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Severity of papular urticaria in children is associated with specific IgG4 anti-salivary gland antigens from *Aedes aegypti*

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Key words

Salivary antigen; Aedes aegypti; papular urticaria; humoral immune response IgG4; children.

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Impact statement

Based on the symptoms and severity of the patients, we hypothesize that elevated IgG4 levels to A. aegypti salivary antigens may be associated with greater disease severity, potentially serving as a severity marker. These findings advance current knowledge of arthropod-bite hypersensitivity and provide a basis for future studies on diagnosis, prognosis, and targeted interventions. However, it is important to emphasize that further studies are required to validate this association and reach a definitive clinical conclusion.

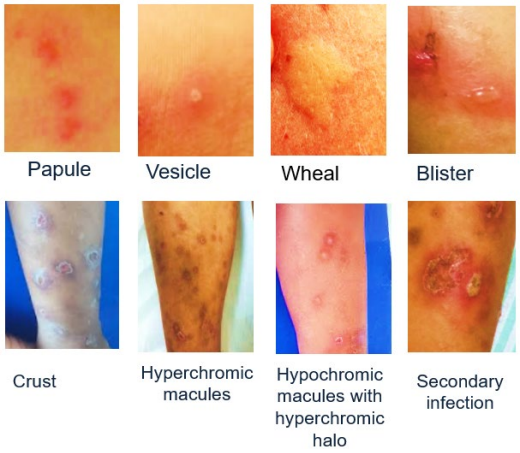
Summary

Background. *Papular Urticaria (PU) is a cutaneous hypersensitivity disorder triggered by hematophagous arthropod bites. Despite being a common condition, especially in tropical environments, many knowledge gaps are observed for this disease. The main objective of this study was to investigate the patterns of humoral immune response to mosquito antigens in children with PU and establish a correlation between this response and the severity of clinical symptoms.* ***Methods.*** *An analytical cross-sectional observational study was carried out. Clinical and sociodemographic data and children's blood samples were collected to measure the specific antibodies from: 1) A. aegypti salivary gland antigens; 2) A. aegypti whole body antigens (both produced in the laboratory of the Center for Health Sciences at the Federal University of Rio de Janeiro). A PU severity score based on clinical data is proposed to correlate disease severity with antibody reactivity signatures.* ***Results.*** *According to the clinical data, 58.9% of children received high severity scores. A significant statistical correlation was found between patients with high PU severity score and the development of symptoms before the age of two ($p = 0.0326$) and high IgG4 anti-salivary gland antigens concentration ($p < 0.05$).* ***Conclusions.*** *It is suggested that PU severity in children is associated with a high concentration of IgG4 anti-salivary gland antigens from Aedes aegypti. Further studies are recommended to deepen the understanding of the mechanisms involved.*

Introduction

Worldwide, skin diseases are observed after insect bites (1). Among these diseases, we highlight Papular Urticaria (PU). PU is defined as a chronic recurrent dermatosis. It manifests itself as diffuse papules and vesicles, with an erythematous base, in different stages of evolution. The papules are extremely pruritic (**figure 1**) (2). Skin diseases caused by insects living in domestic environments have rarely been systematically studied (2). The exact prevalence of PU is still unknown. The number of affected individuals is

Figure 1 *- Aspects of skin lesions observable in patients with PU.*



believed to be high, especially in tropical regions (1). Epidemiological studies have revealed varying prevalence rates of PU in different regions. In Italy, in a total of 105 subjects with dermatitis induced by arthropods in a domestic environment, PU was present in 46.9% of patients (1). In Bogotá, Colombia, 62.9% of children aged 1 to 6 with dermatological conditions had PU (3). In Calcutta, India, the prevalence was 10.6% among children under five (4). In Cameroon, PU was present in 5.4% of children evaluated (5). In Brazil, a study conducted in Curitiba found a 9% rate of PU among patients aged 0 to 14, and 63% were under two years of age (6). A more recent study in Rio de Janeiro reported a PU prevalence of 7.42% among 202 children with dermatoses (7).

Although the diagnosis is generally based on clinical picture and epidemiological information, sometimes it's impossible to determine the insect responsible for the lesions (8).

