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**CORRELATION BETWEEN SERUM LEVELS OF MRGPRX2
AND SOME OF ITS AGONISTS WITH DISEASE SEVERITY
AND TREATMENT RESPONSE OF CHRONIC
SPONTANEOUS URTICARIA**

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INTRODUCTION

1. Rationale of the Study

Chronic spontaneous urticaria (CSU) remains a significant medical challenge due to its prolonged course, unpredictable lesion appearance, and the absence of a definitive cure. The pathogenesis of CSU is intricate, with mast cells playing a pivotal role. Degranulation of these cells results in the release of histamine and other inflammatory mediators, which stimulate sensory nerves, induce vasodilation, promote plasma leakage, and promote chemotaxis of various skin cells. Second-generation H1 antihistamines (sgAH1) constitute the foundational treatment at all stages of CSU management and are the primary first-line therapeutic approach. A pooled analysis indicates a response rate of merely 39% with standard doses of sgAH1 and 60% with higher doses (four times the standard amount). Patients unresponsive to sgAH1 are enrolled in clinical trials investigating novel pharmacological treatments for CSU. Among the sgAH1, bilastine stands out for its numerous advantages, high efficacy, and lower incidence of side effects compared to other sgAH1 inhibitors such as cetirizine, levocetirizine, and fexofenadine.

MRGPRX2 (Mas-related G protein-coupled receptor X2) is an activated receptor located on the mast cell membrane. It represents one of the targets in emerging drug trials aimed at treating refractory sgAH1-resistant CSU. The identification of MRGPRX2 has provided novel insights into mast cell pathophysiology, particularly the IgE-independent degradation pathway. Endogenous agonists of MRGPRX2 identified in CSU include eosinophil proteins such as Eosinophil Peroxidase (EPO) and Major Basic Protein (MBP), as well as the neuropeptide Substance P (SP). Multiple studies worldwide have demonstrated that MRGPRX2 expression on skin mast cells, along with the levels of its agonists (SP, MBP, EPO), are elevated in the serum and/or skin of patients with CSU and are associated with disease severity. However, to date, no research has established a correlation between serum MRGPRX2 concentrations and the levels of SP, MBP, EPO, or the response to sgAH1 treatment, specifically with bilastin CSU.

To date, no studies in Vietnam have investigated the role of MRGPRX2, SP, MBP, and EPO in CSU, nor the correlation between their concentrations and disease activity or treatment response. This has left a group of CSU patients, especially those with severe and/or refractory CSU, with sgAH1, whose causes and effective treatment options remain unknown. Therefore, we conducted the study **"Correlation between serum MRGPRX2 concentrations and some agonists with the severity and treatment response of chronic spontaneous urticaria"** with the following objectives:

1. Determine the correlation between serum MRGPRX2, SP, MBP, and EPO concentrations and the severity of chronic spontaneous urticaria.

2. Determine the correlation between serum MRGPRX2, SP, MBP, and EPO concentrations and bilastin response in the severe chronic spontaneous urticaria group.

2. New contributions of the Dissertation

- Serum concentrations of MRGPRX2 and SP were higher in the severe CSU group compared to the non-severe group and exhibited a positive correlation with the disease activity score UAS7. In Vietnamese patients, the optimal cutoff points for identifying severe CSU based on serum MRGPRX2 and SP concentrations were 11.67 ng/mL and 97.66 pg/mL, respectively, with sensitivities of 75.0% and 66.7%, and specificities of 91.7% and 58.3%. Logistic regression analysis further demonstrated that these two cutoff thresholds for the respective substances were independent risk factors for severe CSU. Serum concentrations of EPO and MBP did not differ between the severe and non-severe groups, nor did they correlate with the UAS7 score.

- Baseline serum concentrations of MRGPRX2, SP, EPO, and MBP were not predictors of treatment response in the severe CSU group. Serum MRGPRX2 levels remained unchanged after 2 months of bilastin treatment, while SP, EPO, and MBP levels tended to decrease. However, the changes differed across groups: SP decreased in the complete response group, while EPO and MBP decreased in the poor response group.

3. Dissertation Structure

The dissertation comprises 117 pages: a two-page introduction, a 31-page literature review, a 20-page examination of research subjects and methodologies, a 29-page report of findings, a 32-page discussion section, a two-page conclusion, and a one-page set of recommendations. It includes 14 tables, 20 figures, 27 charts, and 186 references.

The doctoral candidate has published three articles in reputable scientific journals, including one in Vietnamese and two in English.

CHAPTER 1: LITERATURE REVIEW

1.1. Overview of chronic spontaneous urticaria

1.1.1. Definition

Chronic spontaneous urticaria (CSU) is a condition characterized by the appearance of wheals, angioedema, or both lasting more than six weeks without a specific trigger. Symptoms of CSU may occur daily or recur periodically in episodes. CSU may be isolated (70-90%) or co-occur with chronic induced urticaria (10-30%).

1.1.2. Epidemiology

CSU can manifest in individuals of both sexes at any age. The most prevalent age of onset is 30 to 50 years, with a higher incidence in women. The global prevalence of CSU is approximately 0.5–1%, whereas in Asian countries such as China and South Korea, it ranges from 1.5–3%.

1.1.3. Natural course

The majority of patients diagnosed with CSU experience a disease course

persisting between one and four years. The probability of spontaneous remission increases progressively over time, reaching 17%, 45%, and 73% after 1 year, 5 years, and 20 years, respectively. Nonetheless, the recurrence rate may be as high as approximately 30%.

1.1.4. Pathophysiology

Mast cells in the skin serve as the primary effector cells in CSU. The process of degranulation of these cells results in the stimulation of sensory nerves, vasodilation, extravasation, and the recruitment of basophils, eosinophils, and T lymphocytes. Clinically, this manifests as wheals, pruritus, and angioedema.

There are two mast cell degranulation pathways: the IgE-dependent pathway (accounting for approximately 60%) and the IgE-independent pathway (accounting for approximately 40%). The IgE-dependent pathway is initiated by activation of FcεRI, a receptor with high affinity for IgE. The IgE-independent pathway is triggered by activation of receptors such as MRGPRX2, C5aR, protease-activated receptors 1 and 2 (PAR1, PAR2), chemotactic receptors, and cytokine receptors. Not only do these two pathways differ in their activating receptors on the cell membrane, but they also differ in intracellular signaling, response time, and the quantity, size, and content of inflammatory mediators released from cytoplasmic granules. Currently, researchers have yet to identify the primary factor responsible for activating mast cells during the initial phase of CSU, nor have they determined which key receptor on mast cell membranes is involved throughout the course of the condition.

1.1.5. Disease activity

In CSU, the majority of patients (>90%) have urticaria, but only about 6% have angioedema alone. Therefore, studies assessing disease activity and severity primarily utilize the UAS7 (Urticaria Activity Score over 7 days). The UAS7 reflects the level of urticaria activity over seven consecutive days, calculated based on two indicators: the number of wheals and the intensity of itch, categorized into five levels: inactive (UAS7=0), very mild (UAS7=1–6), mild (UAS7=7–15), moderate (UAS7=16–27), and severe (UAS7=28–42). However, for simplified classification purposes, some studies categorize UAS7 into two groups: non-severe (UAS7 ≤ 27) and severe (UAS7 ≥ 28).

1.1.6. Treatment

Currently, there is no definitive cure for CSU. The primary objective of treatment is to achieve complete symptom control until the condition resolves spontaneously. Management strategies align with guidelines from leading organizations, including the European Academy of Allergy and Clinical Immunology (EAACI), the Global Allergy and Airways Patient Platform (GA²LEN), EuroGuiDerm, and the Asian and Pacific Association of Allergology, Asthma, and Clinical Immunology (APAAACI). Treatment is organized into three tiers: second-generation H1-antihistamines (sgAH1), Omalizumab, and Ciclosporin. These therapeutic levels are adjusted according to the extent of disease control, as assessed by the Urticaria Control Test (UCT) scale.

In initial-line treatment, the proportion of patients with CSU who attained

disease control using standard and high doses of sgAH1 ranged from 39% to 60%, respectively. Patients with CSU unresponsive to sgAH1 constitute the target population for clinical trials of novel pharmacological agents. Regarding second-line (Omalizumab) and third-line (Ciclosporin) therapies, the proportion of patients achieving disease control was approximately 70%.

