihistamines has no benefit in preventing antivenom-induced EARs [38–40]. Skin or conjunctival sensitivity tests are not recommended as there is no predictive value for EAR and delays the antivenom treatment [41]. Another less frequent type of EAR nowadays is the pyrogenic reaction, caused by the administration of an antivenom contaminated with bacterial endotoxins, characterized by fever, rigors, myalgia, headache, nausea, sweating, chills, increase of the heart rate, and vasodilation with secondary fall in blood pressure [32]. Lastly, the frequency of serum sickness, a late adverse event resulting from the deposition of long elimination half-life immune complexes in blood vessels, kidneys and joints, seems to be low, but the true frequency is unknown [32].

There are no accurate predictive factors for antivenom-induced EARs, thus hindering the prevention of side effects from antivenom treatment. During anaphylaxis, a range of mediators is generated, within multiple overlapping pathways and the involvement of several types of cells [42]. Few studies have evaluated the release of specific mediators in cases of antivenom-induced adverse reactions in patients with the immune profile already affected by the envenoming scenario. In Sri Lanka, antivenom-based therapy was demonstrated to enhance the release of inflammatory mediators in the patients, resulting in anaphylactic reactions characterized by high levels of mast cell degranulation but not further complement activation [43]. However, to the best of our knowledge, there are no findings on immune profiles that predict adverse reactions to antivenoms upon patient admission, considering the underlying cytokine and chemokine profile. This study aims to estimate the frequency of EARs of *Bothrops* antivenom therapy in a tertiary unit in the Brazilian Amazon and to determine whether the EARs can be predicted by a characteristic profile of cytokine and chemokine in patients' plasma samples.

2. Methods

2.1. Study design and patients

In this study, a cohort of patients with a diagnosis of *Bothrops* envenomations attended at the *Fundação de Medicina Tropical Dr. Heitor Vieira Dourado* (FMT-HVD), Manaus, Amazonas state, Brazil, was followed from August 2014 to August 2016. The FMT-HVD is a reference center for the management of snakebites in the Brazilian Amazon. All *Bothrops* envenomations were confirmed by identifying the snake brought by the patient to the hospital and/or by using an enzyme immunoassay [84]. Patients who received premedication or antivenom therapy in another hospital prior to FMT-HVD admission were not included. The sample size was calculated by means of an expected frequency of EARs of 23.5%, from a previous study carried out in the Amazon region [35], at an 80% power and 5% of the significance level. A minimum of 186 individuals was needed.

Clinical and epidemiological characterization included demographics, occupation, locality of the snakebite, association with work, the time elapsed from bite to hospital admission, history of previous snakebites, anatomical site of the bite, severity grading, antivenom dosage, and local and systemic manifestations.

Biological samples were collected on admission (T0), before premedication, and 24 h after the antivenom treatment (T1), for whole blood counts, biochemistry, and quantification of cytokines and chemokines. For immune mediators' quantification, samples were processed immediately and plasma was stored at −80 °C until analysis. A T0 plasma aliquot was used to confirm *Bothrops* envenomation by enzyme immunoassay.

2.2. Antivenom treatment

Twenty minutes before antivenom treatment, pre-medication with IV hydrocortisone (500 mg), IV cimetidine (300 mg), and oral dexchlorpheniramine (5 mg), was administered, as standardized according to FMT-HVD guidelines. Antivenom treatment was given to all patients in a dosage corresponding to envenomation severity, classified according to the Brazilian Health Ministry guidelines [13]: *i*) mild cases (2–4vials): local pain, mild edema, and ecchymosis; *ii*) moderate cases (4–8vials): local manifestations without necrosis, minor systemic signs (coagulopathy and bleeding, no shock); *iii*) severe cases (12vials): life-threatening snakebite, with severe bleeding, hypotension/shock and/or acute renal failure. Before administration, each antivenom vial was diluted in 100 mL of saline solution 0.9%. Antivenom administration time was 20 min.

Patients received the *Bothrops* antivenom, which is produced by the three national laboratories (Instituto Butantan, Fundação Ezequiel Dias, and Instituto Vital Brazil), and acquired by the Ministry of Health for distribution free of charge to patients. This antivenom is produced from purified specific immunoglobulins obtained from plasma of hyperimmunized horses with a pool of venoms of *B. jararaca* (50%), *B. alternatus* (12.5%), *B. jararacuçu* (12.5%), *B. moojeni* (12.5%), and *B. neuwedi* (12.5%). Each vial contains heterologous horse F(ab')2, neutralizing at least 50 mg of the reference venom of *Bothrops jararaca*, in mice, phenol (35mgmaximum), and physiological solution 0.85% q.s. 10 mL [13].

Intravenous metamizole was given on demand for pain. The bitten limb was nursed in the most comfortable position, blisters were aspirated, necrotic tissue was surgically debrided, abscesses were drained,

and antibiotic treatment was given accordingly.

2.3. Patients' follow-up and safety outcomes

After antivenom treatment, patients were admitted to the hospital ward for close monitoring for at least 48 h. Primary safety outcome was defined as the presence of EARs related to type I hypersensitivity, including urticaria, asthma-like crisis, laryngeal edema, and shock, shortly after infusion until the first 24 h after administration. The severity of these adverse reactions were classified according to the World Allergy Organization, as follows: Grade I) skin signs: pruritus, erythema, urticaria, and angioedema; Grade II) skin signs and measurable not life-threatening manifestations, including hypotension, tachycardia, and respiratory symptoms such as cough and difficulty breathing; Grade III) life-threatening symptoms, including shock, tachycardia or bradycardia, arrhythmias, and bronchospasm; Grade IV) cardiac and/or respiratory arrest; and Grade V) death [85]. All patients presenting EARs had the antivenom administration interrupted and were treated with and IV anti-histamine. Patients with Grade III EARs were treated also with subcutaneous adrenaline (1:1000, 0.3–0.5 mL), and repeated after 10 min if needed. After the symptoms had subsided, and a new course of premedication was given, and antivenom administration was restored.

The presence of pyrogenic reactions was considered when a patient presented fever and/or chills, associated or not to rigors, myalgia, headache, nausea, sweating, chills, increase of the heart rate, and vasodilation with secondary fall in blood pressure, within the first hour after initiating the antivenom administration [32]. Adrenaline and antipyretics were the standard treatments for this type of reaction.

Patients were asked to attend the hospital seven (D7) and twenty-one days (D21) after discharge. At follow-up visits, clinical examination was carried out in order to investigate the clinical evolution of the envenomations and the occurrence of late adverse reactions to antivenom. The presence of late adverse events was considered if the patient presented fever, myalgia, arthralgia, arthritis, lymphadenopathy, gastrointestinal disorders, neurological and renal complications until D21 [32]. If the patient did not present for the follow-up visits, the investigator contacted the patient by phone, and in case of complaints, planned a domiciliary visit. Patients who did not present to hospital visits and were not found at phone calls were considered lost to follow-up. After D15, patients were advised to return to the hospital or to contact the study physician by phone in case of any signs of complications. In case of late reactions, treatment consisted of analgesics, anti-histamines, and corticotherapy.

2.4. Cytokines and chemokines analysis

The quantification of cytokines and chemokines in the plasma samples of the patients was performed by the flow cytometry technique CBA (Cytometric bead Array) with the BD™ Kit CBA Human Chemokine (Cat. No. 552990, Lot: 7198865, brand BD® Biosciences, San Diego, CA, USA) and Kit BD™ CBA Human Th1/Th2/Th17 Cytokine (Cat. 560484, Lot: 6078753, brand BD® Biosciences, San Diego, CA, USA) following the guidelines described by the manufacturer. The quantified molecules were CXCL-8 (IL-8), CCL-5 (RANTES), CXCL-9 (MIG), CCL-2 (MCP-1), CXCL-10 (IP-10), IL-6, and TNF, IL-2, IL-10, IFN-y, IL-4, and IL-17A. The acquisition of the samples was carried out with the Cytometry-Flow FACS Canto II (Becton, Dickinson and Company, San Jose, CA, USA), at the *Fundação Hospitalar de Hematologia e Hemoterapia do Amazonas* (HEMOAM). The data were obtained in Mean Fluorescence Intensity (MFI) of each molecule with the FCAP-Array™ software (v3).

2.5. Antigenemia analysis by ELISA

The diagnosis of Bothrops envenomation confirmation and antigenemia quantification was performed by ELISA (Enzyme-Linked Immunosorbent Assay) quantitative specific-genus at the Instituto Butantan as described previously [86].

2.6. Statistical analysis

A univariable regression analysis was used to identify factors related to EARs, considering the value of $p < 0.05$ of significance. The odds ratio with the respective confidence intervals (CIs) were calculated. For the laboratory parameters, the continuous values, analyzed using the Mann-Whitney test, were considered and the mean and standard deviation was calculated for both groups. Data normality was performed with the Shapiro-Wilk test and showed a nonparametric distribution. The comparison of the values of cytokine and chemokine levels in plasma at T0 and T1 was made using the Kruskal-Wallis test, with the Dunn post-test for multiple comparisons. Correlations between circulating cytokine and chemokine levels were made with Spearman's correlation test, and molecule networks were built with Cytoscape Consortium software San Diego, CA, USA (v3.7.2). The correlation coefficient was interpreted according to previous studies [28] as being strong ($R \geq 68$), moderate ($R \leq 0.36$ to $R \leq 0.36$), and weak correlation ($R \leq 0.35$). The analysis was performed with the STATA statistical package (V15. 1) and the GraphPad Prism Software (v6.0).

2.7. Eth ical approval

This study was approved by the Research Ethical Board of the Universidade do Estado do Amazonas (approval number 492.892/2014). The adult patients signed an informed consent form after the study was explained to them. Parents or guardians signed the consent of minor patients.