The saliva of various arthropods such as ticks, bed bugs, fleas, and mosquitoes may contain common antigens that can induce an immune response in the patient (9-11). Most studies indicate that PU is a result of an immune response to proteins found in insect saliva (1, 8, 11). Although most of these proteins have unknown functions (12), studies show that they can stimulate the formation of type G, A, M, and E immunoglobulins and/or activate specific CD4+ T lymphocytes in susceptible children (2, 13). Recent studies suggest the participation of more than one immunological mechanism (Gell and Coombs classification) in the PU pathophysiology, but the exact mechanism remains unknown (13).

Among the types of Gell and Coombs hypersensitivity reaction in PU patients three types were found:

In type I Gell and Coombs hypersensitivity reaction, the mosquito saliva protein, upon binding to mast cell-specific IgE, induces the degranulation of vasoactive amines, leading to local manifestations such as the immediate formation of a pruritic wheal (14). The presence of specific IgE can be demonstrated by a positive reaction to a skin test (prick test) or by serum measurement of this antibody (1, 15).

Pustular and hemorrhagic lesions, typical of cutaneous vasculitis, have also been identified in patients with PU. Immunofluorescence examinations conducted on biopsy samples of skin tissue from PU patients revealed the presence of IgG and IgM deposits along with fractions of complement system C1q and C3 on the surface of blood vessels. These findings suggest the formation of immune complexes, with complement system activation, as in Gell and Coombs type III hypersensitivity reactions (14, 15).

Pronouncedly pruritic papules may emerge at the site of insect bite a few days after insect bites and persist for weeks. These late-onset lesions may indicate a cell-mediated reaction, characteristic of Gell and Coombs type IV hypersensitivity (16). Studies conducted by Peng *et al.* revealed that the average lymphocyte proliferation index in response to mosquito allergens was significantly higher in patients with late-onset cutaneous reactions (hardened papules) induced by insect bites (14).

An immunohistochemical study of the cellular infiltrate in the skin lesions of 45 patients with PU revealed a predominance of CD4+ T lymphocytes and eosinophils in papules that emerged after the cutaneous injection of flea antigens (17). The presence of these cells reinforces the theory that both immediate and delayed mechanisms (type I and IV hypersensitivity reactions) are involved in these responses, suggesting the participation of more than one immunological mechanism (15, 18).

The role of immunoglobulins in PU pathophysiology is still unclear. Some authors correlate the presence of specific IgE and IgG with immediate and late reactions. These same authors identified the presence of IgE, IgG1, and IgG4 at high levels in patients with extensive cutaneous manifestations (14). In contrast, others reported that heavily exposed patients produced little or no specific IgG against mosquito antigens (19).

Primary and secondary objectives

In Brazil, no studies on PU have associated clinical information with the specific immune response to insect antigens. The primary objective of this study was to investigate the patterns of humoral immune response to mosquito antigens (*Aedes aegypti*) in children with PU and establish a correlation between this response and the severity of clinical symptoms. The secondary objective was to define the clinical and demographic characteristics of these children.

Materials and methods

Study design

This cross-sectional, observational, and analytical study collected clinical, demographic, and epidemiological data from patients

affected by PU treated at the Allergy and Immunology outpatient clinic of the Hospital Federal de Bonsucesso (Rio de Janeiro, Brazil) from September 2018 to March 2020.

Inclusion and exclusion criteria

The study exclusively enrolled patients under 15 years old with a clinical diagnosis of PU. Schoolchildren and preschoolers who were using antihistamines (for at least five days before the interview), systemic immunosuppressive medication (for at least three months prior to blood sample collection), using immunotherapy, or had any primary or acquired immunodeficiency were excluded from the study.

Papular urticaria severity score

PU is a neglected disease. As such, there are no established criteria to assess its severity. Because of this, a severity scoring system was developed, adapting information from SCORAD (20) (used in atopic dermatitis) to categorize patients into mild, moderate, or severe groups. However, it is essential to highlight that this score has not been validated and has never been used in other populations. The PU severity score developed considers the following clinical data: 1) clinical manifestation of asthma, rhinitis, or atopic dermatitis (skin prick tests for aeroallergens were not conducted), 2) extension of the affected body area, 3) use of systemic antibiotics to treat skin infections, and 4) itching intensity. These four criteria were chosen because they correlate well with the severity of symptoms (**table I**).