1.1.7. Treatment of CSU with bilastin

Bilastin distinguishes itself among sgAH1. While sharing the precise mechanism of action on the histamine H1 receptor—acting as an inverse agonist by binding at a different site than histamine to maintain receptor inactivity—bilastin exhibits a higher affinity for the H1 receptor, which enhances its antihistamine efficacy. The inhibition of urticaria induced by intradermal histamine injection in healthy subjects was more pronounced with bilastin than with desloratadine or rupatadine, at 83%, 38%, and 37%, respectively. In patients with CSU, bilastin demonstrated superior inhibition of urticaria and pruritus compared with cetirizine, levocetirizine, and fexofenadine, as evidenced by head-to-head clinical trials. Moreover, even in cases of CSU resistant to other sgAH1, bilastin remains somewhat effective.

In addition to its high efficacy in treating CSU, bilastin offers several supplementary advantages. Bilastin is rapidly absorbed upon oral administration, undergoes minimal hepatic metabolism, and is predominantly excreted via non-renal pathways. It exhibits the lowest affinity for central nervous system H1 receptors among sgAH1, thereby minimizing central nervous system side effects such as drowsiness, even at elevated doses. Notably, bilastin is among the limited subgroup of sgAH1 that can be safely prescribed to patients engaged in tasks demanding high levels of concentration, such as operating machinery or driving.

1.2. Mas-related G protein-coupled receptors X2 (MRGPRX2)

1.2.1. Origin and history of discovery

MRGPRX2 is a member of the MRGPR family, which is specific to primates and humans, with its initial discovery in 2001. In 2011, it was reported that MRGPRX2 is predominantly expressed on the membrane of human skin mast cells. By 2014, MRGPRX2 was identified as a crucial activating receptor in the IgE-independent mast cell degranulation pathway CSU.

1.2.2. The structure of MRGPRX2 and related signal conduction pathways

MRGPRX2 is a 330-amino acid protein divided into three parts: the extracellular (EC) domain with its N-terminal end and seven transmembrane (TM) chains linked to the three extracellular loops ECL1, ECL2, and ECL3; the three intracellular loops ICL1, ICL2, and ICL3; and the intracellular (IC) domain with its C-terminal end. The EC and the TM near the EC exhibit structural diversity and bind ligands. TM3–6 forms the first accessory vesicle, which is acidic and negatively charged, facilitating MRGPRX2 binding to its cationic ligands. TM1–3 and TM6–7 form the second, hydrophobic accessory vesicle. The IC and the TM near the IC participate in G protein activation, thereby generating downstream signaling within the cell.

Although both processes utilize multiple intracellular protein kinases, such

as ERK1/2, p38, and PI3K, as pathways for mast cell degranulation via FcεRI, mast cell degranulation via MRGPRX2 occurs more rapidly, and the released particles are smaller and more uniform. Additionally, MRGPRX2 exhibits an intrinsic mechanism of self-desensitization. Specific ligands, upon binding to this receptor, can recruit β-arrestin 1 and β-arrestin 2, leading to intracellular trafficking of the MRGPRX2 receptor and consequently diminishing mast cell degranulation via the MRGPRX2 mechanism.

1.2.3. Physiological and pathological functions

MRGPRX2 is characterized by low specificity and affinity, as well as the ability to bind a broad spectrum of endogenous and exogenous agonists with diverse structures. The activation of human skin mast cells via MRGPRX2 binding to its agonists is associated with host defense mechanisms, pseudoallergic reactions, and chronic inflammatory conditions such as CSU, atopic dermatitis, bronchial asthma, non-histaminergic pruritus, neuritis, and inflammatory pain...

1.2.4. Studies on MRGPRX2 in CSU

The role of MRGPRX2 in CSU was first confirmed in 2014 by Fujisawa et al. This study demonstrated increased MRGPRX2 expression on human mast cell membranes in lesional biopsy samples from patients with refractory CSU compared with a healthy group. A 2021 study by Shtessel et al. reported increased skin reactions (wheals, itching) in patients with very mild CSU (average US7 score of 0.5) compared with a healthy group, attributed to MRGPRX2 activation. The latest study in 2025 by Ye et al. focused on the role of MRGPRX2 mutations in CSU: only mutations at the 185A>G site increased MRGPRX2 function and expression on mast cell membranes, as well as serum MRGPRX2 levels and disease activity.

Regarding serum MRGPRX2 levels in CSU, there are currently three studies worldwide with inconsistent results. First, there is no consensus on whether serum MRGPRX2 levels differ between the severe CSU group and the healthy control group. Second, there is no consensus on whether serum MRGPRX2 levels differ between patients with CSU who are severe and those who are non-severe. One reason for this inconsistency is the use of UAS7 scoring in the CSU group at the time of drug discontinuation and during treatment, primarily with sgAH1. Currently, no studies have evaluated changes in serum MRGPRX2 levels after sgAH1 treatment to explain the differences in results across these studies.

1.3. Substance P

1.3.1. Origin

Substance P (SP) is a neuropeptide consisting of an 11-amino acid chain, secreted at the nerve endings of sensory nerves, stimulated explicitly by factors such as leukotrienes, prostaglandins, and histamine.

1.3.2. Physiological and pathological functions

SP serves as a vital mediator in numerous physiological processes, including pain transmission, modulation of inflammatory responses, host defense mechanisms, and wound healing, via its specific receptors, NK-1R (neurokinin-1 receptor) and MRGPRX2. Upon release, SP binds to its receptor on the cell

membrane, forming a ligand-receptor complex that initiates intracellular responses. Excessive signaling through these complexes can result in pathological conditions such as atopic dermatitis, asthma, CSU, pseudoallergic reactions, postoperative pain, and inflammation related to the SP/MRGPRX2 complex, or tissue damage in the lungs, liver, or kidneys due to sepsis, lung damage due to acute pancreatitis, or burns associated with the SP/NK-1R complex.

1.3.3. Studies on SP in CSU

Since 1999, through intradermal injections of SP in patients with CSU, Borici-Mazi et al. have demonstrated that SP can induce mast cell degranulation and histamine release. However, it was not until 2014 that Fujisawa et al. identified the mechanism by which SP induces mast cell degranulation: via the MRGPRX2.

Currently, approximately six studies worldwide have reported serum SP concentrations in CSU; however, the results are inconsistent. Four studies have reported higher serum SP concentrations in the CSU group than in healthy controls. Two studies have demonstrated a correlation between serum SP concentration and disease severity in CSU. Changes in serum SP concentration as a result of treatment were observed in only one study on sgAH1 (no change after 7 days of treatment) and one study on Omalizumab (increased serum SP concentration).

1.4. MBP và EPO

1.4.1. Origin

MBP (Major Basic Protein) and EPO (Eosinophil Peroxidase) are both proteins located within the cytoplasm of eosinophils. MBP manifests in two distinct forms: MBP1, which is present in both eosinophils and basophils, and MBP2, exclusive to eosinophils. MBP1 is the predominant variant, with a molecular weight of 14 kDa and 117 amino acids. EPO is a positively charged protein consisting of 568 amino acids.

1.4.2. Physiological and pathological functions

MBP exerts cytotoxic effects on helminths, bacteria, and mammalian cells through multiple mechanisms, including increased membrane permeability, lipid bilayer disruption, elevated histamine release via mast cell and basophil stimulation, and increased superoxide dismutase production in alveolar tissue macrophages.

EPO participates in defense responses against various types of bacteria through the peroxidase reaction.

1.4.3. Studies on MBP and EPO in CSU

Since 1983, Peter et al. have demonstrated eosinophil infiltration and granulation in the wheal lesions of patients with CSU. Subsequently, Toyoda et al. noted that prolonged wheal lesions showed greater eosinophil granule deposition. However, it was not until 2014 that Fujisawa et al. demonstrated that one mechanism by which eosinophils maintain wheal lesions in CSU is the induction of mast cell granulation via the MRGPRX2 receptor by proteins within these granules, such as MBP and EPO.

Currently, only one study worldwide examines serum MBP and EPO concentrations in CSU. Khanna et al. reported that concentrations of these two

proteins were significantly higher in the patient group than in the control group ($p < 0.05$). However, there was no correlation between peripheral blood eosinophil count and the UAS7 score. This suggests that although eosinophilic granulation occurs in CSU, it may not reflect disease severity. Furthermore, the failure of new drugs targeting eosinophilic cell death in sgAH1-resistant CSU indicates that even complete elimination of these cells, i.e., the proteins in their granules, such as MBP or EPO, does not provide therapeutic efficacy. This indirectly reflects the lack of correlation between EPO and MBP concentrations and the response to sgAH1 treatment in CSU.