3. Results

3.1. Patient characteristics

In total, 186 patients with a diagnosis of *Bothrops* snakebites were followed up. The majority of patients were male (82.3%), from rural areas [87.1%), and aged between 16 and 60 years (75.3%), with an average of 35 years. The lower limbs were predominantly affected (84.4%). Patients mostly initiated the treatment till 6 h after the bite (83.8%). A proportion of 14.0% of the patients reported having suffered a previous snakebite. Snakebite severity was classified as moderate in 47.3% of patients and the majority of them (52.1%) received 7 to 9 vials of *Bothrops* antivenom (Table 1).

3.2. EAR incidence and risk factors

Of a total of 186 patients included in the study, 22 presented EARs related to type I hypersensitivity (11.8%). None patient presented anaphylactic shock or pyrogenic reactions. No demographic or clinical variables presented an association with antivenom-associated EARs (Table 1). Other reported adverse events were nausea (1.6%), chills (1.1%), headache (1.1%), dizziness (0.5%), and vomiting (0.5%).

Manifestations of hypersensitivity included mainly pruritus/urticaria (59.1%), laryngeal irritation (36.4%), angioedema (27.3%), and facial flushing (22.7%). Regarding severity classification, 11 cases (50.0%) were classified as Grade I, 9 (40.9%) as Grade II, and 2 (9.1%) as Grade III (two cases of bronchospasm) (Table 2). The time for the EAR onset ranged from 3 to 60 min after the start of the antivenom treatment, with a median of 20 min. Eleven patients (50%) had EAR before the end of the antivenom treatment and needed the interruption of the procedure. Among patients with EARs, the average number of antivenom vials administered was significantly higher in patients with Grade III (mean of 12 vials) compared with the other groups (mean of 7.4 ± 2.1 vials) ($p = 0.040$). There was no difference in the means of the time (in minutes) until EARs onset between the grades of EARs severity ($p = 0.467$).

Table 1
Characteristics of the patients and factors associated with early adverse reactions to antivenom in *Bothrops*-envenomed patients.

Characteristics	Total	Early adverse reaction		Odds ratio	*p*
		No (n, %)	Yes (n, %)		
Gender					
Female	33 (17.7)	32 (19.5)	1 (4.5)	1	…
Male	153 (82.3)	132 (80.5)	21 (95.5)	5.09 (0.66–39.26)	0.118
Area of occurrence					
Urban	24 (12.9)	24 (14.6)	0 (0.0)	…	…
Rural	162 (87.1)	140 (85.4)	22 (100.0)		
Age group (years)					
1–15	27 (15.5)	25 (15.2)	2 (9.1)	1	…
16–60	140 (75.3)	125 (76.2)	15 (68.2)	1.5 (0.32–6.97)	0.605
>60	19 (10.2)	14 (8.6)	5 (22.7)	4.46 (0.76–26.09)	0.097
Anatomical site of the bite					
Upper limbs	29 (15.6)	25 (12.2)	4 (18.2)	1	…
Lower limbs	157 (84.4)	139 (84.8)	18 (81.8)	0.80 (0.25–2.59)	0.722
Time until medical care					
≤ 6	156 (83.8)	138 (84.2)	18 (81.8)	1	…
> 6	30 (16.2)	26 (15.8)	4 (18.2)	1.17 (0.36–3.76)	0.781
Previous bite					
Yes	26 (14.0)	21 (12.8)	5 (22.7)	2.00 (0.66–6.00)	0.215
No	160 (86.0)	143 (87.2)	17 (77.3)	1	…
Classification of severity					
Mild	80 (43.0)	72 (43.9)	08 (36.4)	1	…
Moderate	88 (47.3)	77 (46.9)	11 (50.0)	1.28 (0.48–3.37)	0.610
Severe	18 (9.7)	15 (9.2)	3 (13.6)	1.80 (0.42–7.58)	0.423
Number of antivenom vials received					
4–6	57 (30.7)	50 (30.5)	7 (31.8)	1	…
7–9	97 (52.1)	86 (52.4)	11 (50.0)	0.91 (0.33–2.50)	0.861
> 9	32 (17.2)	28 (17.1)	4 (18.2)	1.02 (0.27–3.79)	0.976

Table 2
Frequency of signs and symptoms and severity classification in 22 patients presenting early adverse reactions after antivenom treatment in *Bothrops* snakebites.

Signs and symptoms	Number	%
Pruritus/urticaria	13	59.1
Laryngeal irritation	8	36.4
Angioedema	6	27.3
Facial flushing	5	22.7
Multiple skin papules	3	13.6
Dyspnea	3	13.6
Tachycardia	2	9.1
Bronchospasm	2	9.1
Hypertension	1	4.5
Final severity classification		
Grade I	11	50.0
Grade II	10	45.5
Grade III	1	4.5

Moreover, there was no correlation between time (in minutes) until EARs onset and the number of antivenom vials received (Spearman's correlation test; $R = 0.017$; $p = 0.938$).

All patients presenting EARs had the antivenom administration interrupted and were treated with an IV anti-histamine. Two patients with Grade III EARs needed treatment with subcutaneous adrenaline (1:1000, 0.3–0.5 mL). A new course of premedication with IV hydrocortisone (500 mg), IV cimetidine (300 mg), and oral dexchlorpheniramine (5 mg), was administered. After restoring the administration of the antivenom, it was possible to complete the antivenom dosage in all patients, without the need for further interruptions. Fig. 1 shows photographs of patients presenting EARs, represented by pruritus/urticaria. None of the patients presented late adverse reactions in the follow-up.

3.3. Immunological molecules and hematological parameter in EAR patients at admission

The patients were categorized in EAR[+] or EAR[-] according to the presence or absence of the Early Adverse Reaction (EAR), respectively. Levels of the CXCL-8 and IL-2 were stistically lower in patients with EARs when compared to patients without EARs (Fig. 2), although the difference of IL-2 between groups was apparently of modest magnitude. Others chemokines and cytokines did not show significant differences in both *Bothrops* snakebite groups. Furthermore, mean blood cell counts, INR and antigenemia, were similar between EAR[+] and EAR[-] groups (Table 3).

3.4. Networks interaction of cytokines and chemokines at admission

The EAR[-] snakebite envenomed patients demonstrated an interaction of immune mediators different from the EAR[+] (Fig. 3). Correlations in *Bothrops*-envenomed patients show an acute inflammatory response occurring with weak, moderate, and strong interactions between inflammatory chemokines and cytokines. Furthermore, the analyzed cellular components showed more correlations in the ERA[+] group, both among themselves and with the chemokines and cytokines. Patients EAR[-] have a complex and diverse network of interactions of both chemokines and pro-inflammatory/regulatory cytokines, with highlights for molecules CCL-2, CXCL-10, IL-6, IL-10, and IL-17A. Otherwise, patients EAR[+] demonstrated a poor interaction between chemokines and cytokines at T0, with the absence of interaction of the molecules or loss of interaction, mainly in CXCL-8, IL-2, IFN-γ, and IL-4, but many interactions between monocytes, lymphocytes and eosinophils.

3.5. Chemokines and cytokines changes after antivenom therapy

The analysis of the production dynamics of chemokines and cytokines was performed at admission (T0) and 24 h after antivenom therapy (T1). Fig. 4 summarizes the comparison between T0 and T1 in each group, more reliably demonstrating the chemokine and cytokine profiles, beyond changes in immune molecules after antivenom therapy. *Bothrops*-envenomed patients showed decreasing in CXCL-9, CXCL-10, and IL-10, regardless of the presence of EAR in T1. In addition, a significant decrease of CXCL-8, with increased IL-17A, was observed only in patients that did not develop EARs.

4. Discussion

Venomous animals derived toxins are well-known to induce inflammation [44–46], highlighting the involvement of several cytokines. Regarding pit vipers, an early increase in IL-6 and TNF-α after intraperitoneal injection of *B. asper* and *B. jararaca* venoms has already been demonstrated in mice [47]. Moreover, the release of IL-1 and IL-6 was also observed *in vivo* after injection of BaP1, a P1 metalloproteinase isolated from *B. asper* [48]. Further studies have also shown that

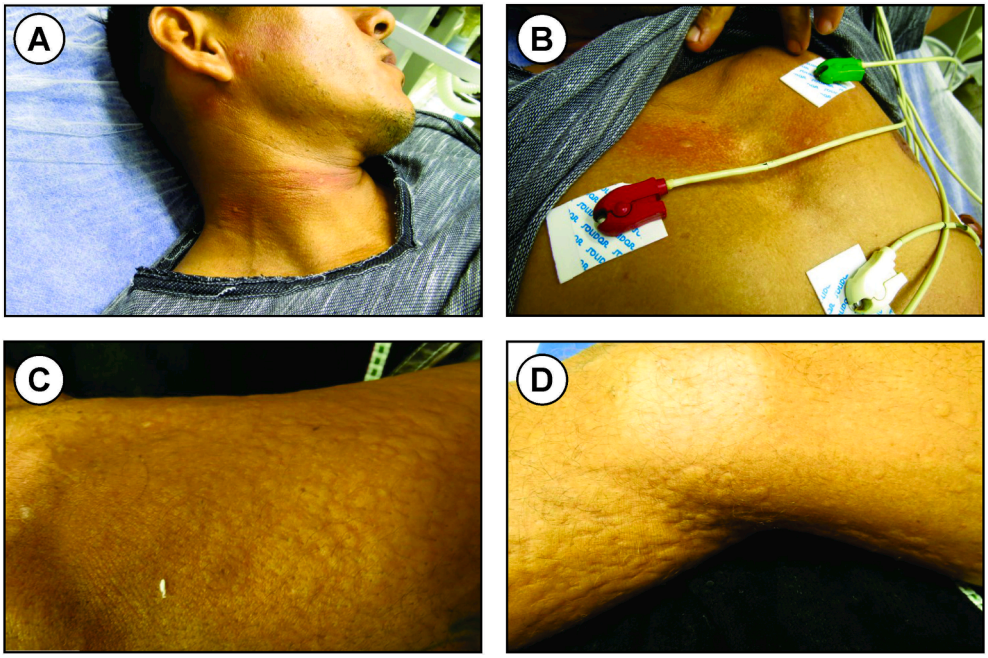


Fig. 1. Envenomed patients presenting early adverse reactions as skin manifestations following antivenom treatment. (A and B) - Men, 23 years old, farmer, suffered a snakebite classified as moderate. After 6 h of the accident, he arrived at the hospital and received 6 vials of *Bothrops* antivenom, and 60 min later, he had pruritus and urticaria, angioedema and edema on the face. (C and D) – Men, a 38-year-old, farmer, suffered a snakebite classified as moderate. After 3 h of the accident, he arrived at the hospital and received 8 vials of *Bothrops* antivenom, and 17 min after the administration, presented multiple papules, pruritus, laryngeal irritation, and angioedema.