A Likert Scale ranging from 0 to 10 was employed to assess itching in the last 48 hours, with 0 signifying the complete absence of itching and 10 indicating severe itching involving nocturnal awakenings (**table I**). The large relative weight of this clinical aspect reflects its contribution to the patient´s discomfort and resulting compromised well-being. The participant PU severity score was calculated by the sum of these values.

The score ranges from 3 to 18 points, with 3 being the least severe and 18 the most severe. We classified the severity of the patient's clinical condition using this scoring method into two groups: one with less severity and the other with greater severity. PU severity score from 3 to 10 were classified as "mild" and scores from 11 to 18 were classified as "moderate to severe".

Preparation of mosquitoes' antigenic extracts

The antigenic extract from *Aedes aegypti*'s salivary gland (AgGlan) was obtained by dissecting adult female mosquitoes (n = 50) six days old before feeding. mosquitoes were anesthetized and treated with 70% alcohol, then phosphate-buffered saline (PBS). Salivary glands were isolated, transferred to microtubes, and frozen at -80 °C. Another antigenic preparation was obtained from the whole body of *Aedes* mosquitoes without glands (AgCor). The salivary gland and abdomen mass were solubilized in PBS with 2% neutral detergent and protease inhibitors. After maceration and freeze-thaw cycles, the suspensions underwent ultrasound treatment, centrifugation, and were stored at -80 °C for later use.

Blood sample collection

The study obtained blood samples from 34 participants via digital puncture on filter paper during outpatient visits, stored in a humidity and light-controlled refrigerator. An image analysis tool was utilized to standardize blood volume. Blood spots were excised, placed in microtubes with PBS, vortexed, and incubated at room temperature for 24 hours. The eluted blood was then pre-diluted to a 1:50 ratio, homogenized, and stored at 2 to 8 °C for further analysis.

Detection of specific immunoglobulin to mosquito antigenic extracts

Initially, 96 well microplates were coated with 20 µg/mL of the antigen extracted and diluted in sodium carbonate and

Table I *- Severity score criteria for papular urticaria patients.*

Clinical aspects	Description	Attributed value
Manifestation of atopic disease (asthma, rhinitis or atopic dermatitis)	No	1
	Yes	2
Body extension	Lower limbs only	1
	Lower limbs and upper limbs	2
	Lower limbs, upper limbs and chest	3
Previous use of antibiotics to treat skin infections	Denies use	1
	Used between 1 and 4 times	2
	Used more than 4 times	3
Intensity of itching in the last 48 hours	Scale from 0 to 10 obtained from the interview with the responsible	0-10

bicarbonate solution at pH 9.6. Subsequently, the wells were blocked with PBS containing 10% Fetal Bovine Serum (FBS). Then, diluted serum (1:50) in PBS with 1% FBS was added in duplicate and incubated. Following incubation, anti-IgG, IgG1, IgG2, IgG3, IgG4, IgA, IgE, or IgM conjugated with HRP were added to each well. A chromogen solution (Orthophenilenediamine + H_2O_2) was then added, and after color development, the reaction was stopped with 2N HCL. The microplate was read at 492 nm, and the number of specific antibodies was determined based on the absorbance value (Enzyme-Linked Immunosorbent Assay).

Data analyses considerations

The study presented categorical data as proportions and continuous data as medians and interquartile ranges (IQR). Statistical comparisons between study groups involved Fisher's exact test or Pearson's chi-square test for categorical variables and the Mann-Whitney U test or Kruskal-Wallis test for continuous variables. Significance was considered when $p < 0.05$. Analysis was conducted using Graph Pad Prism Version 10.0.2. Multidimensional analyses were employed to delineate immunological profiles among participants and their association with clinical data. Patients were categorized into three clusters based on the intensity of antibody recognition towards *Aedes aegypti* gland antigens (AgGlan) and body antigens (AgCor). Cluster 1 exhibited reduced reactivity, cluster 2 moderate, and cluster 3 high reactivity to antigenic preparations. Heatmap matrix analyses of immunoglobulin levels were conducted using Heat mapper®, employing hierarchical clustering with average linkage and Euclidean distance measurement.

Ethical approval

The study was approved by the Research Ethics Committee of Hospital Federal de Bonsucesso under approval number 2.830.938. As all participants were children under 15 years of age, written informed consent was obtained from their parents or legal guardians before inclusion in the study, in accordance with the approved study protocol.