Research gap

Although numerous studies worldwide have observed increased levels of MRGPRX2, SP, MBP, and EPO in patients with CSU compared to healthy controls, with serum levels of MRGPRX2 and SP correlating with disease severity, the findings remain inconsistent. There is a paucity of research examining the correlation between the concentrations of these substances and the response to sgAH1 treatment, as well as the changes in their levels following treatment. Consequently, further investigations are required to elucidate the role of MRGPRX2, SP, MBP, and EPO in the pathophysiology of this condition.

CHAPTER 2: STUDY SUBJECTS AND METHODS

2.1. Study subjects

2.1.1. Subject for objective 1

The study comprised 120 patients with CSU who sought consultation and treatment at the National Hospital of Dermatology and Venereology, Ha Noi, and 30 healthy individuals.

a. Patient group

*** Selection criteria:**

- Diagnosed with CSU.
- Patients aged 16 years and older.
- Refrain from utilizing H1 antihistamines for a minimum of five days, immunosuppressants—including systemic corticosteroids, ciclosporin, etc.—for at least four weeks, and other medications such as NSAIDs and antibiotics for a minimum of one week before the initial serum collection sample.
- Consent to participate in the research project.

*** Diagnostic basis for CSU**

Patients are diagnosed with CSU according to the EAACI/GA2LEN/EuroGuiDerm/ APAAACI criteria.:

- The fundamental lesions consist of bright pink or pale pink edematous papules of diverse sizes, which may merge into large patches exhibiting polyarc-shaped borders and a distinct boundary with healthy skin at any anatomical location. These lesions persist and resolve within 24 hours, leaving no functional remnants: pruritus in the affected or impending area.
- Duration of illness: lasting more than 6 weeks.

- May or may not be accompanied by angioedema.
- The specific causes and triggers of urticaria are not identified.

*** Exclusion criteria:**

- The patient is suffering from other acute illnesses.
- Any skin condition causing chronic itching may affect the evaluation and results of the study.
- Diseases that increase serum levels of MRGPRX2, SP, MBP, and EPO.
- Pregnant and breastfeeding women.

b. Normal control

The group comprised 30 healthy individuals (NC: Normal Control) who met the following criteria: no history of underlying medical conditions, no history of allergies or chronic itching, no infectious diseases or other medical or surgical conditions, and a drug-free period before serum sample collection, similar to the patient group.

2.1.2. Subject for objective 2

All patients with CSU at the first objective exhibited a UAS7 score of ≥ 28 (indicating severe CSU) and had no prior history of bilastine use treatment.

*** Diagnostic basis for severe CSU:**

Patients diagnosed with CSU according to the EAACI/GA2LEN/EuroGuiDerm/APAAACI criteria presented in the first objective and with a UAS7 score ≥ 28 . The UAS7 score is the sum of the number of wheals and the intensity of itching over 7 consecutive days. The pruritus score for each day is calculated on a scale of 0 to 3: 0 points (no pruritus), 1 point (mild pruritus), 2 points (moderate pruritus, causing discomfort but not affecting daily activities), 3 points (severe pruritus, affecting sleep or daily activities). The urticaria score is also calculated on a corresponding scale: 0 points (no urticaria), 1 point (< 20 urticaria/24h), 2 points (20-50 urticaria/24h), 3 points (> 50 urticaria/24h or a large area of urticaria).

2.2. Study Time and Setting

- Time: The activity is scheduled from January 2024 through September 2025. Specifically, the recruitment period for recruits extends from March 2024 to September 2024.
- Setting: Urticaria and Chronic Urticaria Clinic, Outpatient Department, National Hospital of Dermatology and Venereology, Ha Noi.

2.3. Study methods

2.3.1. Study design

- Objective 1: cross-sectional study
- Objective 2: cohort study

2.3.2. Sample size and sampling

2.3.2.1. Sample size and sampling for objective 1

The subjects were selected for the study using a time-ordered purposive sampling method.

Sample size was calculated using a formula that determines the difference in mean serum MRGPRX2 concentrations between two groups of severe and non-severe CSU:

$$n = 2 \left(\frac{Z_{1-\frac{\alpha}{2}}^2 \sigma^2}{d^2} \right)$$

n: the minimum sample size required for each group.

$Z_{1-\alpha/2}$ (confidence coefficient) is a value derived from the normal distribution, calculated based on statistical significance. Choosing $\alpha=0.05$, we have $Z_{1-\alpha/2} = 1.96$.

σ is the common standard deviation of the two groups, calculated using the following formula:

$$\sigma = \sqrt{\frac{(n_1 - 1)s_1^2 + (n_2 - 1)s_2^2}{n_1 + n_2 - 2}}$$

n_1, n_2, s_1, s_2 : Sample size and standard deviation of the group with severe and non-severe CSU. Since Cao et al.'s study did not provide standard deviation, we applied the formula for calculating standard deviation based on the 1st and 3rd quartiles:

$$s \approx \frac{q_3 - q_1}{1.35}.$$

We calculated $s_1=10.4$ and $s_2=10.6$. Therefore, $\sigma^2=111.7$

d is the acceptable margin of error (the allowable deviation between the serum MRGPRX2 concentration obtained from the study sample and the population parameter, as specified by the researcher. We selected $d = 3.8$ ng/mL.

According to the study conducted by Cao et al., a calculated sample size of $n = 59.4$ was determined. The study entailed continuous sample collection until the minimum sample size (n) was achieved for each patient group, comprising individuals with severe or non-severe CSU, with a total of 60 patients. For comparative purposes with the healthy cohort, an additional 30 serum samples were obtained from the control group.

2.3.2.2. Sample size and sampling for objective 2

Given that no studies have yet established a correlation between serum MRGPRX2 concentrations and bilastine response in severe CSU, nor have differences in these concentrations been reported among treatment response groups, we used the entire sample from the severe CSU group for the first objective and, for the second objective, used the same sample ($n=60$).

2.3.3. Research variables and indicators

Bảng 2.1. Research variables

TT	Variable	Definition/Calculation	Classification
1. General information about the patient's characteristics			
1.	Age	Calculated according to the Gregorian calendar.	Continuous
2.	Gender	Patient's gender	Binary
3.	Disease progression time	Number of weeks from the appearance of the wheal lesion to	Continuous

TT	Variable	Definition/Calculation	Classification
		the time of study enrollment	
4.	Angioedema	A sudden, pronounced swelling of deep tissue in the dermis and subcutaneous tissue or mucous membranes, red or skin-colored, with functional symptoms of burning, tightness, and sometimes pain instead of itching, usually lasting longer than a papule (up to 72 hours).	Binary
5.	Autologous serum test ASST	The difference in diameter of the edematous papule at 30 minutes is determined by comparing the intradermal injection site of 0.05 mL of autologous serum with the intradermal injection site of 0.05 mL of physiological saline. A positive result is obtained when the difference is ≥ 1.5 mm.	Binary
6.	CRP test results	Value obtained from the CRP test	Continuous
7.	Classification of CRP concentrations	CRP levels are classified based on a threshold of 5 mg/L	Binary
8.	Total IgE test results	Value obtained from the total IgE test	Continuous
9.	Classification of total IgE concentration	Classification of total IgE concentration based on a threshold of 100 IU/mL	Binary
10.	Results of peripheral blood eosinophil count	Absolute value of the number of eosinophils obtained after testing.	Continuous
11.	Classification of eosinophil count	Eosinophil count is classified based on a threshold of 0.05×10^9 cells/L	Binary
12.	Results of peripheral blood basophil count	Absolute value of the number of basophils obtained after testing	Continuous
13.	Classification of basophil count	Basophil count is classified based on a threshold of 0.01×10^9 cells/L	Binary
14.	Results of the anti-TPO (Thyroid Peroxidase) IgG test	Value obtained from the anti-TPO IgG test	Continuous
15.	Classification of IgG concentrations against TPO	Classification of TPO-resistant IgG concentrations is based on a threshold of 34 U/mL.	Binary

TT	Variable	Definition/Calculation	Classification
2. Objective 1: To determine the correlation between serum MRGPRX2, SP, MBP, and EPO concentrations and the severity of CSU			
16.	Disease activity score (UAS7)	Total pruritic and wheal scores over 7 consecutive days.	Continuous
17.	Disease severity is classified according to the UAS7 scale.	Classification of disease severity according to UAS7	Categorical
18.	MRGPRX2 test results before treatment	Value obtained from the MRGPRX2 test	Continuous
19.	SP test results before treatment	Value obtained from SP test	Continuous
20.	MBP test results before treatment	Value obtained from the MBP test	Continuous
21.	EPO test results before treatment	Value obtained from the EPO test	Continuous
3. Objective 2: To determine the correlation between serum MRGPRX2, SP, MBP, and EPO concentrations and bilastin response in the severe CSU group			
22.	Disease control score UCT	The total score from the four questions determines the level of disease control.	Continuous
23.	Classification of disease control levels based on the UCT scale.	Disease control classification according to UCT	Categorical
24.	MRGPRX2 test results after treatment	Value obtained from the MRGPRX2 test	Continuous
25.	SP test results after treatment	Value obtained from SP test	Continuous
26.	MBP test results after treatment	Value obtained from the MBP test	Continuous
27.	EPO test results after treatment	Value obtained from the EPO test	Continuous

2.3.4. Research materials

- Research equipment includes biochemical and hematological analyzers, a BioTek ELISA washing machine, and a BioTek ELX 808 multi-wavelength ELISA reader; physical urticaria testing devices such as a treadmill, stainless steel ice cube, Temptest, and Frictest; a skin test kit with autologous serum; a serum sample collection and storage kit; and measurement kits for MRGPRX2, SP, MBP, and

EPO concentrations utilizing enzyme-linked immunosorbent assay (ELISA) from MyBioSource, Inc., San Diego, CA, USA. Additionally, a research case report questionnaire is employed for data collection.