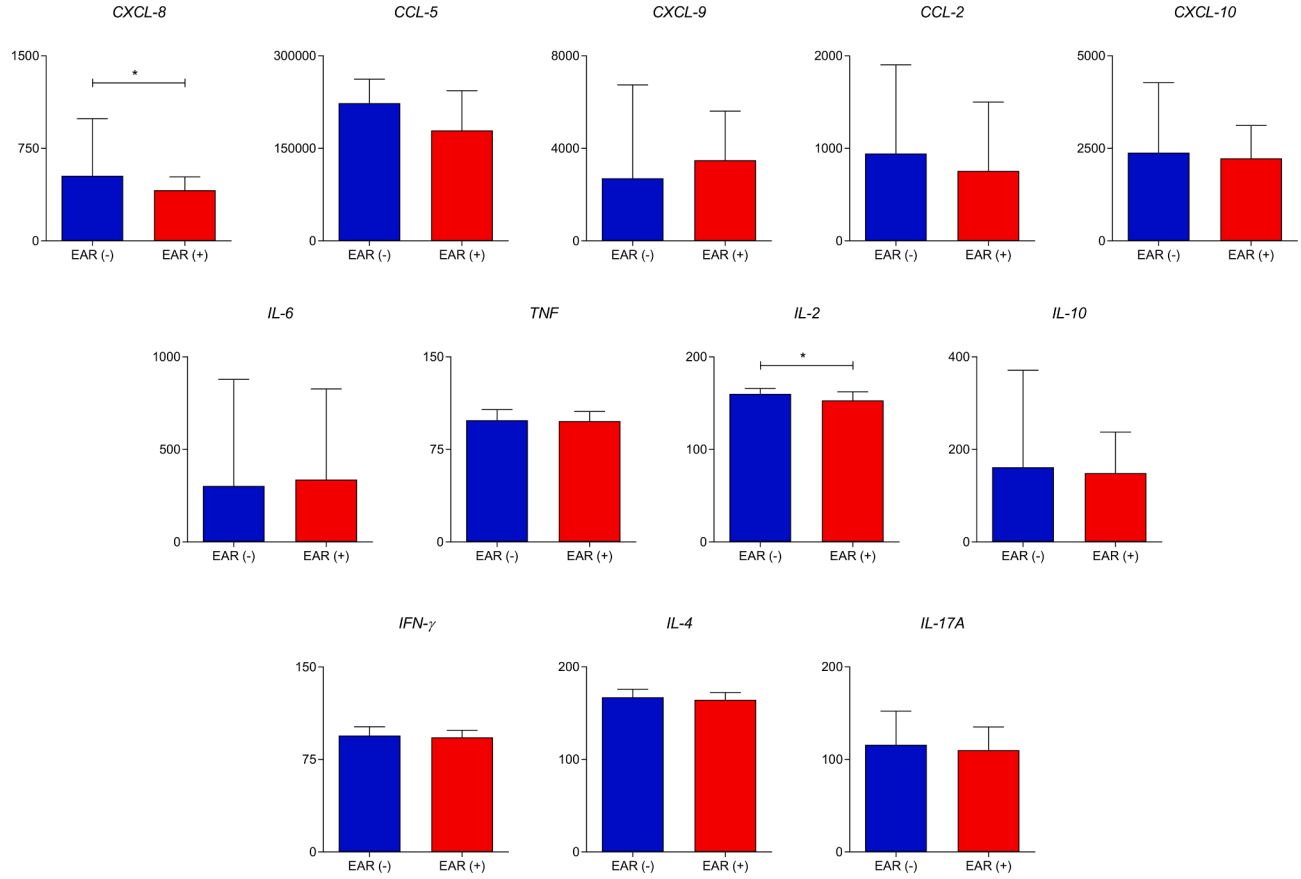


Fig. 2. Cytokine and chemokine plasma levels of patients before the *Bothrops* antivenom administration. Significant differences were presented in $p < 0.05$ (*), $p < 0.01$ (**), and $p < 0.0001$ (***). Quantification of cytokine and chemokine levels was performed using cytometric bead array (CBA). The graphs show the mean and standard deviation of each molecule in the groups, expressed in median fluorescence intensity (MFI). Comparative analyzes between groups were performed using Mann-Whitney test. EAR(-): patients without early adverse reaction (blue). EAR(+): patients with early adverse reaction (red). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 3
Cell profiles and laboratory results of patients presenting or not EARs following antivenom administration.

Laboratory parameter	Early adverse reaction		Total	*p*
	No	Yes		
Leukocytes (x10^3/ mm^3)	11.5 (±3.7)	12.2 (±3.7)	11.54 (±3.7)	0.398
Monocytes (%)	5.2 (±2.6)	5.4 (±2.8)	5.24 (±2.7)	0.765
Lymphocytes (%)	18.9 (±6.9)	21.6 (±7.3)	19.3 (±6.9)	0.109
Neutrophils (%)	71.6 (±12.6)	70.3 (±8.8)	71.44 (±12.2)	0.662
Eosinophils (%)	2.0 (±1.8)	2.6 (±2.0)	2.1 (±1.82)	0.137
Basophils (%)	0.03 (±0.18)	0.02 (±0.22)	0.03 (±0.18)	0.664
Band cells (%)	0.5 (±1.6)	0.9 (±2.2)	0.6 (±1.6)	0.256
Platelets (x10^6/mm^3)	229.9 (±72.2)	243.9 (±76.9)	242.2 (±76.1)	0.655
INR	1.3 (±0.2)	1.2 (±0.3)	1.3 (±0.2)	0.111
Antigenemia (ng/mL) *	4.6 (±10.6)	16.4 (±37.6)	15.0 (±35.6)	0.144

Reference values: eosinophils: 0.0–3.0%; lymphocytes: 20.0–51.1%; leukocytes: 4,000–10,800/mm3; basophils: 0.0–3.0%; monocytes: 1.7–9.3%; neutrophils: 40.0 to 60.0%; band cells: 1 to 5%; INR (International Standardized Ratio): 0.8 to 1.0.*Antigenemia assay was performed with T0 plasma samples.

jararhagin, a P-III metalloprotease isolated from *B. jararaca* venom, induces macrophage expression of mRNA encoding for TNF-α, IL-1, and IL-6 cytokines [49]. In our study we evaluated total TNF, which includes TNF-α and TNF-β(lymphotoxin). Although we did not test IL-1β from patient plasma samples, it is evident that macrophages are important targets for *B. atrox* venom since it is responsible to produce such pro-inflammatory cytokines (IL-1β, IL-6, and TNF-α) [50].

The macrophage innate response is initiated by activation of diverse membrane recognition receptors, such as the Toll-like receptors (TLRs). In inflammatory processes, recognition of pathogen-associated molecular patterns (PAMPs) and endogenous damage-associated molecular patterns (DAMPs) by TLRs (with exception of TLR3) activates cell-signaling pathways mediated by the MyD88 [50]. Venom-derived toxins have also been demonstrated to activate TLRs, which were named venom-associated molecular patterns (VAMPs) [51]. Thus, we postulate that *B. atrox* venom-derived VAMPs stimulate TLRs culminating in the activation of the MyD88-dependent pathway, transcription of NF-κB, resulting in the expression of pro-inflammatory cytokines including IL-6 and TNF-α. In fact, our hypothetical mechanism is highly supported by other studies demonstrating that snake toxins can directly activate TLRs. For instance, TLR2 and MyD88 signaling have a critical role in a PLA_2 from *B. asper* venom (MT-III)-induced inflammatory response in macrophages [52]; and using *B. atrox* venom researchers have proved that TLR2 is responsible to modulate the induced- inflammation [53].

The occurrence of adverse reactions to the use of antivenoms is common in clinical practice, presenting a rate ranging from 3% to 88% [54,55]. This large variation in the prevalence rates of adverse reactions to antivenom is due to the different processes of antivenom production, antibody formats (*e.g.* whole IgG, Fab, $F(ab')_2$), the amount and the velocity of antivenom infusion, and the specific characteristics of the snake venom and the studied population [32].