Results

Sociodemographic and clinical results

A total of 107 children with a clinical diagnosis of PU was included, 64 of these (59.8%) were male. The mean age of the children assisted was 5.9 years ± 3.7 years, ranging from 1.1 to 14 years. Regarding the clinical data, 83 (77.6%) of the patients with PU had clinical manifestations of atopic diseases (asthma, rhinitis, or atopic dermatitis). When the person responsible was asked about the intensity of pruritus in the last two days, on a scale from zero (no pruritus) to ten (severe pruritus, with sleep impairment), 62 (57.8%) reported a value greater than or equal to 6. The mean pruritus intensity was 6.0 ± 3.0. The other sociodemographic and clinical data are described in **table II**.

Table II *- Demographic, clinical, and epidemiological characteristics for the entire PU population (n = 107) and for a group of children evaluated for ELISA-specific antibodies to Aedes aegypti (n = 34).*

Parameter	The entire PU population (n = 107)	PU subgroup evaluated for ELISA-specific antibodies (n = 34)	P-value
Gender			N/S
Male	64 (59.8%)	16 (47.1%)	
Female	43 (40.2%)	18 (52.9%)	
Age			N/S
<5 years	47 (43.9%)	15 (44.1%)	
≥5 <11 years	48 (44.9%)	15 (44.1%)	
≥11 years	12 (11.2%)	4 (11.8%)	
Illness duration			N/S
<6 years	79 (73.8%)	24 (70.6%)	
≥6 years	28 (26.2%)	10 (29.4%)	
Region			N/S
Urban/periurban	83 (77.6%)	28 (82.4%)	
Rural	24 (22.4%)	6 (17.6%)	

Parameter	The entire PU population (n = 107)	PU subgroup evaluated for ELISA-specific antibodies (n = 34)	P-value
Type of insects			N/S
Unaware	2 (1.9%)	2 (5.9%)	
Mosquito	63 (58.9%)	19 (55.9%)	
Mosquito and ant	29 (27.1%)	8 (23.5%)	
Mosquito, ant and flea	9 (8.4%)	3 (8.8%)	
Mosquito and flea	4 (3.7%)	2 (5.9%)	
First symptoms (age)			N/S
< 2 years	74 (69.1%)	23 (67.6%)	
≥2 years	33 (30.9%)	11 (32.4%)	
Body regions affected			N/S
Only superior members	0 (0%)	0 (0%)	
Lower limbs only	66 (61,7%)	19 (55.9%)	
Thorax only	0 (0%)	0 (0%)	
Upper limbs and lower limbs	36 (33.6%)	13 (38.2%)	
Upper limbs, lower limbs and thorax	5 (4.7%)	2 (5.9%)	
Season related to worsening of the condition			N/S
Spring/summer	49 (45.8%)	16 (47.1%)	
Autumn/winter	12 (11.2%)	4 (11.7%)	
No relation	46 (43.0%)	14 (41.2%)	
Intensity of pruritus in the last 2 days			N/S
0	12 (11.2%)	4 (11.8%)	
1	1 (0.9%)	0 (0%)	
2	3 (2.8%)	2 (5.9%)	
3	7 (6.5%)	3 (8.8%)	
4	4 (3.7%)	3 (8.8%)	
5	17 (15.9%)	3 (8.8%)	
6	7 (6.5%)	2 (5.9%)	
7	10 (9.3%)	1 (2.9%)	
8	23 (21.5%)	7 (20.6%)	
9	9 (8.4%)	5 (14.7%)	
10	13 (12.1%)	4 (11.8%)	
Unknown	1 (0.9%)	0 (0%)	
Aspect of injuries			N/S
Papule	19 (17.8%)	3 (8,8%)	
Vesicle	2 (1.9%)	0 (0%)	
Wheal	0 (0%)	0 (0%)	
Crusts	15 (14.0%)	6 (17.7%)	
Papule and cust	25 (23.3%)	9 (26.5%)	
Papule and wheal	2 (1.9%)	1 (2.9%)	
Papule, wheal and crust	5 (4.7%)	2 (5.9%)	