- The drug utilized in the study is bilastine, marketed under the brand name Bilaxten 20mg. These are film-coated tablets containing 20mg of bilastin along with excipients; manufactured by Menarini.

2.3.4. Data collection process

2.3.4.1. With objective 1

Step 1: Diagnosis and confirmation of study subjects

- Patient group: Patients who meet the inclusion criteria of exhibiting a typical wheal persisting for more than six weeks without an identifiable specific trigger, and who have tested negative on provocation tests.
- Control group: thirty healthy individuals, comprising hospital staff and family members of patients, matched by age and gender with the patient group. Participants met the following criteria: no history of underlying medical conditions, allergies, or chronic pruritus; no infectious diseases or other medical or surgical conditions; and adherence to a drug-free period before serum sample collection, comparable to that of the patient group.

Step 2: Gather personal information and medical history

Demographic information, including age, gender, duration of illness, angioedema symptoms, family history, and personal medical history, was recorded in detail using the study case report form.

Step 3: Determine the severity of CSU

- The UAS7 (Urticaria Activity Score over 7 days) is calculated by summing the UAS scores (intensive itching and number of wheals) over 7 consecutive days.:

- Daily Itching Score: 0 points (no itching), 1 point (mild itching), 2 points (moderate itching, uncomfortable but not affecting daily activities), 3 points (very itchy, affecting sleep or daily activities).

- Daily wheal score: 0 points (no wheals), 1 point (< 20 wheals/24h), 2 points (20-50 wheals/24h), 3 points (> 50 wheals/24h or a large area of wheals).

- Disease severity classification according to UAS7:

- Non-sever: UAS7=0-27.

- Severe: UAS7=28-42.

- Select an adequate number of patients based on the calculated sample size: 60 patients with non-severe CSU and 60 patients with severe CSU. The chosen patients had scores ranging from 0 to 42.

Step 4: Collect blood samples and conduct tests.:

- Patients underwent all recommended tests per the EAACI/GA2LEN/EuroGuiDerm/APAAACI guidelines: complete blood count, CRP, total IgE levels, anti-

TPO IgG, and autologous serum skin test (ASST). The first serum sample was collected following the procedures of the National Hospital of Dermatology and Venereology, Ha Noi. Serum samples are stored at -80°C in a freezer for up to 2 months.

- First quantitative measurement of serum MRGPRX2, SP, MBP, and EPO concentrations:

- Testing principle: Enzyme-linked immune-sorbent assay (ELISA), specifically a sandwich ELISA for MRGPRX2, MBP, EPO, and a competitive ELISA with SP.

- Testing procedure: The research team conducted the tests independently, with technical support provided by the Head of Department, Dr. Le Huyen My, and the Chief Technician, Bachelor Nguyen Thi Phuong Hoa, of the Department of Biochemistry, Hematology, and Immunology at the National Hospital of Dermatology and Venereology, Hanoi. All procedures were executed in strict accordance with the manufacturer's instructions. Serum concentrations of MRGPRX2, SP, MBP, and EPO were measured based on the construction of a standard curve.

2.3.4.2. *With objective 2*

Step 1: Diagnosis and confirmation of study subjects

Patients with CSU meeting the study criteria included those with a UAS7 score of at least 28 and who had not previously received bilastine treatment. These patients were administered bilastine (Bilaxten, film-coated tablets, 20 mg, produced by Menarini) at the approved dosage of 20 mg once daily.

Step 2: Determine the level of disease control and adjust medication dosage

- Calculate the UCT (Urticaria Control Test) score at three time points: after 2 weeks (T2), after 4 weeks (T4), and after 8 weeks (T8). The UCT score is calculated as the sum of the scores from four questions. Each question is scored on a scale from 0 to 4.

- Question 1: How bothered have you been by the physical symptoms of urticaria (itching, rashes (plaques), and/or swelling) over the past 2 or 4 weeks? 0 points (very much), 1 point (much), 2 points (somewhat), 3 points (a little), 4 points (not at all).

- Question 2: In the past 2 or 4 weeks, how much has urticaria affected your quality of life? 0 points (very much), 1 point (much), 2 points (somewhat), 3 points (a little), 4 points (nothing at all).

- Question 3: In the past 2 or 4 weeks, has your urticaria treatment ever been insufficient to control your symptoms? 0 points (very often), 1 point (frequently), 2 points (sometimes), 3 points (rarely), 4 points (never).

- Question 4: Overall, how well have your urticaria symptoms been controlled over the past 2 or 4 weeks? 0 points (not at all), 1 point (a little), 2 points (somewhat), 3 points (good), 4 points (excellent).

- Assess disease control levels and adjust medication dosages according to UCT:

- Uncontrolled (poor responders): UCT=0-11. Increase the bilastine dosage by twofold if the patient is currently receiving one tablet, or by fourfold if the patient is currently receiving two tablets.

- Good controlled (good responders): UCT=12-15. Proceed with the current bilastine dosage if there are no side effects.

- Completely controlled (complete responders): UCT=16. Proceed with the current bilastine dosage if there are no side effects.

Step 3: Collect blood samples and conduct tests.

A second serum sample was obtained after eight weeks of treatment (T8), and the concentrations of MRGPRX2, SP, MBP, and EPO were measured using the same methodology as for the initial sample.

2.4. Data Management and Statistical Analysis

Data were imported and managed using REDCap, then transferred to Stata 17.0 for analysis. The analysis included descriptive statistics, such as mean, proportion, and median, and inferential tests, such as Chi-squared, Mann-Whitney, Kruskal-Wallis, and Wilcoxon. Spearman's correlation assessed relationships between MRGPRX2, SP, MBP, EPO, and UAS7 and UCT scores. Univariable and multivariable logistic regression examined associations between MRGPRX2, SP, and CSU severity, adjusting for confounders like age, sex, angioedema, eosinopenia, CRP, total IgE, and anti-TPO IgG, using purposive sampling. Significance was set at $p < 0.05$.

2.5. Bias and Quality Control

The study's biases include sampling inaccuracies, data-collection errors, implementation issues, confounding variables, analytical errors, and publication discrepancies. To address these, it used random sampling with an adequate size, standardized tools, calibration, consistent evaluations, and data management software. Multivariate regression controlled for confounding factors, cross-checking ensured accuracy, and findings were transparently reported in accordance with scientific standards.

2.6. Research Ethics

The study was conducted with approval from the Ethics Committee of Hanoi Medical University, under Decision No. 1145/GCN-HMUIRB.

2.7. Limitations of the study

Firstly, regarding objective 1, the cross-sectional study only helped formulate a hypothesis about the correlation between MRGPRX2, SP, MBP, and EPO concentrations and the severity of CSU.

Secondly, the study did not investigate MRGPRX2 expression on mast cell membranes, nor did it examine SP, MBP, and EPO concentrations at wheal sites, or MRGPRX2 gene mutations in Vietnamese patients with CSU.

Thirdly, this investigation is a single-center study conducted exclusively at the National Hospital of Dermatology and Venereology in Hanoi, which may introduce bias into patient characteristics. Patients who presented at the hospital may have experienced more severe conditions, leading to outcomes that do not entirely represent the actual situation of CSU within the community as a whole.