In this study, we observed a rate of 11.8% of EARs following antivenom administration. Clinical trials have shown values of 18% to 25% [33,40]. Pruritus/urticaria and vomiting were the most observed EARs in our study, with 46.4% and 13.3%, respectively, showing similar prevalence to previous studies with *Bothrops* antivenom [33,35]. All patients were premedicated with corticosteroids and anti-histamines as recommended by the Brazilian Ministry of Health guidelines for EAR prevention [13]. Clinical studies have been conducted in order to evaluate the effectiveness of premedication in the prevention of adverse reactions to horse-derived antivenoms and have not found any protective effect in the use of antihistamines and glucocorticoids [39,40,56,57]. However, other studies have demonstrated the effectiveness of adrenaline when applied subcutaneously, immediately prior to antivenom therapy, indicating that this method significantly reduces the incidence of early reactions [56,58–60]. Use of adrenaline in the prevention of adverse reactions is still controversial, specially in patients with history of heart disease.

There was no significant association between sociodemographic variables and the presence of EARs in our study. The majority of the patients were males from rural areas of an economically active age.

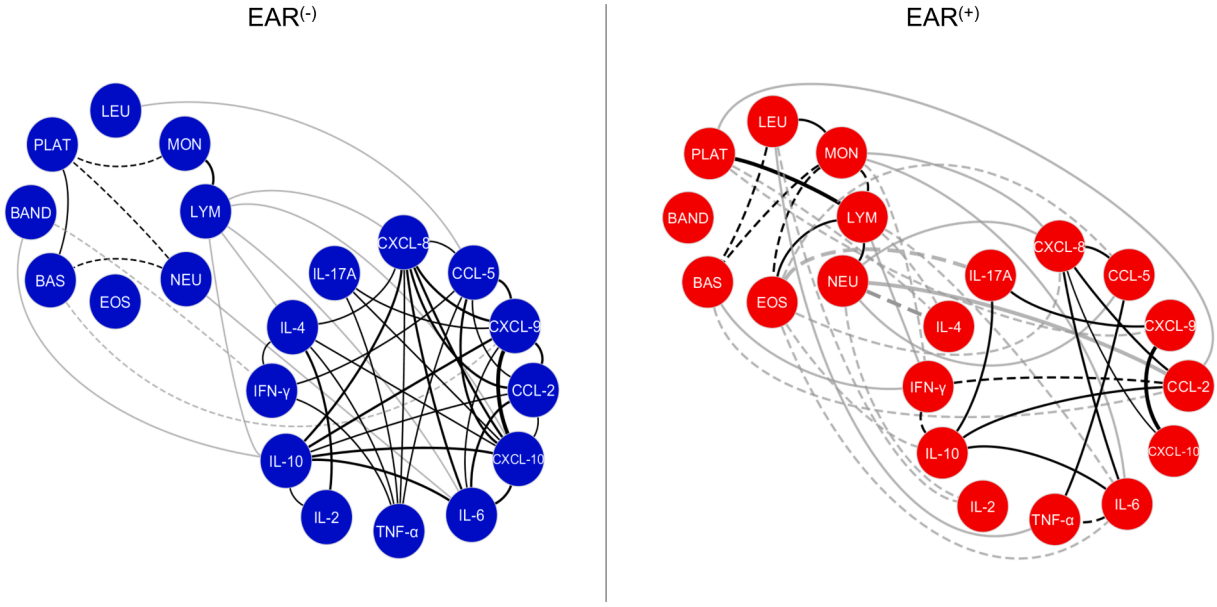


Fig. 3. Network interactions of cytokines and chemokines at T0 in patients presenting or not adverse reactions. The correction index (r) was used to divide the correlations by category according to strength into strong (R ≥ 0.68), moderate (R ≥ 0.36 to R ≤ 0.67), and weak (R ≤ 0.35). Dashed lines show negative correlations and continuous lines indicate positive correlations. Thin lines indicate weak correlation, medium lines show moderate correlation, and thick lines, strong correlation. EAR(-): patients without early adverse reaction (blue). EAR(+): patients with early adverse reaction (red). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

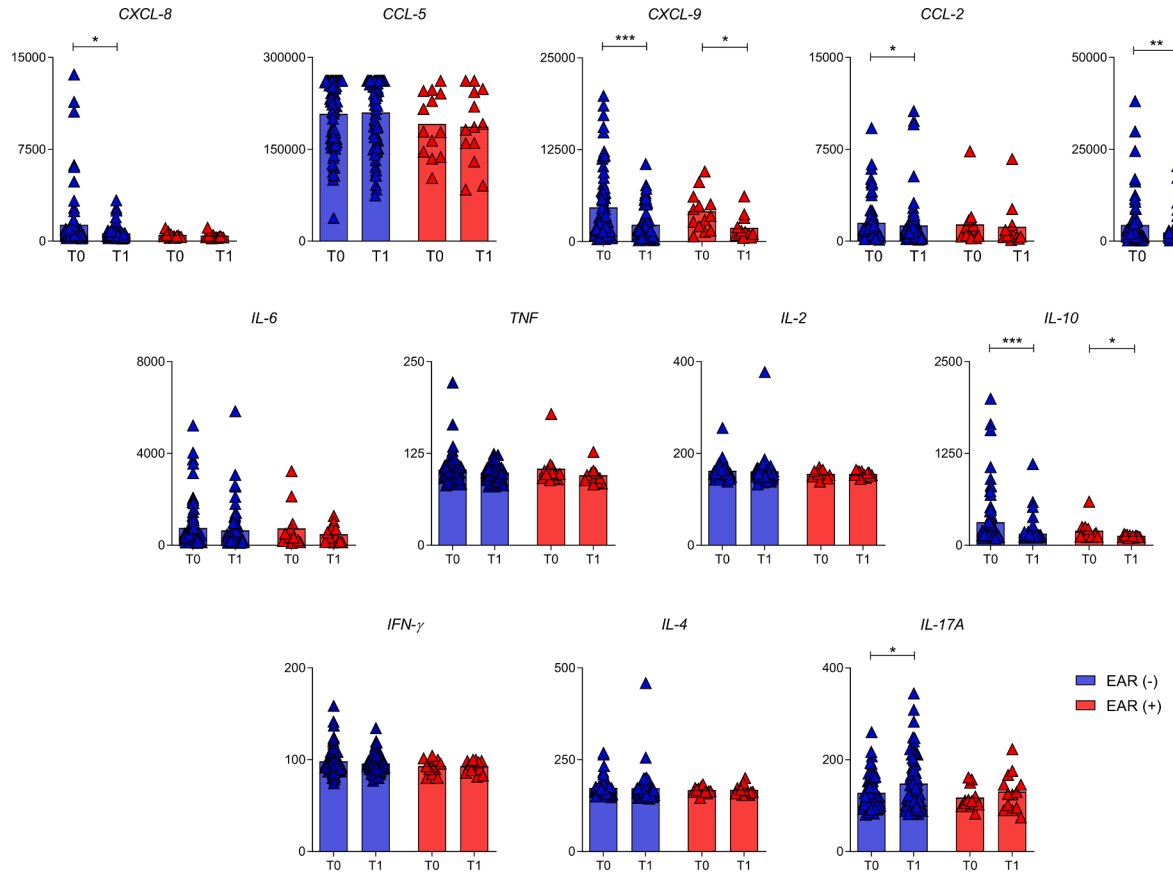


Fig. 4. Cytokine and chemokine plasma levels of patients presenting or not EAR following *Bothrops* antivenom administration at different times. Significant differences were presented in $p < 0.05$ (*), $p < 0.01$ (**), and $p < 0.0001$ (***). Quantification of cytokine and chemokine levels was performed using cytometric bead array (CBA). The graphs show the mean and standard deviation of each molecule in the two groups, expressed in median fluorescence intensity (MFI). Comparative analyzes between times were performed using Wilcoxon test. EAR(-): patients without early adverse reaction (blue). EAR(+): patients with early adverse reaction (red). T0: at patient admission. T1: 24 h after antivenom administration. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Moreover, the lower limbs were the most affected by the snakebite, similar to previous studies conducted in the Brazilian Amazon [61,28,35]. These findings may be related to the greater exposure of these individuals to work and leisure activities [61]. Moreover, snakebite associated variables such as the time until medical care, number of antivenom vials received, envenomation severity, and anatomical site of the bite showed no correlation to the development of EAR, indicating that adverse reactions are more related to patients intrinsic factors. Notably, patients were clinically followed during 30 days after being discharged. The mainly late adverse reaction associated with snakebites following antivenom administration is Serum Sickness. The large amount of antivenom antibodies combined with the human anti-horse antibodies (IgGs and IgMs) may result in the generation of immune complexes (ICs) pesenting long elimination half-life. This can trigger IC deposition in target tissues including blood vessels, glomeruli, and joints (1–2weeksafterantivenomtherapy) and cause Serum Sickness [31]. None of our patients evolved with late reactions.

The early adverse reactions observed in the treatment of snakebite are an association of immune responses to components within antivenom (or with a similar component) which triggers IgE-mediated anaphylactic reactions occurred by prior contact of the individual, where mast cells, basophils and/or eosinophils are sensitized; thus, in the second contact, IgEs induce their degranulation, releasing pharmacological mediators [32,50,62]. Anaphylactic reactions not mediated by IgE are induced by the production of anaphylatoxins (C3a, C4b, and C5a) derived from the cleavage of C3, C4, and C5 zymogens from the complement system [63]. Results observed in the present work showed that patients that reported previous snakebites were not more susceptible to the EAR, indicating that previous sensitization of cells involved in anaphylactic reactions might not be associated. Similar findings have been reported in a previous study that investigated the immune responses to Russell's viper snake envenoming and antivenom treatment, where authors observed EAR in patients who had not been previously exposed to antivenom [42]. The authors have also observed no association of EAR to anaphylatoxins C3a, C4a, and C5a increase, suggesting that complement activation does not mediate early anaphylactic reactions. Their conclusion indicates that antivenom reactions may be triggered by a different pathway, which involves antivenom complexes binding to IgG receptors or non-specifically crosslinking IgE on the surface of mast cells [42].

