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Original article

# Histopathological and clinical evaluation of chronic spontaneous urticaria patients with neutrophilic and non-neutrophilic cutaneous infiltrate

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*Abbreviations:*
CSU, chronic spontaneuous urticaria; IgE, immunoglobulin E; AOSD, adult onset Still disease; CAPS, cryopyrin associated periodic syndromes; X, abnormal tests; N, total test; x/N (%), relation between abnormal tests (x) and total tests (N) followed by the respective percentage in the specific groups; N/E, neutrophilic/eosinophilic group; Ly, lymphocytic group; RV, reference value; TPO-Ab, thyroid peroxidase antibody; TG-Ab, thyroid globulin antibody; ANA, antinuclear autoantibodies; RF, rheumatoid factor; ↑, increased; ↓, decreased; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; TSH, thyroid stimulating hormone; MPV, mean platelet volume; Anti-TBG, anti-tireoglobulin; Anti-TPO, anti-thyroperoxidase; TSH, thyroid stimulating hormone; DIF, direct immunofluorescence; BMZ, basal membrane zone; CB, citoid bodies

ABSTRACT

*Background:* Chronic urticaria has an expressive prevalence in general population, especially in adults, and is defined by the presence of intermittent hives for six weeks or longer. Our study aims to characterize the histological patterns of chronic spontaneous urticaria, based on the inflammatory cell infiltrate, and correlate them to laboratory exams.
*Methods:* It was performed a retrospective analysis of laboratory, histopathology and direct immunofluorescence data of 93 patients with chronic urticaria. For histopathological analysis, cell count was performed in four fields at high magnification (×400) for each specimen. The resulting cell count medians were submitted to statistical analysis and, then, were correlated to laboratorial findings.
*Results:* We found a female predominance (76.34%) of chronic urticaria cases, and an average age of 42.5 years (SD ± 15). Two histological groups were distinctive: 1) chronic urticaria with predominance of neutrophils or eosinophils – N (%) = 39 (42.4%) – and 2) chronic urticaria with predominance of lymphocytes – N (%) = 53 (57.6%). There was not significant correlation between histological groups and laboratorial tests. Moreover, direct immunofluorescence was positive in 21 (33,87%) from 62 patients.
*Conclusions:* There is not enough scientific evidence to support neutrophilic urticaria as a solid, separate entity.


## Introduction

Chronic Spontaneous urticaria (CSU) is characterized by intermittent erythematous-edematous lesions (wheal and flare reaction) that last longer than 6 weeks.[1,2] It has an incidence of 0.5–1.0%

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among general population, occurring predominantly among women (70.6%), with a peak age of onset between 20 and 40 years.[1,3]

Concerning its laboratorial features, it has been described an association with thyroid auto-antibodies. However, it is not always correlated with altered thyroid function.[1,3] Recent studies have also suggested that patients with CSU show signs of thrombin generation and activation of the tissue factor pathway of the coagulation cascade, presenting elevated D-dimer levels.[4,5]

Histologically, it is characterized by dermal capillary dilatation and interstitial edema, in addition to a mixed perivascular inflammatory infiltrate composed by neutrophils, eosinophils, T-helper lymphocytes and activated macrophages.[6–8] Several studies have previously suggested that urticaria with neutrophilic and eosinophilic inflammatory infiltrate predominance might differ in clinic-laboratorial aspects, although it is not already established as a concrete separate entity.[9,10] Although mast cell has classically been described as the major cell involved in the pathophysiology of CSU, the role of those others cells has also been studied.[11]

Considering this context, our study aims to characterize the patterns of inflammatory infiltrate, observed in skin biopsies from CSU patients, as well as their relation to specific altered laboratory exams results.

## Methods

The study was conducted at Hospital das Clínicas of the University of São Paulo Medical School, Brazil. A retrospective analysis of laboratory data, histopathology and direct immunofluorescence was carried out, using the database of our hospital's pathology, immunopathology and laboratory departments, from March 2011 to January 2015.

We selected all urticaria cases that were biopsied in that period. It is important to point that the biopsied wheals had less than 12 h of evolution, according to the department standardization. All patients signed a consent form before joining our protocol on urticarial outcare patients. The term explained the possible risks and outcomes implied to the procedure, besides the aims of the study.