CHAPTER 3: RESEARCH RESULTS

Between March and September 2024, at the National Hospital of Dermatology and Venereology in Hanoi, we collected data from 120 patients with isolated CSU and 30 healthy individuals in the control group. The results are as follows:

3.1. Correlation between serum MRGPRX2, SP, MBP, and EPO concentrations and the severity of CSU

The study was conducted on 120 patients with isolated CSU, divided equally into two groups (severe and non-severe), and 30 healthy individuals of similar age and gender. The control group had an average age of 37.5 ± 12.7 years, with 63.3% being female, identical to the severe and non-severe CSU groups (ANOVA test, $p > 0.05$).

Table 3.1. Some clinical and paraclinical characteristics of severe and non-severe CSU groups

Biến số	Severe CSU (n = 60)	Non-severe CSU (n=60)	p
UAS7 (points)	29.5 [28–35]	20 [14–23.5]	< 0.0001*
Age (years)	40.1±15.2	38,2±15	0.49 ⁺
Female (n, %)	33 (55%)	40 (66.7%)	0.19**
Disease duration (weeks)	20 [12.4–52]	19.5 [11.5–29]	0.31*
Combined angioedema (n, %)	25 (41.7%)	19 (31.7%)	0.26**
Positive ASST (n, %)	34 (56.7%)	36 (61%)	0.63**
Basopenia (n, %) ($<0,01 \times 10^9/L$)	1 (1.7%)	0 (0%)	1.0***
Eosinopenia (n, %) ($<0,05 \times 10^9/L$)	17 (28.3%)	13 (21.7%)	0.40**
Elevated CRP ($>5\text{mg/L}$) (n, %)	8 (13.3%)	6 (10%)	0.57**
Elevated total IgE ($>100\text{IU/mL}$) (n, %)	49 (81.7%)	42 (70%)	0.33***
Elevated anti-TPO IgG ($\geq 34 \text{ U/mL}$) (n, %)	3 (5%)	2 (3.3%)	1.0***

(*Man-Whitney U test, ** χ^2 test, ***Fisher Exact test, ⁺T-test)

The severe and non-severe CSU groups exhibited significant differences in UAS7 scores, with medians of 29.5 [28-35] and 20 [14–23.5], respectively, ($p < 0.0001$). No statistically significant differences were observed in various basic clinical and paraclinical parameters, including mean age, sex ratio, disease duration, the prevalence of angioedema, positive ASST prevalence, basopenia,

eosinopenia, elevated CRP, increased total IgE, and elevated anti-TPO IgG when comparing the severe and non-severe CSU groups ($p > 0.05$).

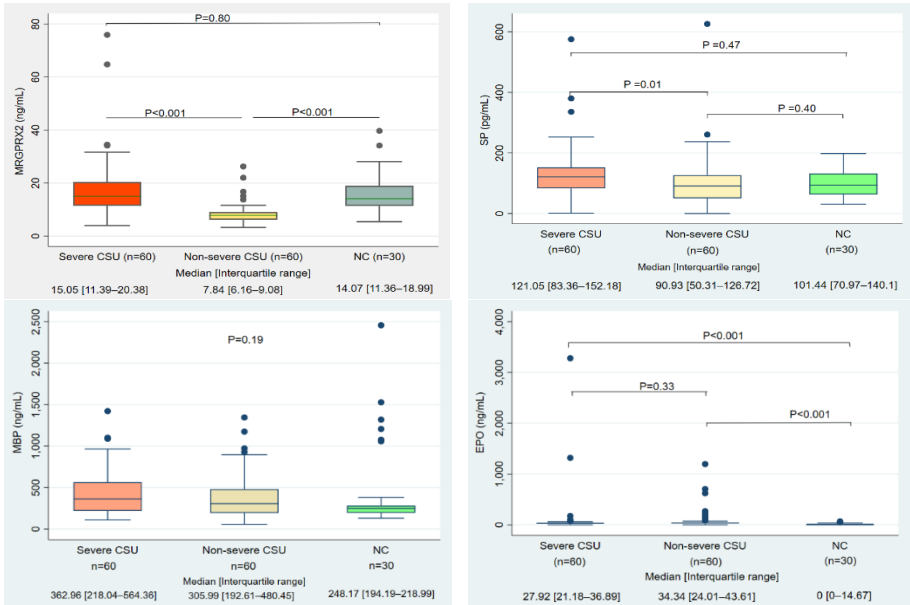


Figure 3.1. Serum concentrations of MRGPRX2, SP, MBP, and EPO between the severe and non-severe CSU groups and the control group (Kruskal-Wallis test followed by Dunn test).

Serum MRGPRX2 and SP concentrations were elevated in the severe CSU group compared with the non-severe CSU group ($p < 0.05$), but did not differ significantly from the control group ($p > 0.05$). Serum MBP concentrations were higher in the severe CSU group relative to the non-severe CSU and control groups; however, this difference was not statistically significant ($p > 0.05$). Meanwhile, serum EPO concentrations in the control group were predominantly undetectable (0 ng/mL), notably lower than in both the severe and non-severe CSU groups ($p < 0.001$). No significant difference was observed in serum EPO concentrations between the severe and non-severe CSU groups ($p > 0.05$).

Table 3.2. Correlation between serum MRGPRX2, SP, MBP, and EPO concentrations and UAS7 disease activity score in CSU

Variables	Spearman' rho	p
MRGPRX2	0.56	<0.001
SP	0.22	0.01
MBP	0.14	0.14
EPO	-0.13	0.17

There was a positive correlation between serum MRGPRX2 and SP concentrations and the UAS7 score ($p < 0.05$), but no correlation was observed between serum MBP and EPO concentrations and the UAS7 score ($p > 0.05$).

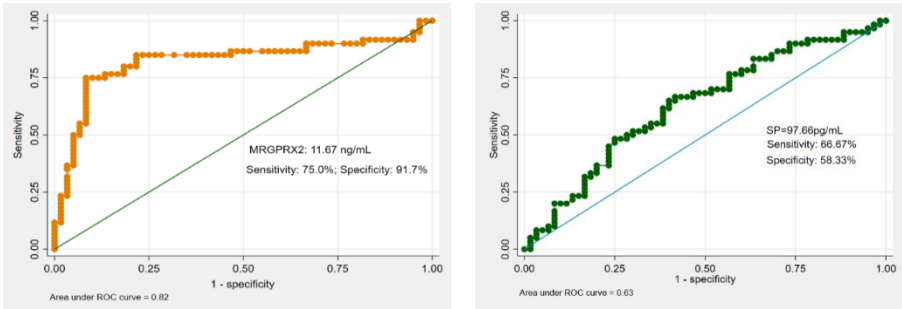


Figure 3.2: ROC curve determining serum MRGPRX2 and SP concentration thresholds differentiating between severe and non-severe CSU

Receiver Operating Characteristic (ROC) curve analysis indicated that a serum MRGPRX2 concentration of 11.67 ng/mL serves as the optimal threshold for the identification of the severe group in CSU (AUC: 0.82; 95% CI: 0.74–0.91), with a sensitivity of 75.0% and a specificity of 91.7%. Similarly, a serum SP concentration of 97.66 pg/mL was determined to be the optimal cutoff point for identifying the severe group in CSU (AUC: 0.63; 95% CI: 0.53–0.73), exhibiting a sensitivity of 66.7% and a specificity of 58.3%.

Table 3.3. Univariate and multivariate logistic regression analysis of risk factors for severe CSU

Biến	Univariate logistic regression			Multivariate logistic regression		
	OR	CI95%	p	OR	CI95%	p
Age (per year)	1.01	0.98–1.03	0.49	1.01	0.98–1.05	0.53
Female (compared to male)	0.61	0.29–1.28	0.19	1.71	0.53–5.57	0.37
Presence of angioedema (compared to absence)	1.54	0.73–3.26	0.26	1.91	0.65–5.59	0.24
Eosinopenia (compared to no eosinopenia)	1.43	0.62–3.29	0.4	1.26	0.39–4.07	0.70
Increased total IgE (compared to no increase)	1.91	0.81–4.49	0.14	2.19	0.61–7.85	0.23
Increased CRP (compared to no increase)	1.38	0.45–4.26	0.57	0.41	0.07–2.43	0.33
Increased anti-TPO IgG (compared to no increase)	1.53	0.25–9.48	0.65	1.61	0.14–18.07	0.70
Serum MRGPRX2 concentration ≥ 11.67 ng/mL (compared to no increase)	30.25	10.28–89.04	<0.001	48.21	13.00–178.82	<0.001
Serum SP concentration ≥ 97.66 pg/mL (compared to no increase)	2.6	1.24–5.44	0.011	3.19	1.10–9.24	0.03

Both univariate and multivariate logistic regression analyses indicated that MRGPRX2 thresholds of 11.67 ng/mL and SP thresholds of 97.66 pg/mL serve as risk factors for severe CSU. The probability of severe CSU was 48.21 times greater in patients with serum MRGPRX2 concentrations ≥ 11.67 ng/mL compared to those with concentrations < 11.67 ng/mL (95% CI 13.00–178.82; $p < 0.001$) under identical conditions. Additionally, the likelihood of severe CSU in patients with serum SP concentrations ≥ 97.66 pg/mL was 3.19 times higher than in patients with serum SP concentrations below this threshold (95% CI 1.1–9.24; $p < 0.05$) under equivalent conditions.