Much is discussed concerning how antivenoms are responsible for adverse reactions, however, neglected attention has been pointed to how can envenomation modulate the EAR induced by antivenoms. Animal venoms are capable of inducing a variety of immune reactions, including innate and adaptive responses, along with hypersensitivity response characterized by exaggerated or inappropriate immunologic reactions involving IgE production and mast cell degranulation [64]. Previous pre-clinical studies have demonstrated that snake venoms are capable of inducing mast cell degranulation and allergic reactions [65–68]. The same has been proven to *Bothrops atrox* venom and its isolated toxins, which mast cell degranulation showed to be involved in inflammatory alterations such as edema and hyperalgesia [69–71]. However, venom-induced immune reactions could interfere with antivenom-induced hypersensitivity reactions. Stone and colleagues

[42] have found that a relevant frequency of patients from Russell's viper snakebite who developed antivenom-related EAR (skin reactions and anaphylaxis) presented increased plasma levels of histamine before antivenom therapy, compared to patients who did not develop EAR. The authors also evidenced elevated plasma levels of the inflammatory cytokines IL-10 and IL-6 from all patients before antivenom, which remained elevated for up to 24 h after the initial dose of antivenom in patients who developed anaphylaxis. The results indicate that previous venom stimulation of mast cell degranulation and inflammation is responsible for priming cells to react to antivenom and can increase the risk for EAR development [42].

Considering the pro-inflammatory behavior of *Bothrops atrox* snakebite found in previous studies [26,27], as well as pre-clinical data referred to crude venom and isolated toxins [23,25,27,53,72], we have then evaluated the inflammatory cytokines and chemokines profile of patients with and without EAR previous and after antivenom therapy. The inflammatory profile from patients previously to antivenom showed that IL-2, and namely CXCL-8, were found significantly decreased in patients who developed EAR (Fig. 5). CXCL-8 is a well-known chemoattractant agent, responsible for the recruitment of neutrophils during the inflammatory process, which can be released from several cell types in response to inflammation, including monocytes, macrophages, neutrophils, intestine, kidney, placenta, and bone marrow cells [73]. IL-2 is produced mainly by CD4 + T helper (Th) cells and by CD8 + T cells, natural killer (NK) cells, and natural killer T (NKT) cells, and has been associated with T-cell growth, augments NK cytolytic activity, and induces the differentiation of regulatory T cells [74]. It has been found that IL-2 and histamine can down-modulate each other, where studies have shown that IL-2 can inhibit histamine release from mast cells and that histamine down-regulates IL-2 gene/protein expression [75,76]. Also, the involvement of IL-2 in allergic reactions has been discussed. It is well established that in the pathogenesis of allergic diseases the histamiactivity on Th2-type immune response is found [77]. Our findings showed that the IL-2 level magnitude difference between the EAR + and EAR- groups was modest, which may not have a relevant impact on the outcome, despite the statistical significant difference. However, a previous study has found that even low doses of IL-2 administration modified the local balance of Th1/Th2 (decreases the predominance of Th2) and decrease the degranulation of basophils and mast cells in an experimental model of food allergy in mice [78]. While for CXCL-8, there is an ambiguous relationship between histamine release and leukocyte degranulation, with studies finding a positive correlation between histamine and increase CXCL-8 production in endothelial cells [79,80], but inhibits histamine release from basophils [81]. Therefore,

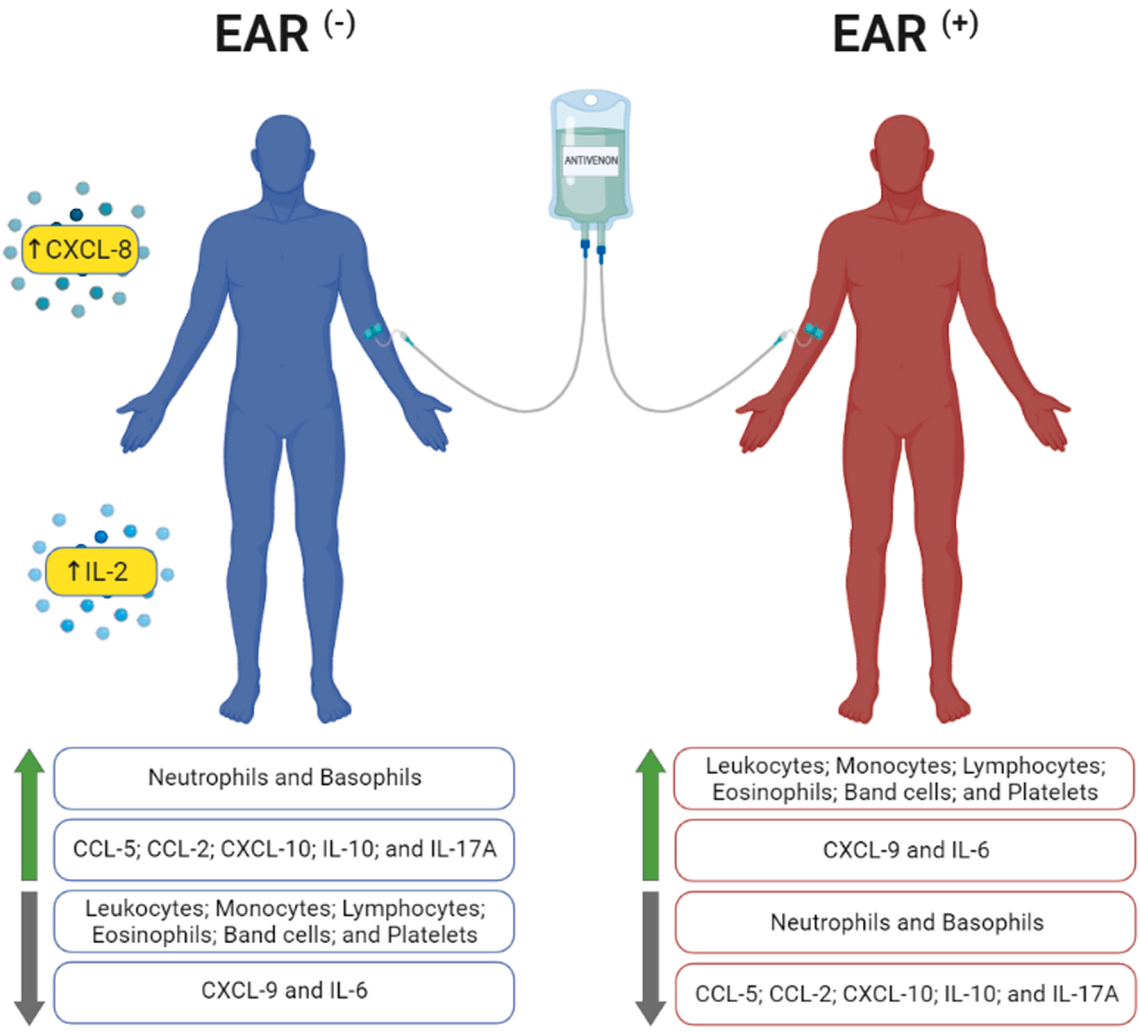


Fig. 5. Chemokine and cytokine profiles of envenomed patients presenting or not early adverse reactions previous *Bothrops* antivenom administration. The left panel in blue represents the immunological profile of envenomed patients that did not present early adverse reactions after antivenom administration – EAR(-). The right panel in red represents the immunological profile of envenomed patients presenting early adverse reactions after antivenom administration – EAR(+). The yellow boxes indicate the immune mediators showing differences between groups (CXCL-8 and IL-2), resulting in potential biomarkers. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

the literature brings the possible protective role of CXCL-8 and IL-2 to hypersensitivity and allergenic reactions, corroborating with the results in our present which both mediators are found increased in patients who did not develop EAR.

Another interesting aspect found in the present work involves the cytokines/chemokines profile found at T1 (24 h after antivenom treatment). Among the major differences observed in patients who developed EAR and not, the mediators CCL-2 and CXCL-8 were found decreased, whereas IL-17 increased following antivenom administration in patients without EAR comparing at T0 and T1. It has been demonstrated that increased levels of CCL-2 are associated with the development of anaphylactic reactions due to a CCL2-dependent chemotactic activity for basophils [82]. As for CXCL-8 mentioned above, its role as an activator or inhibitor of histamine-mediated reactions still unknown due to its dual character. However, the decreased values found in non-EAR patients at T1 could be associated with a resolution phase of the pro-inflammatory response of *Bothrops* snakebite. Exacerbated reactions involving Th-17 type immune response have been strongly associated with autoimmune diseases as psoriasis and rheumatoid arthritis [83]. Therefore, its increased levels at T1 in patients who did not develop EAR is an unlikely result that should be more investigated in the future.

Complement mediators were not measured in the present study, although a previous report by our research group has found that patients victims of *Bothrops* snakebite in the Brazilian Amazon showed activation of complement system components [28]. Therefore, it was not possible to define the participation of complement system activation in the EAR genesis in the present work. Moreover, histamine levels were not investigated, preventing a better description of its correlation with the levels of CXCL-8 and IL-2, which may be the key to the findings of this study.

5. Conclusion

The present study has elucidated some important aspects associated with hypersensitivity reactions due to antivenom therapy against *Bothrops*. The immunological response induced by *Bothrops* snakebite, evaluated by the inflammatory mediators profile, has shown to be directly related to the antivenom-induced EAR, showing that venom components can modulate positively in order to induce these reactions. Moreover, CXCL-8 and IL-2 were found as immune molecules potentially involved in EARs onset following antivenom administration in *Bothrops* envenomings.