Cases without laboratory exams, characterized as acute urticaria or any other confirmed diagnostic hypothesis were excluded (e. g., urticarial vasculitis and inducible urticaria). Cases presenting leukocytoclastic vasculitis, vessel wall fibrinoid necrosis or significant vascular deposition of immunoglobulins, besides systemic symptoms (fever, adenomegaly or arthralgia) were also excluded. The exclusion of patients with leukocytoclastic vasculitis or systemic symptoms was performed in order to avoid including patients diagnosed as neutrophilic urticarial dermatosis, Adult-onset Still disease (AOSD), Schnitzler's disease or cryopyrin associated periodic syndromes (CAPS). Neutrophilic urticarial dermatosis is claimed to be a different type of Neutrophilic Urticaria, with the presence of leukocytoclastic vasculitis, and/or systemic symptoms as a defining features.[9]

Each specimen was examined at high magnification (×400) and evaluated independently by two specialists, a pathologist and a dermatologist. Every biopsy had four fields analyzed, two perivascular and two interstitial, whose neutrophils, lymphocytes and eosinophils were counted. Technical replicates were combined by taking the median. The resulting cell counting of each cell type was tested for significant Spearman correlations by using approximate *t*-tests.

Finally, we aimed to correlate the cellular histopathologic pattern with the laboratorial tests that were performed at the time of biopsy, or after three months at most. Stepwise multiple logistic regression was used as the statistic method, in order to search for associations. Besides histopathologic analysis and direct immunofluorescence results, the following laboratory results were reviewed: total IgE levels, C3 and C4 levels, D-dimer levels, thyroid hormones and auto-antibodies, rheumatologic tests, HIV and hepatitis serology, C-reactive protein, erythrocyte sedimentation rate, parasitological stool sample exam and serum D-vitamin levels.[12]

## Results

Out of a total 133 patients, 40 were excluded: 20 whose biopsy confirmed another diagnosis different from CSU (e.g. eczema, urticarial vasculitis, Sweet syndrome, cutaneous acute erythematous lupus, dermatomyositis, drug reaction, bullous pemphigoid); 5 for presenting direct immunofluorescence with specific deposition of two or more immunoreactants at the vessel wall or at basal membrane zone; 8 for not presenting laboratorial exams and 7 for corresponding to a patient previously included in our database (more than one cutaneous biopsy). After exclusions, we obtained 93 patients for our analysis.

Our patients presented a gender distribution of 71 (76.34%) female and 22 (23.65%) male, with an average age of 42.5 years (SD ± 15).

Concerning histopathologic features, after counting neutrophils, eosinophils and lymphocytes in four fields (Fig. 3, 4), we observed the distribution on chart 1.

On chart 2, we represent the percentage of patients (with each cell predominance) in their biopsy specimens. This classification did not undergo any statistical normalization yet.

The approximate *t*-tests found that the cell counting of neutrophilic and eosinophilic groups were significantly correlated [$t(93) = -1.98$, $p = 0.02$], preventing them to be compared separately. In order to circumvent this limitation, we established then two valid groups for comparison: 1) chronic urticaria with predominance of neutrophils or eosinophils − N (%) = 39 (42.4%) − and 2) chronic urticaria with predominance of lymphocytes − N (%) = 53 (57.6%). Among the 93 patients, one was excluded for presenting the same cell counting, resulting in a total of 92 patients for the next analysis. Finally, we perform a correlation among laboratorial exams results to these two groups. No statistically significant association was found between the proposed groups and the laboratory results, as described on Table 1.

Direct immunofluorescence was performed on 62 patients, from whom 21 were reactive in some pattern, as described on Table 2.

## Discussion

Through literature review, we only found few recent articles approaching the histopathological patterns of CSU. The fact that its diagnosis is roughly clinic, and usually biopsies are not performed, might be an explanation. Considering that our study was conducted in a reference tertiary hospital, which attends difficult to treat patients, atypical clinical presentations of cutaneous diseases and other high complexity patients from all South America, we probably perform more skin biopsies than other health services, resulting in one of the largest South American skin biopsies casuistic related to CSU, recently described.[13]

Winkelman *et al.*[13] firstly described neutrophilic urticaria as a separate entity. Since then, several authors have described a percentage of urticaria patients with a neutrophilic inflammatory infiltrate predominance, which ranges from 15.8% to 48.8%.[9,10,13,14] However, none of previous studies submitted their histopathological analysis to statistical methods, *i.e.*, several neutrophilic patterns were described (such as neutrophilic venulitis, or interstitial infiltrate), but no rough neutrophilic counting was compared with other inflammatory cell counting (eosinophils and lymphocytes) and normalized by statistical methods. Aware of this context, although we observed 45.2% of patients with neutrophil

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