3.2. Correlation between serum MRGPRX2, SP, MBP, and EPO concentrations and bilastin response in the severe CSU group.

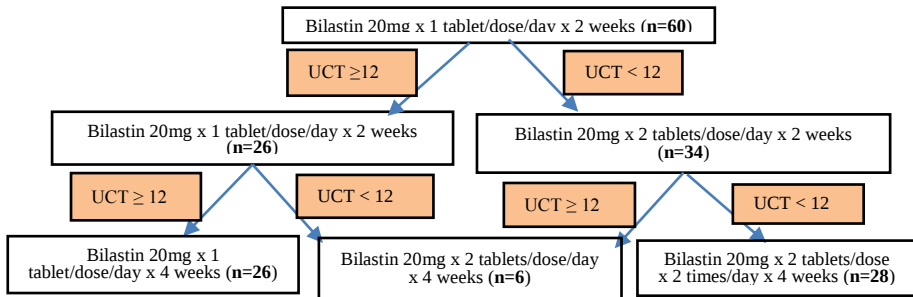


Figure 3.3: Number of patients with severe CSU treated with bilastin according to doses and weeks.

Twenty-six patients with severe CSU, comprising 43.3% of the study population, were subjected to a treatment regimen involving standard-dose bilastin administered over eight weeks. Of the 34 patients who escalated their bilastin dosage to twice the initial amount, only 6 (17.6%) achieved disease control. The remaining twenty-eight patients necessitated a fourfold increase in their bilastin dosage.

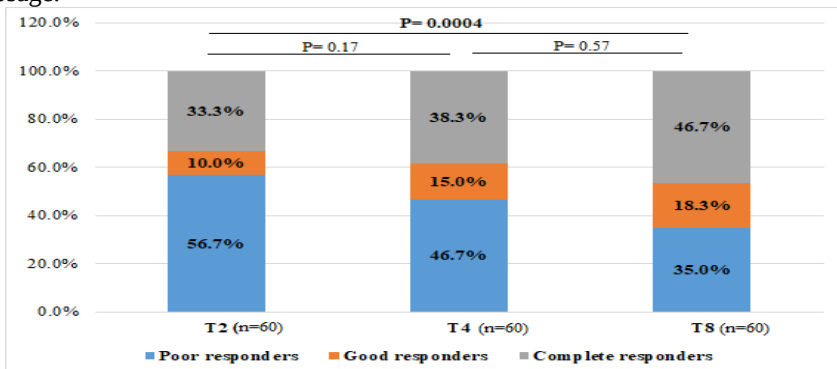


Figure 3.4: Response rates to bilastin over time in the severe CSU group (T2: after 2 weeks, T4: after 4 weeks, T8: after 8 weeks, Friedman test versus Dunn test)

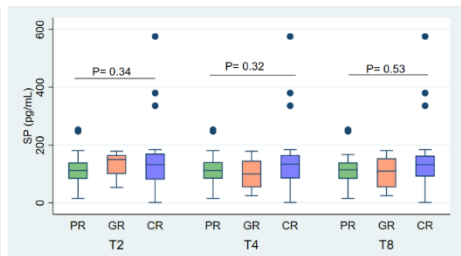
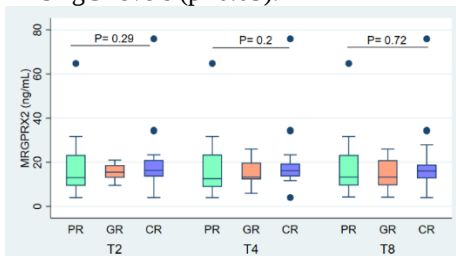
After 2 weeks of treatment with standard-dose bilastin, the rate of complete response in the severe CSU group was only 33.3%; the rate of poor response was 56.7%. After increasing the bilastatin dose in the poor-response group, the 8-week poor-response rate was only 35% ($p<0.001$).

Table 3.4. Some clinical and paraclinical characteristics among groups responding to bilastin at T8 in the severe CSU group

Variables	Poor responders (n = 21)	Good responders (n=11)	Complete responders (n=28)	p
Age (years)	39.5±16.6	37.6±15.5	41.5±14.5	0.81 ⁺⁺⁺
Female (n, %)	11 (52.4)	4 (36.4)	18 (64.3)	0.28 ^{***}
Baseline UAS7 (points)	35.5±5.6	29.2±2.3	31.4±4.8	0.02⁺⁺⁺
Duration of urticaria (weeks)	18 [10–39]	52 [13–60]	20 [13–46]	0.45 ⁺⁺
Positive ASST (n, %)	11 (52.4)	6 (54.6)	17 (60.7)	0.83 ^{**}
Combined angioedema (n, %)	9 (42.9)	5 (45.5)	11 (39.3)	0.93 ^{**}
Basopenia ($<0,01 \times 10^9/L$) (n, %)	1 (4.8)	0 (0)	0 (0)	0.53 ^{***}
Eosinopenia ($<0,05 \times 10^9/L$) (n, %)	7 (33.3)	1 (9.1)	9 (32.1)	0.33 ^{***}
Elevated CRP ($>5\text{mg/L}$) (n, %)	3 (14.3)	1 (9.1)	4 (14.3)	1.0 ^{***}
Elevated total IgE ($>100\text{IU/mL}$) (n, %)	17 (80.9)	8 (72.7)	24 (85.7)	0.32 ^{***}
Elevated anti TPO IgG (≥ 34 U/mL) (n, %)	1 (4.8)	0 (0)	2 (7.1)	1.0 ^{***}

(^{**} χ^2 test, ^{***}Fisher Exact test, ⁺⁺⁺Anova test, ⁺⁺Krusal Wallis test)

The group with severe CSU who responded poorly to bilsatine had a higher UAS7 score than the group with a good and complete response ($p<0.05$). No significant differences were observed among the three groups in other clinical and paraclinical characteristics, including age, sex, disease duration, angioedema rate, ASST-positive rate, eosinophilia, basophilia, total IgE levels, CRP levels, and anti-TPO IgG levels ($p>0.05$).



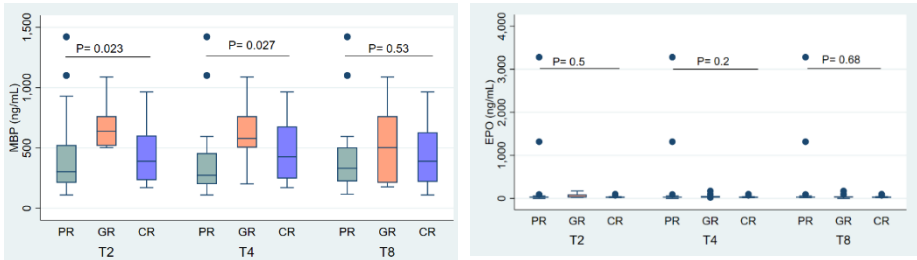


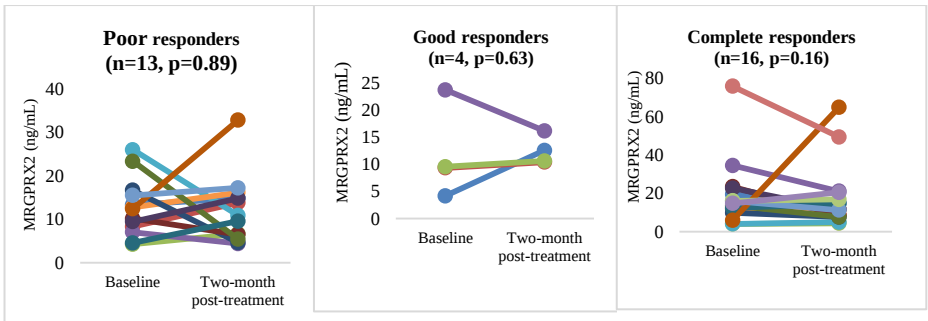
Figure 3.5. Baseline serum concentrations of MRGPRX2, SP, MBP, and EPO among bilastin-responsive groups over time in the severe CSU group (Kruskal-Wallis test) (T2: after 2 weeks, T4: after 4 weeks, T8: after 8 weeks, PR: Poor responders, GR: Good responders, CR: Complete responders)

There were no differences in baseline serum MRGPRX2, SP, and EPO concentrations between the poor, good, and complete responders to bilastine at the three time points, T2, T4, and T8 ($p>0.05$). In MBP, baseline serum MBP concentrations in the poor responders were lower than those in the other two groups at T2 and T4 ($p<0.05$), but there was no difference at T8 ($p>0.05$).