Parameter	The entire PU population (n = 107)	PU subgroup evaluated for ELISA-specific antibodies (n = 34)	P-value
Papule and vesicle	4 (3.7%)	2 (5.9%)	
Papule, vesicle and crust	2 (1.9%)	1 (2.9%)	
Papule, vesicle, wheal and crust	2 (1.9%)	1 (2.9%)	
Absent	31 (28.9%)	9 (26.5%)	
Macules pigmentation			N/S
Hyperchromic	25 (23.3%)	13 (38.2%)	
Hypochromic	11 (10.3%)	4 (11.8%)	
Both	66 (61.7%)	15 (44.1%)	
Absent	5 (4.7%)	2 (5.9%)	
Manifestation of atopic disease			N/S
Yes	83 (77.6%)	27 (79.4%)	
No	24 (22.4%)	7 (20.6%)	
Type of atopic diseases			N/S
Asthma	4 (3.7%)	1 (2.9%)	
Atopic dermatitis	5 (4.7%)	1 (2.9%)	
Rhinitis	44 (41.1%)	16 (47.1%)	
Rhinitis and asthma	20 (18.7%)	9 (26.5%)	
Rhinitis and asthma and atopic dermatitis	8 (7.5%)	0 (0%)	
Rhinitis and atopic dermatitis	2 (1.9%)	0 (0%)	
Absent	24 (22.4%)	7 (20.6%)	
Previous antibiotic use			N/S
Yes	64 (59.8%)	15 (44.1%)	
No	43 (40.2%)	19 (55.9%)	
Family members affected (father, mother and brothers)			p<0.001
Yes	47 (43.9%)	15 (44.1%)	
No	56 (52.3%)	7 (20.6%)	
Unknown	4 (3.7%)	12 (35.3%)	
Pattern of injuries at exam			N/S
In activity	15 (14.0%)	7 (20.6%)	
Scarring	48 (45.0%)	10 (29.4%)	
Both	42 (39.2%)	17 (50.0%)	
Have not been evaluated	2 (1.8)	0 (0%)	

N/S = non-significant.

Table III shows how the severity of symptoms varies with gender, age, season of the year, clinical parameters and other variables. Regarding the severity score, we observed that 63 children (58.9%) had moderate to severe PU, as evidenced by the clinical severity score developed for this study. Among these, we observed that 78% had the onset of symptoms before two years of age (p = 0.032). We did not verify statistical significance for any other association.

Clusters were defined based on the intensity of recognition of antibodies specific to the different antigenic preparations. When we evaluated the reactivity of antibodies to *Aedes aegypti* AgGlan in the heat map, we identified that the signature generated by cluster 3 was associated with a greater PU severity (median = 12.50; IQR = 11.0 and 15.0) in relation to cluster 2 (median = 9.0; IQR = 4.5 and 13.5; p < 0.05). We could highlight that augmented reactivity of IgG4 and IgG specific to AgGlan was

Table III *- Severity levels for different variables.*

Variable	Intensity level mild (score < 11) n = 44 (41.1%)	Intensity level moderate to severe (score ≥ 11) n = 63 (58.9%)	P-value
Gender	19 (43.0%)	24 (38.0%)	N/S*
Female	25 (57.0%)	39 (62.0%)	
Male			
Age			N/S*
<5 years	19 (43.0%)	28 (44.0%)	
≥ 5 <11 years	19 (43.0%)	29 (46.0%)	
≥11 years	06 (14.0%)	06 (10.0%)	
Illness duration			N/S*
< 6 years	33 (75.0%)	46 (73.0%)	
≥ 6 years	11 (25.0%)	17 (27.0%)	
Region			N/S*
Rural	9 (20.0%)	15 (24.0%)	
Urban/peri-urban	35 (80.0%)	48 (76.0%)	
First symptoms (age)			*p=0.032**
<2 years	25 (57.0%)	49 (78.0%)	
≥2 years	19 (43.0%)	14 (22.0%)	
Season of the year related to worsening of the condition			N/S*
Spring/summer	23 (52.0%)	26 (41.0%)	
Autumn/winter	2 (4.5%)	10 (16.0%)	
No relation	19 (43.5%)	27 (43.0%)	
Manifestations of atopic disease (asthma, atopic dermatitis, rhinitis)			N/S*
Yes	32 (73.0%)	51 (81.0%)	
No	12 (27.0%)	12 (19.0%)	
Family members affected			N/S **
Father, mother or sibling	22 (50.0%)	21 (33.3%)	
Other members	6 (13.6%)	18 (28.6%)	
Unknown	16 (36.4%)	24 (38.1%)	

N/S = non-significant; *Fischer's exact test; **chi-square test.

