Parasympathetic nervous system: a key role in control and mood disorders in patients with asthma

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Abbreviations/Acronyms

ACT, Asthma Control Test; ANOVA, analysis of variance; ANS, autonomic nervous system; BMI, body mass index; ELISA, enzyme-linked immunosorbent assay; ECG, electrocardiogram; FeNO, fraction of exhaled nitric oxide; FEV_1, forced expiratory volume in the first second; FVC, forced vital capacity; HADS, Hospital Anxiety and Depression Scale; HF, high frequency; HFP, high-frequency power; HRV, heart rate variability; IgE, immunoglobulin E; LABA, long-acting beta2 agonist; LAMA, Long-acting muscarinic antagonists; LF, low frequency; MiniAQLQ, Asthma Quality of Life Questionnaire (short version); NN, normal-to-normal; PANS, parasympathetic autonomic nervous system; PLF, low-frequency power; PLFnr, non-respiratory-related HRV power; pNN50, percentage of NN intervals greater than 50 ms; Pr, respiratory-related HRV power; QoL, quality of life; RMSSD, root mean square of successive differences; RR, respiratory rate; SABA, Short-acting beta-2 agonists; SD, Standard Deviation; SDNN, standard deviation of the difference between consecutive NN intervals; SNS, sympathetic nervous system; TP, total power.

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INTRODUCTION

The important role played by the autonomic nervous system (ANS) in asthma pathophysiology and symptomatology has long been known.[1] In addition to regulating important airway functions, such as bronchial smooth muscle tone, secretions, blood flow, and microvascular permeability, the ANS also intervenes in the migration and release of inflammatory mediators.[2–5] Inflammatory phenotypes in asthma can usually be distinguished based on the presence of eosinophils or neutrophils, using non-invasive procedures such as exhaled nitric oxide and induced sputum.[6] However bronchoconstriction is not always mediated by bronchial inflammation, as evidenced by a significant proportion of patients with asthma (40%) in whom bronchial inflammation is not detected.[7,8] However, it is suspected that bronchoconstriction may be caused by strictly airway diameter-related mechanical mechanisms induced by nerve stimulation (the cholinergic reflex).[9] This complex interaction between inflammation and neuronal airway control, with effects on inflammatory mediators in neurotransmitters, modulates the inflammatory response (hypersecretion, edema, and the release of pro-inflammatory mediators such as mast cells)[3] by activating the cholinergic reflex.[4,5]

Evidence-based literature refers specifically to mental and emotional health in people with severe asthma.[10] Anxiety and depression are 1.5-2.4 times more common in people with asthma than in people without asthma,[11,12] and the impact is even greater in people with severe or uncontrolled asthma.[13,14] Anxiety and depression, which often occur together, affect a person's ability to function and are associated with various behavioural, cognitive, and physiological changes.[15] There is growing awareness of the shared relationship between

mental health and asthma course, as both interact directly on the pathogenic mechanisms of the respiratory tract and affect the appearance and evolution of asthma.[10,15] Although the relationship is not fully understood, anxiety and depression can negatively affect asthma course, which could be related to the overlap in autonomic mechanisms that appear to play a role in asthma are also involved in activation and regulation of the physiological response to emotional disorders related to asthma.[16,17]

Decreased heart rate variability (HRV), a neurobiological marker of the autonomic nervous system (ANS), is associated with a variety of negative physical and psychological outcomes.[18] Given that individuals with asthma tend to have a dysregulated heart rate compared to individuals without asthma, some authors suggest that autonomic control of airway calibre in asthma may be accompanied by a change in heart rhythm, suggesting altered activity of the parasympathetic ANS (PANS).[19-21] In fact, variations in PANS activity and HRV have been observed in children with asthma and allergy, with the baseline parasympathetic tone associated with altered HRV.[22]

Mood disorders, including depression and anxiety, are prevalent psychiatric disorders and a common comorbidity in people with asthma,[15] and their impact on psychophysiological markers of health and wellbeing, such as HRV, has been documented.[23] The mechanism underlying the relationship between physical and mental health may, in part, be related to impaired vagus nerve activity, leading to dysregulation of inflammatory processes.[24]

