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TESTING THE HYPOTHESIS REGARDING THE CHOLINERGIC PHENOTYPE IN ASTHMA

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Objective *– This study aims to test the hypothesis of a cholinergic phenotype in asthma, which is characterized by increased vagal tone and heightened sensitivity to anticholinergic drugs. Primary care physicians regularly treat patients with asthma, and current recommendations are based on a classic step-by-step approach to inhaled therapy. While this method has clear advantages, it fails to consider the various phenotypes of asthma, which differ in their underlying mechanisms and thus influence patients' responses to specific medications. A phenotype-based approach to asthma therapy appears promising, particularly in relation to the autonomic nervous system (ANS), as the main classes of asthma medications exert their effects through this system.*

Material and Methods*. We examined 60 patients whose asthma remained uncontrolled despite using a combination of inhaled corticosteroids and long-acting beta-agonists. Among these patients, 44 were male with a mean age of 35.6 ± 4.4 years, and 16 were female with a mean age of 38.1 ± 5.8 years. The control group consisted of 30 healthy subjects (HS) with a mean age between 35 and 65 years (23 males and 7 females). After introducing a long-acting muscarinic antagonist into their treatment, asthma control improved in 29 of the 40 patients (72.5%). We analyzed the state of the ANS based on short-term heart rate variability (HRV) data obtained using a single-channel chest ECG sensor Polar H10 (Polar Electro Oy, Finland). HRV parameters were calculated using Kubios HRV Scientific software version 3.5.0, applying both time-domain and frequency-domain methods. These included the square root of the variance of R-R intervals, the square root of the mean square of differences between consecutive R-R intervals, as well as measures in very low frequency, low frequency, high frequency, normalized low frequency, normalized high frequency, and their ratios.*

Results. *A strong correlation (ρ = 0.814) was observed between parasympathetic activity (indicated by high HRV frequency) and the effectiveness of the patients' response to the muscarinic antagonist, as determined by the Asthma Control Questionnaire and spirometry. Patients exhibiting high parasympathetic activity generally showed better asthma control when treated with muscarinic antagonists.*

Conclusions. *These findings support the concept of a cholinergic phenotype in asthma. Family physicians can utilize short-term HRV assessments to evaluate the ANS state and predict the effectiveness of anticholinergic drugs in asthma patients.*

ПЕРЕВІРКА ГІПОТЕЗИ ЩОДО ХОЛІНЕРГІЧНОГО ФЕНОТИПУ АСТМИ

Височина І.Л., Березуцький І.В.

Ключові слова: *бронхіальна астма, антагоністи мускаринових рецепторів тривалої дії, варіабельність серцевого ритму, вегетативна нервова система, сімейна практика.*

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Мета роботи *– перевірити гіпотезу холінергічного фенотипу астми, який характеризується підвищеним тонусом блукаючого нерва та чутливою реакцією на антихолінергічні препарати. Лікарі первинної медичної допомоги щоденно мають справу з пацієнтами з астмою. Сучасні рекомендації ґрунтуються на класичному східчастому плані інгаляційної терапії. Хоча цей підхід має очевидні переваги, він не враховує різних фенотипів астми. Ці фенотипи відрізняються своїми основними механізмами, які впливають на те, як пацієнти реагують на конкретні ліки. Патогенетичний підхід (на основі фенотипу) до терапії астми видається перспективним, особливо з урахуванням стану автономної нервової системи (АНС), бо саме через неї здійснюють свій вплив основні групи препаратів від астми.*

Оригінальні дослідження

Матеріал і методи. *Обстежено 60 пацієнтів, астма яких була неконтрольованою на тлі прийому комбінації інгаляційних кортикостероїдів та β-агоністів тривалої дії. Чоловіків було 44, середній вік (35.6±4.4) років, жінок - 16, середній вік (38.1±5.8) років. До групи контролю увійшли 30 умовно здорових осіб віком від 35 до 65 років (23 чоловіки та 7 жінок). Після введення мускаринового антагоніста тривалої дії до схеми лікування контроль астми покращився у 29 із 40 пацієнтів (72,5%). Ми аналізували стан АНС за даними короткочасної варіабельності серцевого ритму (ВСР) за допомогою нагрудного одноканального екг-датчика Polar H10 (Polar Electro Oy, Finland). Розрахунок показників ВСР виконувався у програмному забезпеченні Kubios HRV Scientific версії 3.5.0. Для аналізу ВСР ми використовували методи як часової, так і частотної зони: квадратний корінь із дисперсії інтервалів R-R, квадратний корінь із середньоквадратичних різниць послідовних інтервалів R-R, дуже низька частота, низька частота, висока частота, нормалізована низька частота, нормалізована висока частота та їх співвідношення.*