Table 3.5: Correlation between serum MRGPRX2, SP, MBP, and EPO concentrations and UCT scores over time in the severe CSU group (T2: after 2 weeks, T4: after 4 weeks, T8: after 8 weeks) (Spearman's correlation)

Time	MRGPRX2		SP		MBP		EPO	
	r	p	r	p	r	p	r	p
T2	0.09	0.51	0.17	0.21	0.17	0.19	-0.1	0.44
T4	0.2	0.13	0.11	0.42	0.26	0.041	-0.11	0.4
T8	0.04	0.76	0.1	0.43	0.1	0.45	-0.1	0.43

No correlation was observed between serum MRGPRX2, SP, and EPO concentrations and UCT scores at three time points, T2, T4, and T8 ($p>0.05$). Serum MBP concentration demonstrated a weak positive correlation with UCT scores at T4 ($p<0.05$); however, no correlation was observed at T2 or T8 ($p>0.05$).



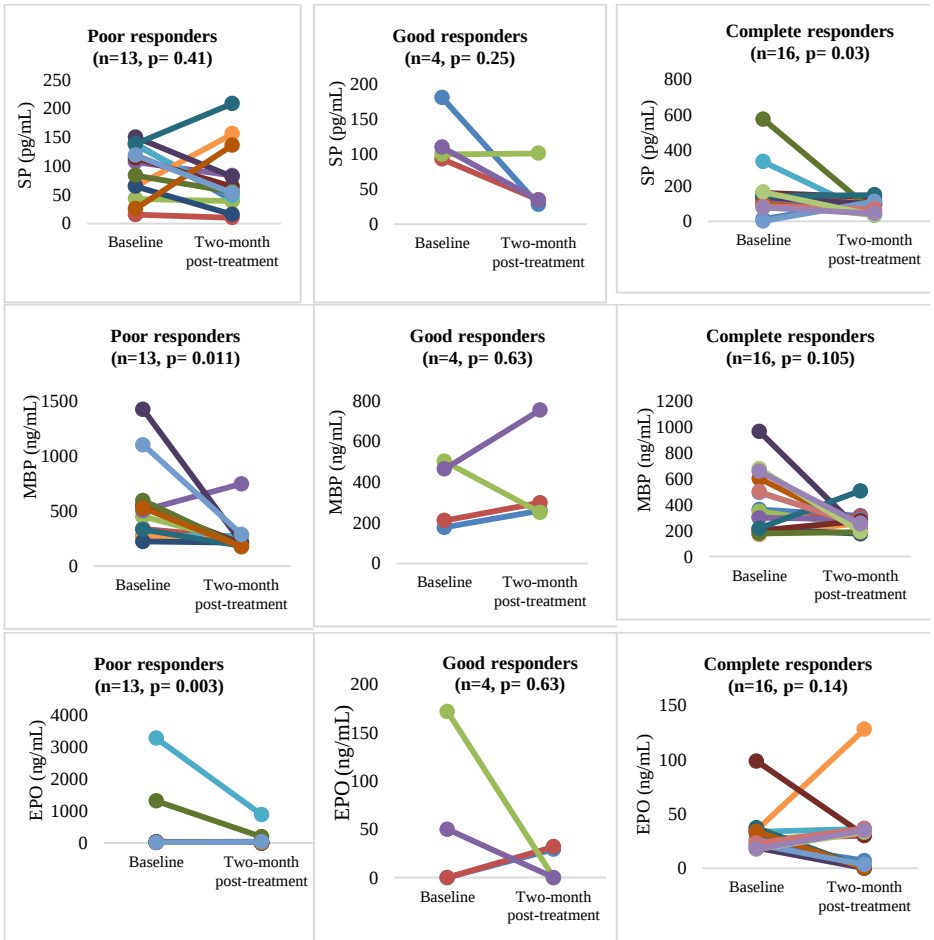


Figure 3.6: Changes in serum MRGPRX2, SP, MBP, and EPO concentrations after bilastin treatment in the severe CSU group (Wilcoxon signed-rank test)

At T8, a total of 33 serum samples were obtained: 13 from poor responders, 4 from good responders, and 16 from complete responders. No significant variation in serum MRGPRX2 concentrations was observed among these groups ($p > 0.05$). Notably, serum SP concentration diminished in the complete responders. Conversely, reductions in MBP and EPO concentrations were observed in poor responders ($p < 0.05$).

CHAPTER 4: DISCUSSION

4.1. Correlation between serum MRGPRX2, SP, MBP, and EPO concentrations and the severity of CSU

4.1.1. Some clinical and paraclinical characteristics of the study subjects

In this study, the majority of patients with CSU were working-age (average age 40), predominantly female. These findings are notably consistent with those observed in

Vietnam and internationally. The distribution of age and gender in CSU holds considerable clinical and social significance, as it influences patients' quality of life and imposes a substantial burden on healthcare systems and society.

Our study found no significant differences between the severe and non-severe CSU groups in clinical and paraclinical characteristics, including age, gender, disease duration, angioedema rate, CRP, total IgE, eosinopenia, basopenia, or anti-TPO IgG levels. Globally and within Vietnam, research hasn't reached a consensus on these biomarkers for distinguishing severity. Fox et al.'s review also states that no biomarker can yet replace the UAS7 score for assessing disease activity in CSU.

4.1.2. The correlation between serum MRGPRX2 levels and the severity of CSU

Our research indicates no significant difference in serum MRGPRX2 levels between patients with severe CSU and healthy controls. Prior investigations into serum MRGPRX2 levels within these groups have yielded inconsistent findings. A plausible explanation is the variability in selection criteria. Nonetheless, the discrepancies across these studies suggest that serum MRGPRX2 levels are not a reliable biomarker for distinguishing the severe CSU group from healthy individuals. Additional research indicates that structural alterations in MRGPRX2 may play a crucial role in this distinction.

Our research demonstrated that serum MRGPRX2 concentrations were elevated in the severe CSU group compared with the non-severe group and correlated positively with the UAS7 score. Additionally, the study identified an optimal serum MRGPRX2 threshold of 11.67 ng/mL for distinguishing severe disease in Vietnamese patients with CSU. Only one prior study reported this threshold in Korean patients, at 12 ng/mL. Further research involving diverse ethnic groups is needed to determine the most appropriate serum MRGPRX2 threshold for identifying severe CSU cases.

4.1.3. The correlation between serum SP levels and the severity of CSU

Our study revealed that serum SP concentrations in both severe and non-severe CSU groups did not differ significantly from those in the healthy control group. Prior research examining serum SP concentrations across these three groups has yielded inconsistent results. Nevertheless, this does not undermine the role of SP in histamine release, as SP is primarily released locally. Elevated and very elevated serum SP concentrations were observed exclusively in the CSU group, and not in the healthy control group.

Our study demonstrated that serum SP concentrations were elevated in the severe CSU cohort compared with the non-severe cohort and correlated positively with the UAS7 score. Moreover, this constitutes the initial investigation to establish a threshold serum SP concentration of 97.66 pg/mL in Vietnamese patients with CSU to determine disease severity.

4.1.4. The correlation between serum MBP and EPO levels and the severity of CSU

Our study demonstrated that serum EPO concentrations were significantly elevated in the patient group compared to the control group ($p < 0.001$). Additionally, serum MBP concentrations were higher in the patient group than in the control group; however, this difference did not attain statistical significance ($p > 0.05$). Our research results once again confirm eosinophil degranulation in CSU, leading to increased serum concentrations of these two proteins in patients. Nonetheless, no significant differences in protein concentrations were observed between severe and non-severe CSU groups, and no correlation was identified with the UAS7 score or peripheral blood eosinophil count. This aligns with the observations of Khanna et al., who reported that eosinophils are not the primary effector cells in CSU.