Non-invasive PANS is evaluated via HRV according to measurement, physiological interpretation, and clinical use standards as described in guidelines of the working group of the European and American Society of Cardiology and

Electrophysiology, which recommends measuring HRV with the electrocardiogram (ECG) as a means for non-invasively evaluating the PANS.[18]

Because publications are few and yield different results, we aimed to comprehensively evaluate the role played by activation of the PANS in uncontrolled asthma and related mood disorders (specifically anxiety and depression). We hypothesize that ANS dysregulation, and particularly PANS dysregulation, plays a role in both uncontrolled asthma and mood disorders. Although studies have evaluated the association of both disorders separately, to our knowledge, no study has comprehensively related whether greater anxiety and/or depression leads to worse asthma control or vice versa objectively by analysing the PANS.

Methods

Study population

A proof-of-concept cross-sectional study was conducted to assess the PANS in relation to uncontrolled asthma, anxiety, and depression.

Forty-two patients diagnosed with asthma were recruited from the pneumology and allergy outpatient~~'s~~ clinic at the Hospital Santa Creu i Sant Pau, Barcelona (Spain). Twelve declined to participate and 30 agreed to be included. All 30 patients complied with the inclusion and exclusion criteria. Inclusion criteria were age ≥18 years, and an asthma diagnosis based on Spanish asthma guidelines (GEMA)[25] and *Global Initiative for Asthma* (GINA) criteria.[26] Exclusion criteria were upper respiratory tract infection or asthma exacerbation within the previous 4 weeks, concomitant respiratory disease (bronchiectasis, fibrosis, etc), and any other major comorbidity (according to investigator criteria), such as diabetes,

psychiatric or neurological disease, and systemic inflammatory or immunological disease.

The research complied with the principles of the Declaration of Helsinki (18th World Medical Assembly, 1964) and was approved by the Hospital Santa Creu i Sant Pau Hospital Clinical Research Ethics Committee (NTC02836691).

Clinical assessment

Patients were informed about the purposes of the study and signed their informed consent before inclusion. The 30 patients meeting the inclusion criteria attended a single visit for ECG measurement of HRV. All asthma medications could be used, but short acting beta - 2 agonist (SABA) use had to be avoided at least 6 hours before. Patients completed specific asthma and anxiety-depression questionnaires, and demographic and clinical data collected included data on asthma severity, asthma control, lung function, and inflammatory cells in induced sputum, and mental and emotional health (specifically depression and anxiety).

Heart rate variability measurement

HRV analysis has been widely used for non-invasive ANS characterization.[18] Traditionally, a distinction has been made between analysis in the time and frequency domains, each with advantages and disadvantages. In this study, both domain types were considered.[27] Measurements were conducted with participants seated and motionless: they were asked to breathe naturally and avoid talking during recording, and following a 2-minute stabilization period, they were recorded for 10 minutes. Time-domain analysis was based on calculating different statistics from the HRV signal, which, in our study, were the following: the normal-to-normal (NN) interval; the time between consecutive beats (a

measure of the average heart rate); SDNN, standard deviation of the difference between consecutive NN intervals (a global measure of HRV); RMSSD, root mean square of successive differences (a measure of short-term variability reflecting parasympathetic regulation); and pNN50, the percentage of consecutive NN intervals that differ by more than 50 ms (a widely used HRV measure).[18]

The resting HRV spectrum is characterized by 2 main components: a high-frequency (HF) component in the 0.15-0.4 Hz band, and a low-frequency (LF) component in the 0.04-0.15 Hz band. While the HF band has been related to parasympathetic activity, the LF band has been related to both sympathetic and parasympathetic activity, so the power in each of the bands is related to different ANS branches. The following parameters were considered: PLF, PHF, and TP, i.e., LF power, related to both sympathetic and parasympathetic activity, HF power, mainly related to parasympathetic activity, and total power (i.e., PLF and PHF summed), respectively. [21,28]