Результати. *Виявлена сильна кореляція між парасимпатичною активністю (висока частота ВСР) та силою реакції пацієнтів на мускариновий антагоніст (ρ = 0.814), що визначалася за даними опитувальника контролю астми та спірометрії. Пацієнти з високою парасимпатичною активністю демонстрували кращий контроль астми при лікуванні мускариновими антагоністами.*

Висновки. *Результати підтверджують концепцію холінергічного фенотипу при астмі. Сімейні лікарі можуть використовувати короткочасну ВСР для оцінки стану АНС та прогнозування ефективності антихолінергічних препаратів пацієнтам із астмою.*

Introduction. Effectively managing asthma in primary care settings can be challenging due to various factors that lead to inadequate control. To address this issue, it is essential to identify the reasons behind the ineffectiveness of triple inhaler therapy (TIT). Current asthma management guidelines recommend adding long-acting muscarinic receptor antagonists (LAMAs) when the combination of inhaled corticosteroids (ICS) and prolonged long-acting beta-2 agonists (LABAs) is not sufficient [1]. Recent studies indicate that TIT is effective in more than two-thirds of cases [2]. The necessity to enhance the effectiveness of TIT has become clear, leading to an increasing recommendation for its use in patients with moderate persistent asthma [3]. Between 2024 and 2025, researchers focused on patients whose asthma was not well-controlled with the combination of ICS and LABAs, but the addition of a LAMA solved the problem [4].

The analysis of this patient group aimed to identify predictors of the effectiveness of TIT, specifically the effectiveness of LAMAs. The results concluded that LAMAs were particularly effective in patients with high bronchial hyperreactivity and persistent airway limitations [5]. These are indirect, but quite obvious signs of increased activity of the parasympathetic division of the autonomic nervous system (PANS). It would seem that such findings should have forced researchers to propose a differentiated approach to the prescription of LAMAs. However, this did not occur. The success of TIT inspired researchers to propose the 5T (Triple Therapy Targeting Treatable Traits) approach. This method recommends utilizing TIT for all asthma cases, not just severe ones. It also involves using LAMAs in combination with ICS and LABAs earlier than current guidelines suggest [6]. Further improvement of TIT in our time is anticipated by exploring the most effective combinations of different representatives of ICS, LABAs, and LAMAs, along with various dosages of ICS [7].

The traditional stepwise approach to pharmacological therapy for asthma fails to consider the individual differences among patients. This method does not acknowledge the heterogeneity of asthma, which results in varying mechanisms of bronchospasm and inflammation. Consequently, this leads to different levels of effectiveness of treatment in patients with diverse underlying causes of the disease. We believe that a more promising alternative would be a differentiated approach to asthma therapy. This would involve identifying the different pathogenic phenotypes of the disease [8].

In recent years, phenotyping of asthma has seen significant development. Some phenotypes have already been identified, while many others remain under discussion [9]. One of the most promising approaches is phenotyping based on ANS status. The importance of differentiating patients in this way is indisputable. First, the ANS plays a significant role in the mechanism of bronchoconstriction. Second, the primary classes of bronchodilators-LABAs and LAMAs – exert their effects through the ANS [10].

Since long-acting muscarinic antagonists (LAMAs) promote bronchodilation by suppressing cholinergic activity, we hypothesized that they would be most effective in patients with excessive PANS tone. This hypothesis has been recognized for some time; it is well-established that increased parasympathetic tone can be one of the mechanisms contributing to bronchoconstriction [11]. LAMAs have been used for over two decades, with the first LAMA, tiotropium (Spiriva). They were developed to suppress excess cholinergic activity [12].

In 2016, Gennaro Liccardi and co-authors proposed *'a hypothesis that increased cholinergic tone in asthma might*

serve as a marker for a cholinergic phenotype, which shows a better response to anticholinergics' [13]. In the same year, the use of an increased cholinergic tone in asthma patients as a predictor for a positive response to LAMAs has been offered [14]. In 2021, evidence was provided regarding the usefulness of noninvasive assessment of autonomic activity for stratifying asthma control [15]. In 2024, Gennaro Liccardi and co-authors noted that *'phenotyping is still an unmet need',* as no studies have been conducted to confirm or refute this hypothesis [16].

Our study aimed to test two related hypotheses: first, whether a *cholinergic phenotype* exists *that responds better to anticholinergics*, and second, whether increased cholinergic tone in asthma can predict a positive response to LAMAs. If these hypotheses are confirmed, the guidelines for prescribing LAMAs in step-down asthma therapy may need to be revised.