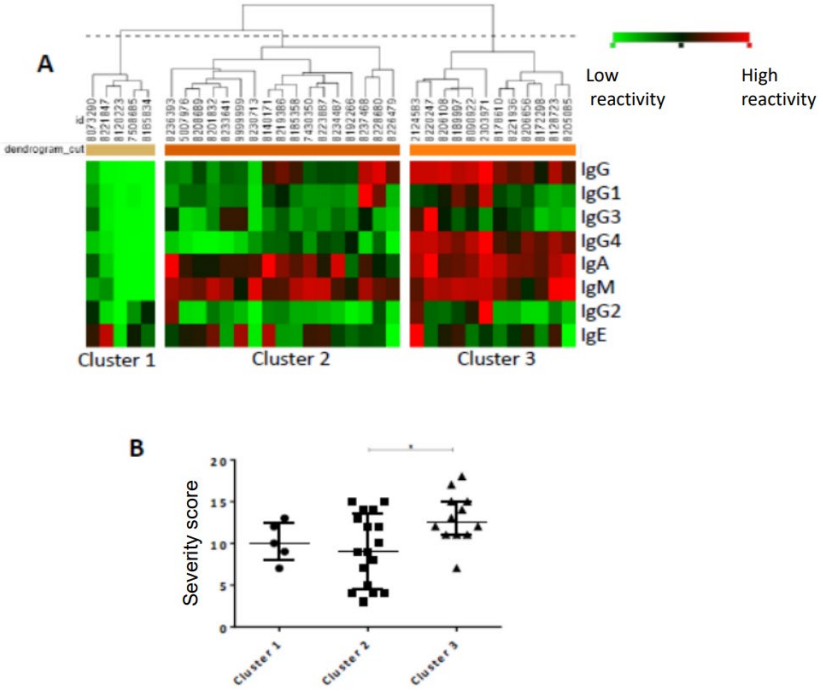
observed for the signature associated with greater severity. There was no greater reactivity of specific IgE to AgGlan that could be related to greater PU severity (**figure 2**). Cluster 1 showed a profile of low antibody reactivity to AgGlan, and although the median severity score presented by cluster 1 patients (median = 10.0; IQR = 8.0 and 12.5) was smaller than cluster 3, the difference was not significant.

The reactivity profile of specific antibodies to the *Aedes aegypti* body antigen (AgCor) didn't show a statistically significant association with PU severity score. Specific IgE reactivity was not determinant in the definition of signatures and was not associated with PU severity.

Discussion and conclusions

In our study, most patients (88.8%) were under 11 years old and had symptoms for less than 6 years (73.8%), suggesting, as in several studies, a natural desensitization over the years (21, 22). Furthermore, our study found that most patients had a personal history of allergic disease (77.6%) with rhinitis and asthma being the most prevalent.

Figure 2 *- Association between the PU patient's severity score and the immune response profile based on the reactivity index of specific antibodies to AgGlan (n = 34).*



(A) Heat map showing reactivity indices of antibody isotypes (low reactivity = green; high reactivity = red) specific to AgGlan. **(B)** Analysis of the severity score between clusters (1, 2 and 3) based on the reactivity indices of specific antibodies to AgGlan.

Unlike what is seen in the literature, where the patient is the only one affected in the family (11), our study revealed a high number of family members affected: 43.9% of the children had at least one close relative with the same symptoms. Because of this finding, one can consider the possibility of a genetic predisposition in addition to environmental exposure.

Children whose symptoms appeared before 2 years of age had a higher severity score when compared to those who started after 2 years of age ($p = 0.032$). This finding suggests that the age of onset of symptoms may be related to the severity of PU. Genetic causes that interfere with immune tolerance mechanisms, causing impaired production of regulatory cytokines (IL-10) could be present, as Cuéllar *et al.*, showed in patients with PU due to flea bites. Low levels of regulatory cytokines may favor the secretion of pro-inflammatory T2 cytokines that contribute to the generation and maintenance of cutaneous hypersensitivity reactions generated by insect bites during childhood (23). Other clinical correlations with score severity did not show statistical significance (**table III**).