Author Contributions

LCLF, AGC, WMM, and JS designed the experiments. FGSS, HNI, IMS, EFN performed the experiments. FGSS, MAS, AM, MBP, FAC, FHW, AGC, WM, and JS analyzed data and wrote the manuscript. MAS, FHW, AMMS, MBP, WM, and JS revised the manuscript. LCLF, WM, and JS conceived and supervised the project. All authors read and approved the final manuscript.

Funding

We thank *Conselho Nacional de Desenvolvimento Científico e Tecnológico* (CNPq), for the scholarships to MBP (n. 307184/2020–0), AMMS (n. 303958/2018–9) and WMM (n. 309207/2020–7). AGC and WMM acknowledges funding support from *Fundação de Amparo à Pesquisa do Estado do Amazonas* (FAPEAM) (Pró-Estado Program-#002/2008, #007/2018, and #005/2019, PAMEQ Program-#004/2019, PAPAC Program-#005/2019, and POSGRAD Program-#006/2020). MBP acknowledges funding support from Hamish Ogston Foundation - Global Snakebite Initiative. AMMS is a research fellow from FAPEAM (PVN-II, Pró-Estado Program - #002/2008, #007/2018 and #005/2019).

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

[1] J.M. Gutiérrez, J.J. Calvete, A.G. Habib, R.A. Harrison, D.J. Williams, D.A. Warrell, Snakebite envenoming, Nat. Rev. Dis. Prim. 3 (1) (2017), https://doi.org/10.1038/nrdp.2017.63.

[2] Lancet T. Snakebite — emerging from the shadows of neglect. Lancet. 2019;393:2175.

[3] Brasil. Ministério da Saúde. SINAN - Sistema de Informação de Agravos de Notificação. Acidente por Animais Peçonhentos [Internet]. 2019. 2020 [cited 2020 Feb 24]. p. 1. Available from: http://www2.datasus.gov.br/DATASUS/index.php?area=0203&id=29878153&VObj=http://tabnet.datasus.gov.br/cgi/deftohtm.exe?sinannet/cnv/animais.

[4] Hui Wen F, Monteiro WM, Moura da Silva AM, Tambourgi D V., Mendonça da Silva I, Sampaio VS, et al. Snakebites and Scorpion Stings in the Brazilian Amazon: Identifying Research Priorities for a Largely Neglected Problem. PLoS Negl Trop Dis [Internet]. 2015;9(5):e0003701. Available from: https://doi.org/10.1371/journal.pntd.0003701.

[5] Feitosa EL, Sampaio VS, Salinas JL, Queiroz AM, Silva IM, Gomes AA, et al. Older age and time to medical assistance are associated with severity and mortality of snakebites in the Brazilian Amazon: A case-control study. PLoS One [Internet]. 2015;10(7):1–15. Available from: 10.1371/journal.pone.0132237.

[6] Bourguignon S, Paes F, Calheiros E, Juliano M, Aguiar A, Melgarejo A, et al. Bothrops Moojeni Venom Peptides Containing Bradykinin Potentiating Peptides Sequences. Protein Pept Lett [Internet]. 2005;8(1):21–Available from: 10.2174/0929866013409689.

[7] S.V. Pierini, D.A. Warrell, A. De Paulo, R.D.G. Theakston, High incidence of bites and stings by snakes and other animals among rubber tappers and amazonian indians of the Juruá Valley, Acre State, Brazil, Toxicon 34 (2) (1996) 225–236.

[8] T. Matsui, Y. Fujimura, K. Titani, Snake venom proteases affecting hemostasis and thrombosis, Biochimica et Biophys. Acta (BBA) Protein Struct. Mol. Enzymol. 1477 (1-2) (2000) 146–156.

[9] S.M.T. Serrano, R.C. Maroun, Snake venom serine proteinases: sequence homology vs. substrate specificity, a paradox to be solved, Toxicon 45 (8) (2005) 1115–1132.

[10] T. Ogawa, T. Chijiwa, N. Oda-Ueda, M. Ohno, Molecular diversity and accelerated evolution of C-type lectin-like proteins from snake venom, Toxicon 45 (1) (2005) 1–14.

[11] J.W. Fox, A brief review of the scientific history of several lesser-known snake venom proteins: l-amino acid oxidases, hyaluronidases and phosphodiesterases, Toxicon 62 (2013) 75–82.

[12] Moura-Da-silva AM, Contreras-Bernal JC, Gimenes SNC, Freitas-De-sousa LA, Portes-Junior JA, Peixoto P da S, et al. The relationship between clinics and the venom of the causative amazon pit viper (Bothrops atrox). PLoS Negl Trop Dis [Internet]. 2020;14(6):1–17. Available from: 10.1371/journal.pntd.0008299.

[13] Brasil. Ministério da Saúde. Fundação Nacional de Saúde. Manual de diagnóstico e tratamento de acidentes por animais peçonhentos. 1ª. Ministério da Saúde, editor. Brasília: Ministério da Saúde; 2001. 120 p.

[14] I.M.B. Francischetti, H.C. Castro, R.B. Zingali, C.R. Carlini, J.A. Guimarães, Bothrops sp. Snake Venoms: Comparison of Some Biochemical and Physicochemical Properties and Interference in Platelet Functions, Comparative Biochem. Physiol. Part C Pharmacol. Toxicol. Endocrinol. 119 (1) (1998) 21–29.

[15] J.M. Gutiérrez, G. León, G. Rojas, B. Lomonte, A. Rucavado, F. Chaves, Neutralization of local tissue damage induced by Bothrops asper (terciopelo) snake venom, Toxicon 36 (11) (1998) 1529–1538.

[16] H.C. Castro, M. Fernandes, R.B. Zingali, Identification of Bothrojaracin-like proteins in snake venoms from Bothrops species and Lachesis muta, Toxicon 37 (10) (1999) 1403–1416.

[17] K.U. Camey, D.T. Velarde, E.F. Sanchez, Pharmacological characterization and neutralization of the venoms used in the production of Bothropic antivenom in Brazil, Toxicon 40 (5) (2002) 501–509.

[18] W.M. Monteiro, J.C. Contreras-Bernal, P.F. Bisneto, J. Sachett, I. Mendonça da Silva, M. Lacerda, A. Guimarães da Costa, F. Val, L. Brasileiro, M.A. Sartim, S. Silva-de-Oliveira, P.S. Bernarde, I.L. Kaefer, F.G. Grazziotin, F.H. Wen, A. M. Moura-da-Silva, Bothrops atrox, the most important snake involved in human envenomings in the amazon: How venomics contributes to the knowledge of snake biology and clinical toxinology, Toxicon X. 6 (2020) 100037, https://doi.org/10.1016/j.toxcx.2020.100037.

[19] Silva FS, Ibiapina HNS, Neves JCF, Coelho KF, Barbosa FBA, Lacerda MVG, et al. Severe tissue complications in patients of Bothrops snakebite at a tertiary health unit in the Brazilian Amazon: clinical characteristics and associated factors. Rev Soc Bras Med Trop [Internet]. 2021;54:1–5. Available from: 10.1590/0037-8682-0374-2020.

[20] S.S. Oliveira, E.C. Alves, A.S. Santos, J.P.T. Pereira, L.K.S. Sarraff, E.F. Nascimento, J.D. De-Brito-Sousa, V.S. Sampaio, M.V.G. Lacerda, J.A.G. Sachett, I.S. Sano-Martins, W.M. Monteiro, Factors Associated with Systemic Bleeding in Bothrops Envenomation in a Tertiary Hospital in the Brazilian Amazon, Toxins (Basel) 11 (1) (2019) 22, https://doi.org/10.3390/toxins11010022.

[21] Oliveira SS, Alves EC, Santos AS, Nascimento EF, Pereira JPT, Silva IM, et al. Bleeding Disorders in Bothrops atrox Envenomations in the Brazilian Amazon: Participation of Hemostatic Factors and the Impact of Tissue Factor. Toxins (Basel) [Internet]. 2020;12(9). Available from: 10.3390/toxins12090554.

[22] Alves EC, Sachett J de AG, Sampaio VS, Sousa JD de B, Oliveira SS de, Nascimento EF do, et al. Predicting acute renal failure in Bothrops snakebite patients in a