Objective of the study: to test two related hypotheses: first, whether a cholinergic phenotype exists that responds better to anticholinergics, and second, whether increased cholinergic tone in asthma can predict a positive response to LAMAs. If these hypotheses are confirmed, the guidelines for prescribing LAMAs in step-down asthma therapy may need to be revised.

Material and methods. To achieve this goal, we investigated the correlation between the ANS status of patients with uncontrolled asthma and their response to adding a LAMA to their existing treatment of inhaled corticosteroids (ICS) and long-acting beta-agonists (LABAs). To do this, we examined 60 patients at the allergy center in Dnipro, Ukraine, during the 2024-2025. Among the 60 patients, 44 were men (73.3%) with a mean age of 35.6±4.4 years, while 16 were women (16.7%) with a mean age of 38.1±5.8 years. The duration of asthma in patients ranged from 3 to 9 years, with an average 6.5±4.4. We only included research participants who were willing to provide written consent (patient informed consent for the study code: 22253/2024). Control group consisted of 30 healthy subjects (HS) aged 35 to 65 years (23 males and 7 females). The study was approved by the ethics committee of the Dnipro State Medical University. We excluded patients with other diseases like hypertension, diabetes, thyroid disorders, and ischemic heart disease. Smokers, alcoholics and pregnant women also were excluded from our study.

All patients underwent clinical examinations and investigations between 9:00 AM and 12:00 PM to avoid any confounding effects of circadian rhythm on heart rate variability (HRV). On the day of the HRV study, patients did not receive any treatment. Asthma activity was evaluated for the month leading up to the patients' assessment using the Asthma Control Questionnaire (ACQ-7). A score of 0.75 or lower was used to differentiate between controlled asthmatic (CA) patients and uncontrolled asthmatic (UA) patients [17]. Lung function was assessed using a computerized spirometer (Spirolab III® MIR), and the Forced Expiratory Volume in 1 second (FEV1) was measured. Statistical analysis was performed using SPSS version 27 software. An independent Student's t-test was conducted to compare the patient groups. Additionally, Spearman's correlation coefficients were calculated to assess correlations between the variables.

The autonomic efferent fibers of the cardiovascular and respiratory systems share a common central origin. As a result, cardiovascular autonomic modulation is influenced by the autonomic control of the respiratory system. HRV is therefore recognized as a reliable method for diagnosing autonomic dysfunction in both the heart and respiratory systems.

ANS status was assessed using Heart Rate Variability (HRV). The ANS supplies nerves to both the heart and lungs. It regulates the heart rate and the force of contraction in the heart, as well as bronchial smooth muscle constriction and mucus hypersecretion in the lungs. There is a connection between these two components. Therefore, HRV is recognized as a reliable method for diagnosing autonomic dysfunction in both the myocardium and respiratory systems. Non-invasive assessments of the ANS are conducted using HRV based on established measurement standards, physiological interpretations, and clinical uses outlined in the guidelines from the working group of the European and American Society of Cardiology and Electrophysiology [18]. In 2024, the Psychophysiological Research Societies Committee released an updated report on Heart Rate and Heart Rate Variability. This report provides guidelines for methodology, interpretation, and reporting in HRV research. It supports the use of mobile HRV recording devices and encourages the application of short-term HRV measurements [19].

Recent studies have shown the validity and reliability of short-term HRV parameters in assessing the status of the ANS [20]. HRV parameters were derived from 5-min electrocardiogram recordings by the Polar H10 sensor chest strap device (Polar Electro Oy, Kempele, Finland) in the supine position after ensuring clean ECG signals, absence of movement artifacts and comfortable breathing. After extraction of the 1-channel ECG data and RR data (exported from the Elite HRV app) import into Kubios HRV Scientific Software version 3.5.0 (Biosignal Analysis and Medical Imaging Group, Department of Physics, University of Kuopio, Kuopio, Finland) was conducted. For the HRV analysis, we used both the time domain and frequency domain methods: heart rate (MHR), the square root of the variance of RR intervals (SDNN), the square root of the mean squared differences of successive RR intervals (RMSSD); very low frequency (VLF), low frequency (LF), high frequency (HF), normalized low frequency (LF Norm), normalized high frequency (HF Norm), and the LF/HF ratio [19].

The assessment of HRV, lung function (FEV1) and asthma control (ACQ-7) was performed within the first 2 days of inpatient care (before starting LAMA) and again: since 7-10 days after taking LAMA inhalation therapy.