4.1.5. Logistic regression model of risk factors for severe CSU

Our study demonstrates that both univariate and multivariate logistic regression analyses have validated that MRGPRX2 and SP thresholds of 11.67 ng/mL and 97.66 pg/mL,

respectively, in Vietnamese individuals are significant risk factors for severe CSU. This finding further substantiates the role of MRGPRX2 in the pathogenesis of severe CSU. Elevated expression of MRGPRX2, along with mast cell-nervous system interactions, facilitates increased mast cell degranulation via an IgE-independent pathway, leading to histamine and other inflammatory mediator release; consequently, this exacerbates wheals and pruritus and heightens disease severity in CSU.

4.2. Correlation between serum MRGPRX2, SP, MBP, and EPO concentrations and bilastine response in the severe CSU group

4.2.1. Results of treating severe CSU with bilastine

sgAH1 is the first-line treatment for CSU. Fok et al. found UAS7 is a biomarker for predicting response to sgAH1. No definitive UAS7 threshold exists, but higher scores often indicate poor response. Patients with uncontrolled symptoms despite sgAH1 are targets for new drug trials. We studied severe CSU patients (UAS7 $\geq$ 28) responding to bilastine, a highly effective, well-tolerated sgAH1. About 60% had suboptimal control at standard doses, but this decreased to 35% at higher doses. Higher doses improved control significantly ($p < 0.05$). Patients with poor response had higher UAS7 scores than responders ($p < 0.05$), with no other clinical differences. These findings align with reviews on the efficacy of high-dose sgAH1 and the correlation between UAS7 response and CSU.

4.2.2. Correlation between serum MRGPRX2 levels and bilastine response in the severe CSU group

Our study found no significant differences in baseline serum MRGPRX2 levels among the three groups responding to bilastine at T2, T4, and T8 ($p > 0.05$). MRGPRX2 levels also did not correlate with UCT scores, indicating they are not indicative of treatment response in this context of CSU.

Our study shows serum MRGPRX2 levels are unaffected by bilastine. Histamine H1 receptors are absent on mast cells; thus, sgAH1 cannot directly target them or influence serum MRGPRX2 levels. Few studies have examined changes in serum MRGPRX2 levels. Even Omalizumab, a monoclonal anti-IgE antibody that partly inhibits mast cell degranulation via Fc ϵ RI, did not alter serum MRGPRX2 levels. Only Rilzabrutinib, a BTK inhibitor blocking mast cell degranulation via Fc ϵ RI, has been shown to reduce serum MRGPRX2 levels.

4.2.3. Correlation between serum SP levels and bilastine response in the severe CSU group

Our study showed no significant differences in baseline serum SP levels across the three groups (complete, good, and poor responders) at three time points (T2, T4, and T8), and no correlation with UCT scores. These findings suggest SP levels do not predict response to bilastine in CSU.

The study showed that serum SP levels decreased after 2 months of bilastine treatment in the complete response group, likely because bilastine kept histamine H1 receptors on sensory neurons inactive, thereby preventing neuropeptide release, including SP. In the poor response group, serum SP levels stayed the same or increased in 3 of 13 patients, supporting that other inflammatory mediators from mast cells can stimulate sensory neurons to release SP and maintain lesions in CSU less responsive to treatment sgAH1.

4.2.4. Correlation between serum MBP and EPO levels and bilastine response in the severe CSU group

Our study is the first to compare serum EPO and MBP concentrations with the response to bilastine treatment for CSU. Results showed that after 2 months, there were no differences among the response groups in baseline serum EPO and MBP levels, nor was there a correlation with the UCT score ($p > 0.05$). This indicates these substances are not predictors of treatment response. However, two severe CSU cases with very high serum EPO and MBP (> 1000 ng/mL) responded poorly, suggesting that eosinophils have a limited role in a small subset of refractory CSU patients.

Our study showed that serum levels of EPO and MBP decreased after bilastine,

especially in poor responders. Piperidine-type sgAH1, like bilastine, can inhibit eosinophil chemotaxis, indirectly reducing eosinophil degranulation and serum MBP and EPO levels. However, this reduction didn't affect treatment response, indicating eosinophils may not be the main effectors in sgAH1-resistant CSU. This could explain the limited efficacy of eosinophil-targeting agents in phase IIb trials for these patients with CSU.

CONCLUSION

1. Correlation between serum MRGPRX2, SP, MBP, and EPO concentrations and the severity of CSU

There were no significant differences between the severe and non-severe CSU groups in age, disease duration, sex, angioedema occurrence, ASST positivity rate, CRP elevation, total IgE elevation, or anti-TPO IgG elevation. The UAS7 score in the severe CSU group was 29.5 [28-35] points, whereas in the non-severe group, it was 20 [14-23.5] points.

Serum concentrations of MRGPRX2 and SP were elevated in the severe CSU group compared with the non-severe CSU group; however, they did not differ significantly from those in the control group. There was a positive correlation between serum MRGPRX2 and SP concentrations and the UAS7 score. An optimal diagnostic cutoff value for serum MRGPRX2 was determined to be 11.67 ng/mL, demonstrating a sensitivity of 75.0% and specificity of 91.7%. Similarly, the optimal cutoff for serum SP was identified as 97.66 pg/mL, with a sensitivity of 66.7% and a specificity of 58.3%. Both univariate and multivariate logistic regression analyses indicated that threshold values of 11.67 ng/mL for MRGPRX2 and 97.66 pg/mL for SP constitute risk factors for severe disease CSU.

Serum MBP levels were elevated in the severe CSU cohort compared with the non-severe CSU and control groups; however, this difference was not statistically significant. Serum EPO levels in the control group were predominantly undetectable and lower than those observed in both the severe and non-severe CSU groups. No significant difference in serum EPO levels was identified between the severe and non-severe CSU cohorts. Furthermore, serum levels of MBP and EPO showed no correlation with the UAS7 score.

2. Correlation between serum MRGPRX2, SP, MBP, and EPO concentrations and bilastine response in the severe CSU group

After 2 weeks of standard-dose bilastine treatment, the rates of complete response, good response, and poor response were 33.3%, 10.0%, and 56.7%, respectively. Increasing the bilastine dose (twice and four times) effectively reduced the poor response rate to 46.7% after 4 weeks and 35% after 8 weeks.

The groups with complete response, good response, and poor response differed only in baseline UAS7 scores, without differences in age, disease duration, sex, prevalence of angioedema, positive ASST, elevated CRP, elevated total IgE, or elevated anti-TPO IgG. The baseline UAS7 score in the poor response group was 35.5 ± 5.6 points, higher than the other two groups.

Initial concentrations of MRGPRX2, SP, MBP, and EPO did not predict response to bilastine in severe CSU. Serum levels of MRGPRX2 remained stable following a two-month treatment period with bilastine, whereas levels of SP, MBP, and EPO tended to decrease. Notably, serum SP levels were significantly reduced in the cohort demonstrating complete responsiveness to bilastine in CSU. Conversely, reductions in MBP and EPO levels were primarily observed in the poor-response group.

RECOMMENDATION

Consider conducting additional research to evaluate the correlation between the expression of the MRGPRX2 receptor on mast cell membranes at the wheal lesions, the concentrations of SP, MBP, and EPO, and the serum levels of MRGPRX2, SP, MBP, and EPO in CSU.

Review of research on MRGPRX2 gene mutations in CSU among Vietnamese subjects.

LIST OF PUBLICATIONS RELATED TO THE DISSERTATION

1. Nguyễn Thị Kim Cúc, Lê Huyền My, Trần Thu Hà Phương, Lê Hữu Doanh, Phạm Thị Lan, Vũ Nguyệt Minh. Nồng độ chất P huyết thanh trong bệnh mày đay mạn tính tự phát. Tạp chí Nghiên cứu Y học, Tập 187 số 2 ngày 27 tháng 2 năm 2025, trang 1-10, doi:10.52852/tcncyh.v187i2.2983
2. Nguyen Thi Kim Cuc, Vu Nguyet Minh, Pham Thi Lan, Le Huyen My, Le Huu Doanh. Serum MRGPRX2 and substance P levels are biomarkers of disease activity rather than an antihistamine response in chronic spontaneous urticaria. *Scientific Reports*. Volume 15 – Issue 1, 2025-03-23, Pages 10014. doi:10.1038/s41598-025-94841-1.
3. Nguyen Thi Kim Cuc, Pham Thi Lan, Le Huyen My, Le Huu Doanh, Vu Nguyet Minh. The relationship between serum Eosinophil Peroxidase and Major Basic Protein levels in relation to severity and response to H1 antihistamines in chronic spontaneous urticaria. *PLOS ONE*. Volume 20 – Issue 11, Pages e0336118. doi:10.1371/journal.pone.033611