tertiary reference center, Western Brazilian Amazon. Burdmann EA, editor. PLoS One. 2018 Aug 17;13(8):12–6.
[23] D.L. Menaldo, C.P. Bernardes, K.F. Zoccal, A.L. Jacob-Ferreira, T.R. Costa, M.P.F. M. Del Lama, R.M.Z.G. Naal, F.G. Frantz, L.H. Faccioli, S.V. Sampaio, Immune cells and mediators involved in the inflammatory responses induced by a P-I metalloprotease and a phospholipase A 2 from Bothrops atrox venom, Molecular Immunology 85 (2017) 238–247.
[24] V. Moreira, C. Teixeira, H. Borges da Silva, M.R. D'Império Lima, M.C. Dos-Santos, The crucial role of the MyD88 adaptor protein in the inflammatory response induced by Bothrops atrox venom, Toxicon 67 (2013) 37–46.
[25] V. Moreira, M.C. Dos-Santos, N.G. Nascimento, H.B. da Silva, C.M. Fernandes, M. R. D'Império Lima, C. Teixeira, Local inflammatory events induced by Bothrops atrox snake venom and the release of distinct classes of inflammatory mediators, Toxicon 60 (1) (2012) 12–20.
[26] Teixeira CFP, Zamunér SR, Zuliani JP, Fernandes CM, Cruz-Hofling MA, Fernandes I, et al. Neutrophils Do Not Contribute To Local Tissue Damage , But Play a Key Role in Skeletal Muscle Regeneration, in Mice. Muscle Nerve [Internet]. 2003;1(1): 449–59. Available from: 10.1002/mus.10453.
[27] D.L. Menaldo, C.P. Bernardes, A.L. Jacob-Ferreira, C.G. Nogueira-Santos, T. M. Casare-Ogasawara, L.S. Pereira-Crott, S.V. Sampaio, Effects of Bothrops atrox venom and two isolated toxins on the human complement system: Modulation of pathways and generation of anaphylatoxins, Mol. Immunol. 80 (2016) 91–100.
[28] Ibiapina HNS, Costa AG, Sachett JAG, Silva IM, Tarragô AM, Neves JCF, et al. An Immunological Stairway to Severe Tissue Complication Assembly in Bothrops atrox Snakebites. Front Immunol [Internet]. 2019;10:1–12. Available from: 10.3389/fimmu.2019.01882.
[29] Wellmann IAM, Ibiapina HNS, Sachett JAG, Sartim MA, Silva IM, Oliveira SS, et al. Correlating Fibrinogen Consumption and Profiles of Inflammatory Molecules in Human Envenomation's by Bothrops atrox in the Brazilian Amazon. Front Immunol [Internet]. 2020;11(August):1–12. Available from: 10.3389/fimmu.2020.01874.
[30] WHO – World Health Organization. Guidelines for the Production Control and Regulation ol Snake Antivenom Immunoglobulins [Internet]. WHO. 2010 [cited 2021 Mar 9]. p. 1. Available from: https://www.who.int/bloodproducts/snake_antivenoms/snakeantivenomguide/en/.
[31] A.H. Laustsen, J. María Gutiérrez, C. Knudsen, K.H. Johansen, E. Bermúdez-Méndez, F.A. Cerni, J.A. Jürgensen, L. Ledsgaard, A. Martos-Esteban, M. Øhlenschlæger, U. Pus, M.R. Andersen, B. Lomonte, M. Engmark, M.B. Pucca, Pros and cons of different therapeutic antibody formats for recombinant antivenom development, Toxicon 146 (2018) 151–175.
[32] G. León, M. Herrera, Á. Segura, M. Villalta, M. Vargas, J.M. Gutiérrez, Pathogenic mechanisms underlying adverse reactions induced by intravenous administration of snake antivenoms, Toxicon 76 (2013) 63–76.
[33] P.P. Pardal, S.M. Souza, M.R. Monteiro, H.W. Fan, J.L. Cardoso, F.O. França, et al., Clinical trial of two antivenoms for the treatment of Bothrops and Lachesis bites in the north eastern Amazon region of Brazil, Trans. Royal Soc. Tropical Med. Hygiene 98 (1) (2004) 28–42.
[34] Otero R, Le G, Rojas G, Abel D, Barona J, Carlos J, et al. Efficacy and safety of two whole IgG polyvalent antivenoms, refined by caprylic acid fractionation with or without beta-propiolactone, in the treatment of Bothrops asper bites in Colombia. Trans R Soc Trop Med Hyg [Internet]. 2006;100(1):1173—1182 available. Available from: 10.1016/j.trstmh.2006.01.006.
[35] Mendonça-da-Silva I, Magela Tavares A, Sachett J, Sardinha JF, Zaparolli L, Gomes Santos MF, et al. Safety and efficacy of a freeze-dried trivalent antivenom for snakebites in the Brazilian Amazon: An open randomized controlled phase IIb clinical trial. PLoS Negl Trop Dis [Internet]. 2017;11(11):1–21. Available from: https://doi.org/10.1371/journal.pntd.0006068.
[36] Isbister GK, Shahmy S, Mohamed F, Abeysinghe C, Karunathilake H, Ariaratnam A. A Randomised Controlled Trial of Two Infusion Rates to Decrease Reactions to Antivenom. PLoS One [Internet]. 2012;7(6):e38739. Available from: 10.1371/journal.pone.0038739.
[37] S.K. Sharma, E. Alirol, A. Ghimire, S. Shrestha, R. Jha, S.B. Parajuli, D. Shrestha, S. J. Shrestha, A. Bista, D. Warrell, U. Kuch, F. Chappuis, W.R.J. Taylor, Acute Severe Anaphylaxis in Nepali Patients with Neurotoxic Snakebite Envenoming Treated with the VINS Polyvalent Antivenom, J. Tropical Med. 2019 (2019) 1–12.
[38] A.G. Habib, Effect of Pre-Medication on Early Adverse Reactions Following Antivenom Use in Snakebite: A Systematic Review and Meta-Analysis, Drug Safety 34 (10) (2011) 869–880.
[39] F. Bucarethi, J.L. Douglas, M.R.C.C. Fonseca, F.A.D. Zambrone, R.J. Vieira, Envenenamento ofídico em crianças: frequência de reações precoces ao antiveneno em pacientes que receberam pré-tratamento com antagonistas H1 e H2 da histamina e hidrocortisona, Rev. Inst. Med. Trop. S. Paulo 36 (5) (1994) 451–457.
[40] H.W. Fan, L.F. Marcopito, J.L.C. Cardoso, F.O.S. Franca, C.M.S. Malaque, R. A. Ferrari, R.D.G. Theakston, D.A. Warrell, Sequential randomised and double blind trial of promethazine prophylaxis against early anaphylactic reactions to antivenom for bothrops snake bites, BMJ 318 (7196) (1999) 1451–1452.
[41] P. Cupo, M.M. Azevedo-Marques, J.B.d. Menezes, S.E. Hering, Reações de hipersensibilidade imediatas após uso intravenoso de soros antivenenos: valor prognóstico dos testes de sensibilidade intradérmicos, Rev. Inst. Med. trop. S. Paulo 33 (2) (1991) 115–122.
[42] S.F. Stone, S.G.A. Brown, Mediators Released During Human Anaphylaxis, Curr Allergy Asthma Rep 12 (1) (2012) 33–41.
[43] Stone SF, Isbister GK, Shahmy S, Mohamed F, Abeysinghe C, Karunathilake H, et al. Immune Response to Snake Envenoming and Treatment with Antivenom ; Complement Activation , Cytokine Production and Mast Cell Degranulation. PLoS Negl Trop Dis [Internet]. 2013;7(7):1–9. Available from: 10.1371/journal.pntd.0002326.
[44] C.d.F.P. Teixeira, C.M. Fernandes, J.P. Zuliani, S.F. Zamuner, Inflammatory effects of snake venom metalloproteinases, Mem. Inst. Oswaldo Cruz 100 (suppl 1) (2005) 181–184.
[45] M.B. Reis, K.F. Zoccal, L.G. Gardinassi, L.H. Faccioli, Scorpion envenomation and inflammation: Beyond neurotoxic effects, Toxicon 167 (2019) 174–179.
[46] E. Voronov, R.N. Apte, S. Sofer, The systemic inflammatory response syndrome related to the release of cytokines following severe envenomation, J. Venom. Anim. Toxins 5 (1) (1999) 5–33.
[47] V.L. Petricevich, C.F.P. Teixeira, D.V. Tambourgi, J.M. Gutiérrez, Increments in serum cytokine and nitric oxide levels in mice injected with Bothrops asper and Bothrops jararaca snake venoms, Toxicon 38 (9) (2000) 1253–1266.
[48] A. Rucavado, T. Escalante, C.F.P. Teixeira, C.M. Fernándes, C. Díaz, J.M. Gutiérrez, Increments in cytokines and matrix metalloproteinases in skeletal muscle after injection of tissue-damaging toxins from the venom of the snake Bothrops asper, Mediat. Inflamm. 11 (2) (2002) 121–128.
[49] P.B. Clissa, G.D. Laing, R.D.G. Theakston, I. Mota, M.J. Taylor, A.M. Moura-da-Silva, The effect of jararhagin, a metalloproteinase from Bothrops jararaca venom, on pro-inflammatory cytokines released by murine peritoneal adherent cells, Toxicon 39 (10) (2001) 1567–1573.
[50] Abbas AK, Lichtman AH, Pillai S. Cellular and Molecular Immunology. 9th Edition. Elsevier, editor. Philadelphia; 2017. 608 p.
[51] Zoccal KF, Bitencourt CDS, Paula-Silva FWG, Sorgi CA, de Castro Figueiredo Bordon K, Arantes EC, et al. TLR2, TLR4 and CD14 Recognize Venom-Associated Molecular Patterns from Tityus serrulatus to Induce Macrophage-Derived Inflammatory Mediators. Talamas-Rohana P, editor. PLoS One [Internet]. 2014 Feb 7;9(2):e88174. Available from: 10.1371/journal.pone.0088174.
[52] Leiguez E, Giannotti KC, Moreira V, Matsubara MH, Gutiérrez JM, Lomonte B, et al. Critical Role of TLR2 and MyD88 for Functional Response of Macrophages to a Group IIA-Secreted Phospholipase A2 from Snake Venom. Bozza PT, editor. PLoS One [Internet]. 2014 Apr 9;9(4):e93741. Available from: 10.1371/journal.pone.0093741.
[53] Moreira V, Teixeira C, Borges da Silva H, D'Império Lima MR, Dos-Santos MC. The role of TLR2 in the acute inflammatory response induced by Bothrops atrox snake venom. Toxicon [Internet]. 2016 Aug;118:121–8. Available from: 10.1016/j.toxicon.2016.04.042.
[54] A. Thiansookon, P. Rojnuckarin, Low incidence of early reactions to horse-derived F(ab')2 antivenom for snakebites in Thailand, Acta Tropica 105 (2) (2008) 203–205.
[55] Amin M, Mamun S, Rashid R, Rahman M, Ghose A, Sharmin S, et al. Anti-snake venom: use and adverse reaction in a snake bite study clinic in Bangladesh. J Venom Anim Toxins incl Trop Dis [Internet]. 2008;14(4):660–72. Available from: https://doi.org/10.1590/S1678-91992008000400009.
[56] D.J. Williams, S.D. Jensen, B. Nimorakiotakis, R. Müller, K.D. Winkel, Antivenom use, premedication and early adverse reactions in the management of snake bites in rural Papua New Guinea, Toxicon 49 (6) (2007) 780–792.
[57] Kularatne SAM, Weerakoon K, Silva A, Maduwage K, Walathara C, Rathnayake I, et al. Efficacy of intravenous hydrocortisone administered 2–4 h prior to antivenom as prophylaxis against adverse drug reactions to snake antivenom in Sri Lanka: An open labelled randomized controlled trial. Toxicon [Internet]. 2016;120:159–65. Available from: 10.1016/j.toxicon.2016.08.011.
[58] Premawardhena AP, De Silva CE, Fonseka MMD, Gunatilake SB, De Silva HJ. Low dose subcutaneous adrenaline to prevent acute adverse reactions to antivenom serum in people bitten by snakes: Randomised, placebo controlled trial. Br Med J [Internet]. 1999;318(7190):1041–3. Available from: 10.1136/bmj.318.7190.1041.
[59] G.K. Isbister, S.G. Brown, E. MacDonald, J. White, B.J. Currie, Current use of Australian snake antivenoms and frequency of immediate-type hypersensitivity reactions and anaphylaxis, Medical J. Australia 188 (8) (2008) 473–476.
[60] H.A. de Silva, N.M. Ryan, H.J. de Silva, Adverse reactions to snake antivenom, and their prevention and treatment: Adverse reactions to snake antivenom, and their prevention and treatment, Br. J. Clin. Pharmacol. 81 (3) (2016) 446–452.
[61] E. Moreno, M. Queiroz-Andrade, R.M. Lira-da-Silva, J. Tavares-Neto, Características clínicoepidemiológicas dos acidentes ofídicos em Rio Branco, Acre, Rev. Soc. Bras. Med. Trop. 38 (1) (2005) 15–21.
[62] Cruce JM, Lewis RE. Types I, II, III, and IV Hypersensitivity. In: Atlas of Immunology. 2nd ed. 1999. p. 225–6.
[63] Morais VM, Massaldi H. Snake antivenoms: adverse reactions and production technology. J Venom Anim Toxins incl Trop Dis [Internet]. 2009;15(1):2–18. Available from: https://doi.org/10.1590/S1678-91992009000100002.
[64] León G, Sánchez L, Hernández A, Villalta M, Herrera M, Segura A, et al. Immune response towards snake venoms. Inflamm Allergy - Drug Targets. 2011;10(5): 381–98.
[65] A.G.C. Bonavita, A.S. da Costa, A.L.A. Pires, A.G.C. Neves-Ferreira, J. Perales, R.S. B. Cordeiro, M.A. Martins, P.M.R. e Silva, Contribution of mast cells and snake venom metalloproteinases to the hyperalgesia induced by Bothrops jararaca venom in rats, Toxicon 47 (8) (2006) 885–893.
[66] C.R. de Medeiros, K.C. Barbaro, M.S. Lira, F.O.S. França, V.L. Zaher, C.M. Kokron, J. Kalil, F.F.M. Castro, Predictors of Bothrops jararaca venom allergy in snake handlers and snake venom handlers, Toxicon 51 (4) (2008) 672–680.
[67] J.-F. Wei, X.-L. Wei, Y.-Z. Mo, S.-H. He, Induction of mast cell accumulation, histamine release and skin edema by N49 phospholipase A2, BMC Immunol. 10 (1) (2009), https://doi.org/10.1186/1471-2172-10-21.
[68] J. Rivera, Snake bites and bee stings: the mast cell strikes back, Nat. Med. 12 (9) (2006) 999–1000.