Results and Discussion. In Table 1 we observe that after adding LAMA (tiotropium) to current therapy (ICS+LABA), asthma became controlled in 36 out of 60 patients (60.0%): 24 men and 12 women. For convenience we named this group of patients - group No. 1. In 24 patients, asthma remained uncontrolled: 10 men and 14 women (group No. 2). Overall Score ACQ7 in the group No. 1 was 0.55±0.15, while in the group No. 2 this indicator was 2.18±0,55 ($p < 0.01$). In the group No. 1

FEV1 was 83.4 ± 11.4 %, while in the group No. 2 this indicator was 71.2 ± 10.8 (p < 0.01). Assessing the dynamics of the Overall Score ACQ7 and FEV1 in the asthmatic patient groups after the introduction of LAMA therapy would be inappropriate, as Table 1 presents only the mean baseline values of Overall Score ACQ7 and FEV1 for all asthmatic patients. FEV1 was 91.4±12.7 % in a control group, which is 32 % higher, than group No. 2 (p < 0.01) and 8% higher than group No. 1 (p > 0.05) (Table 1).

Table 1

Characteristics of studied subjects

	All asthma patients on admission to hospital (Uncontrolled asthma)	All asthmatic patients after a week of hospital treatment		Control group (healthy persons)
		group No. 2 Uncontrolled asthma	group No. 1 Controlled asthma	
Inhaled therapy	LABA & ICS	LABA & ICS + LAMA		
N (men/women)	44/16	24 (10/14)	36 (24/12)	30 (23/7)
Asthma duration (years)	27.9±7.1	27.5±6.4	28.5±7.4	
Age (men/women)	47.8±10,8/ 48.9±12.5	45.6±10,2/ 48.4±12.6	52.5±11,4/ 49.3±10.6	45.6±11,2/ 48.4±13.5
Inhaled therapy	LABA & ICS	LABA & ICS + LAMA	LABA & ICS + LAMA	
FEV1 (%)	64.4 ± 8.7	71.2 ± 10.8	83.4 ± 11.4	91.4±12.7
ACQ-7	4.12±0,85	2.18±0,55	0.55±0.15	

Table 2

Characteristics of asthmatic patients at the time of hospitalisation (before LAMA treatment)

	All asthma patients on admission to hospital (Uncontrolled asthma)		Control group (healthy persons)
	group No. 2	group No. 1	
Inhaled therapy	LABA & ICS		
N (men/women)	44/16		30 (23/7)
	24 (10/14)	36 (24/12)	
Asthma duration (years)	27.9±7.1		
	27.5±6.4	28.5±7.4	
Age (men/women)	47.8±10,8/ 48.9±12.5		45.6±11,2/ 48.4±13.5
	45.6±10,2/ 48.4±12.6	52.5±11,4/ 49.3±10.6	
FEV1 (%)	64.4 ± 8.7		91.4±6,4
	68.2 ± 9.8	61.4 ± 8.5	
ACQ-7	3.72±0,85		
	2.98±0,55	4.55±0.35	

Thus, asthmatic patients in group No. 1 demonstrated very positive response to LAMA therapy comparing to group No. 2. Since a better response to anticholinergics' [13]. Is one of the key features of cholinergic phenotype of asthma, we concentrated our efforts on searching other key features of that phenotype. Our task was to analize distinquished features of patients on group No. 1 and group No. 2, including state ANC using HRV. From the data in Table 2 we see that the two groups did not differ significantly in age or in the duration of asthma. In group No. 1, males predominated (66.6%), and in group No. 2 females predominated (58.35%).

FEV1 in group No. 1 was lower, than in group No. 2: 67.4 ± 8.5% and 61.2 ± 9.8 % (p < 0.05). Overall Score ACQ7 also shows that in group No. 1 asthma control is worse: 4.55±0.35 в group No. 1 against 2.98±0.55 in group No. 2 (p < 0.01). (Table 2).

Table 3 represents the HRV values of asthmatic patients and the control group at the time of hospital admission. All 60 patients had uncontrolled asthma, and the HRV values indicated a marked predominance of PANS: tendency to bradycardia (63.2±7.1 bpm), increased RMSSD (49.8±17.6 ms), decreased absolute and normalized LF power (306.5±212.8 ms2 and 37.8±14.2 n.u, respectively), increased absolute and normalized HF (916.7±378.5 ms2 and 59.4±17.2 n.u, respectively), low LF/HF Ratio (0.65±0.38). All HRV parameters of asthmatic patients differed significantly from those in the group of healthy subjects, in whom they corresponded to a balanced ANS status (p < 0.01).