In the heat map, the signature of cluster 3 antibody reactivity to AgGlan was associated with a higher PU severity score compared with cluster 2 ($p < 0.05$). Notably, higher specific IgG4 reactivities were observed in the most severe patients. This observation suggests that IgG4 could be associated with a more severe symptom. Researchers in Finland have already identified the presence of IgE and IgG4 against proteins present in *Aedes communis* saliva in the serum of adults with immediate and delayed skin cutaneous reactions to mosquito bites. These antibodies were not identified in unexposed infants, suggesting that specific IgE and IgG4 may play a critical role in the pathogenesis of hypersensitivity reactions to mosquito bites (24).

The study of Palosuo *et al.* verified an increase in the production of IgE, IgG1 and IgG4 against the proteins in the saliva of *Aedes* found in the serum of volunteers after the mosquitos' season. This finding suggests the association of IgG4 with the pathophysiology of PU (25).

On the other hand, a study by Srivastava *et al.*, in India, in sensitized patients demonstrated an improvement in cutaneous reactivity associated with reduced IgE and increased specific IgG4 against whole-body mosquito extracts of *Culex quinquefasciatus* after receiving specific immunotherapy for one year (26).

The role of IgG4 in the pathophysiology of reactions to mosquito bites is still uncertain. This association suggests two pos-

sibilities: 1) either this increase in IgG4 specific to AgGlan can be interpreted as a marker for the severity of PU, or 2) it can be considered as a confounding factor, *i.e.*, part of a compensatory mechanism of the immune system indicating that the patient is evolving to immune tolerance.

However, based on the symptoms and severity of patients in our study, with elevated IgG4 levels to *A. aegypti* AgGlan, we hypothesize that this antibody may be associated with greater disease severity, potentially serving as a severity marker. Additionally, based on the authors' experience and considering that other mosquitos have several antigens in common, it is possible to infer that these findings can be generalized to bites of different species of mosquitos. Nevertheless, further studies are required to confirm this hypothesis.

Regarding the immunoglobulin profile, the low reactivity of the immunoglobulins to *A. aegypti* AgGlan observed in cluster 1 patients can be explained by different stages of the disease for each individual or because antigens from insect species other than *A. aegypti* induce PU. In some patients, the immune response related to the pathogenesis of PU is possibly mediated by antigens of different insect species, especially in children living in households where more than one type of insect is present. What we see in our results may be one part of the complex process of the immune system's response to different antigens associated with the pathogenesis of PU.

This original article examines clinical and laboratory data from patients with PU in South America, particularly Brazil. The study highlights the need for a validated scoring system to improve patient care and support future research. Furthermore, it is worth highlighting the substantial correlation identified between IgG4 levels to *A. aegypti* AgGlan and the severity of the clinical condition.

We emphasize that we could not evaluate the reactivity index to *Culex* mosquito antigens, which, depending on the geographic location in the city of Rio de Janeiro, is much more prevalent than *Aedes* (27). Another limitation is this study is the exclusive use of patients from a single center. Future research should involve a larger multicenter sample with a control group to understand the disease-immunoglobulin isotype relationship. Moreover, the use of a non-validated clinical score developed by researchers presents a challenge when conducting comparative studies. It is critical to note that the need for validated severity scores and biomarkers poses significant challenges for future research.

In conclusion, the results show that a statistically significant correlation exists between the severity of PU in children and a high concentration of IgG4 anti-salivary gland antigens of *Aedes aegypti*. This investigation also highlights the importance of developing a globally validated severity scoring system to validate comparative studies worldwide. Finally, we aspire to encourage the search for a biological marker that helps diagnose and assess the severity of patients with PU.

Further studies on PU will deepen our understanding of the mechanisms involved in this prevalent, uncomfortable, and often neglected hypersensitivity manifestation, not only in South America, but also in other continents.

Fundings

None.

Contributions

AGS, RTA: conceptualization, data curation, formal analysis, writing – original draft. AGS, SAS, RTA: methodology. RTA: investigation. AGS: resources. AGS, RTA, FPM, SAS, ESG: visualization. AGS, ESG, FPM: supervision. RTA, ESG, FPM, SAS, AGS: writing – review & editing.

Conflict of interests

The authors declare that they have no conflict of interests.

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