[69] L.G.B. De Toni, D.L. Menaldo, A.C.O. Cintra, M.J. Figueiredo, A.R. de Souza, W.M. A. Maximiano, M.C. Jamur, G.E.P. Souza, S.V. Sampaio, Inflammatory mediators involved in the paw edema and hyperalgesia induced by Batroxase, a metalloproteinase isolated from Bothrops atrox snake venom, Int. Immunopharmacol. 28 (1) (2015) 199–207.
[70] Mendonça-Franqueiro EDP, Alves-Paiva RDM, Sartim MA, Callejon DR, Paiva HH, Antonucci GA, et al. Isolation, functional, and partial biochemical characterization of galatrox, an acidic lectin from Bothrops atrox snake venom. Acta Biochim Biophys Sin (Shanghai) [Internet]. 2011;43(3):181–92. Available from: 10.1093/abbs/gmr003.
[71] de Souza CA, Kayano AM, Setúbal SS, Pontes AS, Furtado JL, Kwasniewski FH, et al. Local and systemic biochemical alterations induced by Bothrops atrox snake venom in mice. J Venom Res [Internet]. 2012;3:28–34. Available from: http://www.ncbi.nlm.nih.gov/pubmed/23487552%0Ahttp://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=PMC3595125.
[72] M.A. Sartim, T.B. Riul, C.-A. Del, S.R. Stowell, M. Dias-baruffi, S.V. Sampaio, Galatrox, a C-type lectin in Bothrops atrox snake venom, selectively binds LacNAc-terminated glycans and possesses the capacity to induce acute inflammation, Glycobiol. Adv. Access. (2014).
[73] Brennan K, Zheng J. Interleukin 8. In: xPharm: The Comprehensive Pharmacology Reference [Internet]. Elsevier; 2007. p. 1–4. Available from: https://linkinghub.elsevier.com/retrieve/pii/B9780080552323619166.
[74] O. Boyman, J. Sprent, The role of interleukin-2 during homeostasis and activation of the immune system, Nat. Rev. Immunol. 12 (3) (2012) 180–190.
[75] A.R. Rezai, J.F. Salazar-Gonzalez, O. Martínez-Maza, J. Bramhall, R. Afrasiabi, V. Kermani-Arab, Histamine Blocks Interleukin 2 (IL-2) Gene Expression and Regulates IL-2 Receptor Expression, Immunopharmacol. Immunotoxicol. 12 (3) (1990) 345–362.
[76] Tasaka K, Hamada M, Mio M. Effect of Hyperthermia on Histamine Release from Rat Mast Cells. Jibi Inkoka Tembo [Internet]. 1993;36(5):563–71. Available from: 10.11453/orltokyo1958.36.563.
[77] K.V. Poojary, Y.-C. Kong, M.A. Farrar, Control of Th2-Mediated Inflammation by Regulatory T Cells, American J. Pathol. 177 (2) (2010) 525–531.
[78] B. Bonnet, J. Vigneron, B. Levacher, T. Vazquez, F. Pitoiset, F. Brimaud, G. Churlaud, D. Klatzmann, B. Bellier, Low-Dose IL-2 Induces Regulatory T Cell–Mediated Control of Experimental Food Allergy, J. Immunol. 197 (1) (2016) 188–198.
[79] Jeannin P, Delneste Y, Gosset P, Molet S, Lassalle P, Hamid Q, et al. Histamine induces interleukin-8 secretion by endothelial cells. Blood [Internet]. 1994;84(7):2229–33. Available from: 10.1182/blood.v84.7.2229.2229.
[80] Y. Li, L. Chi, D.J. Stechschulte, K.N. Dileepan, Histamine-Induced Production of Interleukin-6 and Interleukin-8 by Human Coronary Artery Endothelial Cells Is Enhanced by Endotoxin and Tumor Necrosis Factor-α, Microvascular Res. 61 (3) (2001) 253–262.
[81] Kuna P, Reddigari SR, Kornfeld D, Kaplan AP. IL-8 inhibits histamine release from human basophils induced by histamine-releasing factors, connective tissue activating peptide III, and IL-3. J Immunol. 1991;147(6):1920–4.
[82] R. Vantur, M. Rihar, A. Koren, M. Rijavec, P. Kopac, U. Bidovec-Stojkovic, R. Erzen, P. Korosec, Chemokines during anaphylaxis: the importance of CCL2 and CCL2-dependent chemotactic activity for basophils, Clin. Transl. Allergy 10 (1) (2020), https://doi.org/10.1186/s13601-020-00367-2.
[83] J.M.G. Ruiz de Morales, L. Puig, E. Daudén, J.D. Cañete, J.L. Pablos, A.O. Martín, C.G. Juanatey, A. Adán, X. Montalbán, N. Borruel, G. Ortí, E. Holgado-Martín, C. García-Vidal, C. Vizcaya-Morales, V. Martín-Vázquez, M.Á. González-Gay, Critical role of interleukin (IL)-17 in inflammatory and immune disorders: An updated review of the evidence focusing in controversies, Autoimmunity Rev. 19 (1) (2020) 102429, https://doi.org/10.1016/j.autrev.2019.102429.
[84] Sachett JAG, da Silva IM, Alves EC, Oliveira SS, Sampaio VS, do Vale FF, et al. Poor efficacy of preemptive amoxicillin clavulanate for preventing secondary infection from Bothrops snakebites in the Brazilian Amazon: A randomized controlled clinical trial. PLoS Negl Trop Dis [Internet]. 2017;11(7):1–21. Available from: https://doi.org/10.1371/journal.pntd.0005745.
[85] World Allergy Organization. Perioperative Allergic Reactions [Internet]. 2019 [cited 2019 Jun 27]. Available from: https://www.worldallergy.org/education-and-programs/education/allergic-disease-resource-center/professionals/allergy-to-anesthetic-agents.
[86] M. Colombini, Reatividade antigênica cruzada entre os venenos de Bothrops atrox e Lachesis muta muta e desenvolvimento de um teste imunoenzimático diferencial para acidentes causados por essas serpentes, São Paulo: Universidade de São Paulo (2003).