Although all asthmatic patients showed a predominance of PANS activity upon admission, patients in group No. 1 demonstrated higher cholinergic activity compared to patients in group No. 2 (Table 3). RMSSD in group No. 1 was 55.4±15.8 ms versus 44.5±14.6 in group No. 2 (p < 0.05). LF/HF Ratio in group No. 1 was 0.55±0.26 versus 0.75±0.32 in group No. 2 (p < 0.05). Thus, patients in group No. 1 demonstrated another key

feature of the cholinergic phenotype: increased cholinergic tone anticholinergics' [13].

SDNN in the groups of asthmatic patients did not have significant differences and was low, which is usually regarded as a decrease in the adaptive capacity of the ANS: 21.2 ± 8.3 ms in group No. 1 versus 22.6 ± 8.6 ms in group No. 2 ($p > 0.05$). In the control group, SDNN was 2.5 times higher: 55.6 ± 16.4 ms ($p < 0.01$). With regard to asthmatic patients, low SDNN can be regarded as one of the criteria of uncontrolled asthma. The presence of this feature in all patients with uncontrolled asthma (Table 3) and its absence in patients with controlled asthma (Table 4) allows us to regard this criterion as sufficiently reliable.

Table 4 represents the dynamics of HRV parameters in groups of asthmatic patients during TIT. The table shows that in both groups, PANS activity decreased after inclusion in LAMA inhalation therapy. However, while in Group 1, ANS status radically changed from pronounced vagotonia to moderate sympathicotonia, in Group 2, only a moderate decrease in PANS activity was observed.

Table 3

HRV in groups of asthmatic patients at the time of hospital admission (before LAMA therapy)

	All asthma patients on admission to hospital (Uncontrolled asthma)		Control group (healthy subjects)
	group No. 2	group No. 1	
N (men/women)	44/16		30 (23/7)
	24 (10/14)	36 (24/12)	
MHR (bpm)	63.2±7.1		72,7±7,2
	67.4±7.5	58.2 ± 6.6	
SDNN, (ms)	21.8± 9.4		55.6±16.4
	22.6± 8.6	21.2± 8.3	
RMSSD, (ms)	49.8±17.6		35.6 ±12.6
	44.5±14.6	55.4±15.8	
LF (ms^2)	306.5±212.8		522.6±204.7
	342.6±197.5	286.4±178.2	
HF (ms^2)	916.7±378.5		647.5±224.5
	865.7±315.8	976.8±386.8	
LF Norm (n.u., normalized units)	37.8±14.2		44.5 ± 12.8
	42.8±15.7	31.2±13.7	
HF Norm (n.u.)	59.4±17.2		41.2 ± 11.5
	55.1±16.3	65.6±18.3	
LF/HF Ratio	0.65±0.38		1.58±0.79
	0.75±0.32	0.55±0.26	

Table 4

HRV in groups of asthmatic patients during TIT

	group No. 2		group No. 1		Control group (healthy persons)
	LABA & ICS	LABA & ICS+ LAMA	LABA & ICS	LABA & ICS+ LAMA	
N (men/women)	24 (10/14)		36 (24/12)		30 (23/7)
MHR (bpm)	67.4±7.5	71.4±7.8	58.2 ± 6.6	88.3 ± 9.7	72,7±7,2
SDNN, (ms)	22.6± 8.6	23.6± 8.6	21.2± 8.3	44.4± 11.7	55.6±16.4
RMSSD, (ms)	44.5±14.6	40.5±15.8	55.4±15.8	21.5±6.8	35.6 ±12.6
LF (ms^2)	342.6±197.5	733.6±238.2	286.4±178.2	839.2±186.3	522.6±204.7
HF (ms^2)	865.7±315.8	805.7±326.8	976.8±386.8	432.4±152.2	647.5±224.5
LF Norm (n.u., normalized units)	42.8±15.7	49.2±14.9	31.2±13.7	62.1±21.4	44.5 ± 12.8
HF Norm (n.u.)	55.1±16.3	51.6±16.3	65.6±18.3	28.5±14.4	41.2 ± 11.5
LF/HF Ratio	0.75±0.32	0.95±0.42	0.55±0.26	2.15±1.44	1.58±0.79

The assessment of HRV dynamics parameters revealed significant differences between the two asthma patient groups. MHR in group No 1 during TIT increased from 58.2 ± 6.6 to 88.3 ± 9.7 ($p<0.01$). While in group No. 2 heart rate dynamics was insignificant: с 67.4±7.5 до 71.4±7.8 ($p > 0.05$).

The SDNN dynamics in group No. 1 indicated the restoration of the adaptive capacity of the ANS: with UA, SDNN was 21.2±8.3 ms, and upon achieving asthma control, it doubled to 44.4±11.7 ms ($p<0.01$). However, SDNN in group No. 1 was still lower than in the control group: 55.6±16.4 ms ($p<0.05$). In patients in group No. 2, the SDNN dynamics were insignificant: 22.6±8.6 ms with LABA & ICS and 23.6±8.6 ms with TIT ($p>0.05$).

The RMSSD, an indicator of parasympathetic activity, в group No. 1 decreased more than twofold: from 55.4±15.8 to 21.5±6.8 (p<0.01). B group No. 2 RMSSD also slightly decreased: from 44.5±14.6 t 40.5±15.8 ms (p > 0.05), which indicated the continued predominance of PANS activity [21].

The HF component of HRV, which reflects vagal efferent activity, в group No. 1 decreased by more than half (HF Norm) from 65.6±18.3 to 28.5±14.4 n.u. (p<0.01). B group No. 2 HF component of HRV slightly decreased from 55.1±16.3 to 51.6±16.3 n.u. (p > 0.05).

Normalization eliminates the effects of overall HRV differences between individuals or conditions, facilitating a more straightforward comparison of the relative contribution of HF and LF to the overall autonomic balance. The LF/HF ratio, which reflects the balance between sympathetic and vagal activity, in group No. 1 increased from 0.55±0.26 to 2.15±1.44 (p<0.01), which indicated a change from a significant predominance of PANS to a moderate predominance of SANS [21]. In group No. 2 LF/HF ratio increased from 0.75±0.32 до 0.95±0.42 (p > 0.05), which indicated the maintenance of a moderate predominance of PANS activity. Fig 1 shows the dynamics of the ANC balance in groups of asthmatic patients with the inclusion of LAMA therapy. Thus, patients in group No. 1 *shows a better response to anticholinergics'* [13], which is the third key feature of the cholinergic phenotype of asthma.

Spearman's coefficient indicated a strong correlation between HRV parameters related to parasympathetic tone and the Overall Score on the ACQ7. It also showed a strong direct correlation with FEV1. Specifically, the correlation between RMSSD and the Overall Score on ACQ7 was ρ = 0.746 (Figure 2. A), while for the HF component of HRV and the Overall Score on ACQ7, it was ρ = 0.814 (Figure 1. B). In contrast, the correlation between RMSSD and FEV1 was ρ = 0.687 (Figure 2. C), and between HF and FEV1, it was ρ = 0.728 (Figure 2. D). In other words, greater PANS activity increases the effectiveness of tiotropium in controlling asthma, as indicated by both the ACQ-7 and spirometry (FEV1).

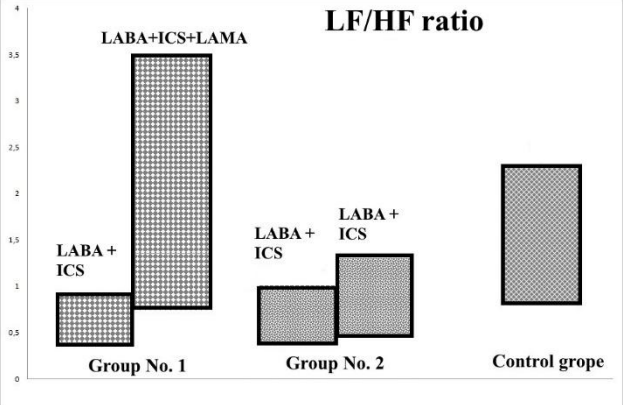

Fig. 1. LF/HF ratio in groups of asthmatic patients and in control group

Relationship between RMSSD and the Overall Score on ACQ7	Relationship between HF component of HRV and the Overall Score on ACQ7
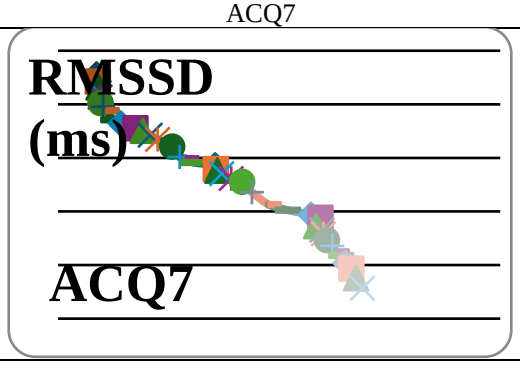	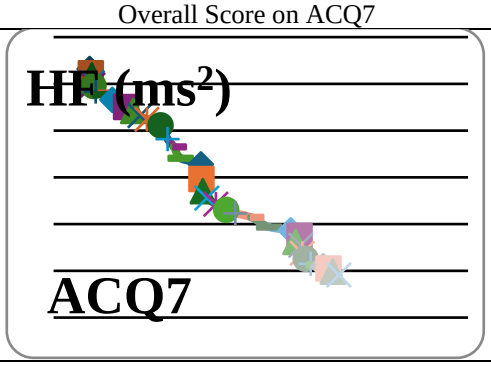
Figure 1. A.	Figure 1. B
Relationship RMSSD and FEV1	Relationship between HF component of HRV and FEV1
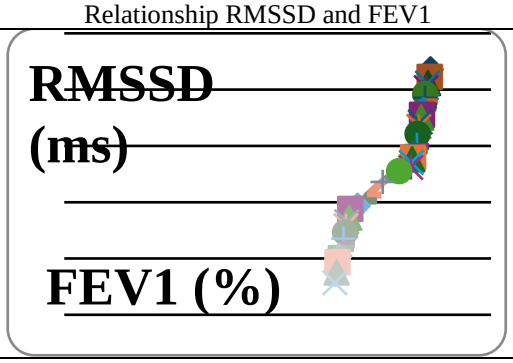	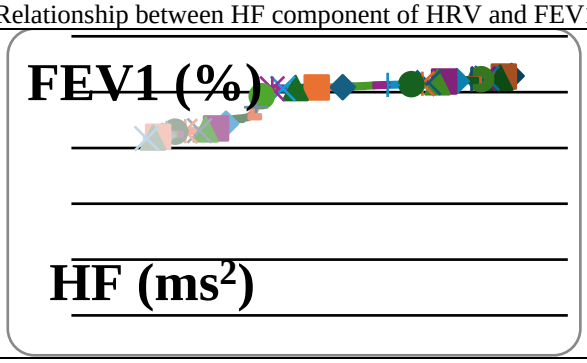
Figure 1. C	Figure 1. D

Fig. 2. A = Relationship between RMSSD and the Overall Score on ACQ7; B = relationship between HF component of HRV and the Overall Score on ACQ7; C = relationship RMSSD and FEV1; D = relationship between HF component of HRV and FEV1.

To our knowledge, this study is the first to test the cholinergic phenotype hypothesis of asthma.

Antimuscarinics were used for the maintenance treatment of asthma over a century ago. After a long interval, in 2014, their use was recommended again as an add-on therapy with LAMAs for the maintenance treatment of asthma [22]. Despite the widely acknowledged importance of the vagus nerve in the development of bronchospasm, as well as the anti-inflammatory and anti-fibrotic effects of LAMAs, asthma guidelines recommend that LAMA be added as a third controller alongside the combination of ICS and LABA before considering the initiation of biological therapies [1]. All current and previous asthma guidelines follow a stepwise approach to inhalation therapy for asthma. Phenotype-driven and endotype-driven approaches to asthma management have been actively developed by researchers since the early 2010s. In 2012 Ioana Agache and co-authors in a paper entitled "Untangling Asthma Phenotypes and Endotypes" stated: '*Phenotype' has been used extensively in asthma but is still largely indiscriminate'* [23]. In 2025, Ioana Agache et al. in a paper titled "The Bronchodilator and Anti-Inflammatory Effect of Long-Acting Muscarinic Antagonists in Asthma" acknowledge that '*the relatively low success rate of LAMA in severe asthma might arise from the lack of a phenotype-guided prescription'* [1]. Most researchers in 2025 acknowledge the underuse of LAMAs in asthma therapy [1, 5, 6], and believe that '*further studies are needed to identify the LAMA responsive therapy, and thus to position LAMA in the personalised and cost-efficient strategies for the management of asthma patients'* [1]. Almost all scientific papers devoted to LAMAs 2025 analyze the possibilities of predicting their effectiveness based on clinical, laboratory or instrumental signs [1, 5, 6]. We have developed a straightforward and cost-effective method for predicting the effectiveness of LAMAs using short-term HRV. This method does not require any additional equipment, making it easy to implement, even in primary care settings.

In our study, we screened patients whose asthma was not controlled by the ICS+LABA combination and found that 60.0% (44 out of 60 patients) exhibited signs of the cholinergic asthma phenotype. All these patients responded positively to LAMA treatment. Such a large proportion of patients with signs of the cholinergic phenotype does not allow us to judge its prevalence. Patients admitted to hospital for treatment of asthma exacerbations represent only that portion of asthmatic patients in whom the LABA & ICS combination has been ineffective. Early detection of the cholinergic asthma phenotype could significantly reduce healthcare costs for treating these patients and improve their quality of life. Implementing such screening on a national scale is therefore very appealing for the healthcare system. Additionally, since vagal tone increases with age [1], it can be inferred that the longer a patient has had asthma, the more relevant LAMA treatment may become.

When analyzing the results of the HRV assessment in the context of triple therapy, it is important to acknowledge that we cannot completely eliminate the influence of inhaled LABAs and LAMAs on the study outcomes. Although the study conditions stipulated that patients did not take prolonged-release medications on the day of the HRV assessment, their use in the days leading up to the assessment could still affect the status of the autonomic nervous system (ANS). Additionally, patients with uncontrolled asthma were permitted to use salbutamol. Hajar Ali et al. reported that administering salbutamol within two hours before HRV testing, or using a LABA in the two weeks prior, was linked to decreased PNS activity and increased SNS activity in asthma patients [24]. Ching-Huei Tsou et al report that '*the HRV parameters in both the time and frequency domains showed significant changes indicating sympathetic activation of the autonomic balance immediately after inhaling the beta2-agonist (Berotec 200 mcg)'* [25]. Sarah Elhage et al. showed in their study that LABAs and LAMAs do not have a significant effect on HRV in asthma [26]. Lorena Soto-Retes et al., after reviewing the latest research on the state of the ANS in asthmatic patients, concluded that '*given the lack of clarity, nonetheless, further studies are needed on the pharmacological effects of LABAs or LAMAs and their influence on HRV outcomes in asthma'* [27]. It is extremely difficult to even speculate about the effect of TIT on the ANS. It is noteworthy that studies in recent years have provided contradictory results regarding ANS dysfunction in asthma. Some researchers report increased PANS activity in patients with uncontrolled asthma [28], while others, on the contrary, report a predominance of SNS [29]. The contradictory results may be influenced by the difficulty in excluding the impact of prolonged use of bronchodilators on heart rate variability (HRV). Additionally, a patient's emotional state can also affect HRV. Although the study excluded patients with psychiatric conditions, such as anxiety and depression, long-term asthma is often associated with these disorders, which can be challenging to identify [27].

Thus, the influence of LABAs and LAMAs on HRV in our patients cannot be excluded, since they received triple therapy. We found that all patients whose asthma remained uncontrolled after the addition of LAMA to therapy demonstrated pronounced SNS activity, whereas patients whose asthma was controlled after the addition of LAMA to therapy showed a predominance of PANS. That is, these patients showed a full set of features of the cholinergic phenotype: increased cholinergic tone and a better response to anticholinergics [13].

The limitations. Present study has some limitations. First, the single-center study was limited by its relatively small sample size, which may have affected the reported outcomes. Additionally, the breathing frequency, known to influence HRV analysis, was not recorded in this study. However, efforts were made to minimize any potential impact. The strength of this study lies in the fact that all our patients had uncontrolled asthma prior to switching to TIT, which includes the addition of a LAMA. In other words, LABAs proved ineffective for this asthma phenotype. This serves as an ideal criterion for testing the hypothesis of a cholinergic asthma phenotype.

Conclusions. ANS in asthmatic patients in the exacerbation phase of asthma (with uncontrolled asthma) is characterized by the predominance of PANS activity, and in the remission phase (controlled asthma) – by the predominance of SANS.

1. Patients with high vagal tone responded positively to the addition of a LAMA to the combination of ICS and

LABA. This suggests the existence of a *cholinergic phenotype* of asthma that *shows a better response to anticholinergics*.

2. A strong correlation between indicators of PANS activity, based on HRV data, and signs of effective asthma control indicates that increased cholinergic tone could serve as a predictor of a positive response to LAMAs.

3. A significant percentage of patients with high cholinergic activity (60.0%) while using the ICS+LABA combination highlights the limitations of a stepwise approach to inhalation therapy for asthma. If ANS status had been assessed prior to selecting inhalation therapy, asthma control could have been achieved much earlier in 44 out of the 60 patients included in the study.

Short-term HRV (by the Polar H10 sensor chest strap device or standard ECG recorder) enables rapid and accurate assessment of ANS status and predicts the efficacy of LAMAs in asthma.

Prospects for further research is needed to develop precise diagnostic criteria for the cholinergic phenotype based on HRV data, since all asthmatic patients (not only those with the cholinergic phenotype) demonstrate a predominance of PANS activity during an exacerbation, and a predominance of SANS activity when control is achieved. In addition, the cause-and-effect relationship between the state of the ANS and asthma control is unclear: does the relief of asthma exacerbation lead to the predominance of SANS activity or, conversely, does the achievement of the predominance of SANS activity as a result of treatment lead to the achievement of asthma control?

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