In the frequency domain, when the respiratory rate (RR) is low, HRV analysis is compromised. This is because the information contained in the HF band is mainly related to respiration, so if the RR is so low as to be contained within the limits of the LF band, the HF band ends up empty, and consequently, physiological interpretation according to traditional measures is not feasible. One way to overcome this problem is to use orthogonal sub-space projection,[27] which combines respiratory signal and HRV information and separates the HRV part due to respiration from that due to other effects. From this decomposition, we obtained the following indices: PLFnr, non-respiratory-related HRV power; and Pr, respiratory-related HRV power.[28]

Generated through the ECG, those signals distinguish between the influence of the sympathetic ANS and the PANS.[18] This method, previously developed and studied by our team, has been adapted to patients with asthma using 12 leads, a respiratory band, and a pulse oximeter. Encephalan-EEGR-19/26 (Medicom) software was used for recording over 10 minutes and registration.[28]

Clinical variables, atopic status, lung function, and inflammatory tests

Collected data were as follows: demographic and anthropometric data; smoking status; forced expiratory volume in the first second (FEV_1) and forced vital capacity (FVC),[29,30] asthma severity (according to GEMA[25] and GINA[26]); and fraction of exhaled nitric oxide (FeNO) (chemiluminescence sensor N-6008R SIR; Madrid, Spain).[31,32] Atopic status was determined using skin prick tests for common aeroallergens such as dust mites, grass pollen, animal dander, and common fungi (Leti Pharma, Madrid, Spain),[33] with positivity defined as the presence on at least 1 weal $\geq$3 mm.

Induced sputum

Cell count was analysed by microscopy following the method described by Belda et al.[34] and Pin et al.[35] Patients were classified by bronchial inflammatory phenotype according to European Respiratory Society recommendations, as follows: paucigranulocytic (eosinophils <3%, neutrophils <65%), neutrophilic (eosinophils <3%, neutrophils ≥65%), eosinophilic (eosinophils ≥3%, neutrophils <65%), and mixed (eosinophils ≥3%, neutrophils ≥65%).[36]

Questionnaires

To establish clinical asthma control level, a validated Spanish version of the Asthma Control Test (ACT) questionnaire was administered,[37] patients

completed the ACT, which comprises five questions to assess activity limitation, shortness of breath, nighttime symptoms, use of rescue medication, and patient overall rating of asthma control over the previous 4 weeks. The questions are scored from 1 (worst) to 5 (best), and the ACT score is the sum of the responses, giving a maximum best score of 25. An ACT score of 19 or less is the cutoff point defining uncontrolled asthma. To evaluate quality of life (QoL), a validated Spanish version of the short version of the Asthma Quality of Life Questionnaire (MiniAQLQ) was administered,[38,39] includes 15 items divided into four dimensions: symptoms (5 items), activity limitation (4 items), emotional function (3 items), and environmental stimuli (3 items). The 15 items are scored on a seven-point Likert scale, with scores 1 to 7 corresponding to maximum limitation and absence of limitation (worst and best possible QoL), respectively. Finally, administered to asses anxiety-depression was the Hospital Anxiety and Depression Scale (HADS),[40] a 14-point self-assessment scale used to screen for clinically significant anxiety and depression (7 points each). Each item is rated on a 4-point scale: 0 indicating not at all; 1, sometimes; 2, often; and 3, all the time. This gives a maximum subscale score of 21 for anxiety and depression, respectively. We considered the HADS questionnaire because it is very simple and explores both anxiety and depression. In the validation of the questionnaire, a score greater than 7 (in the 2 subscales) has been found to define anxiety or depression.

Peripheral blood test

Biological samples were collected (using BD-Vacutainer tubes) to determine complete blood count and total immunoglobulin E (IgE) by enzyme-linked immunosorbent assay (ELISA; UNICAP, Pharmacia, Uppsala, Sweden).

Statistical analysis

Descriptive baseline values were reported as percentages and frequencies for qualitative data, and as mean and standard deviation (SD) values for quantitative data. Severity groups were compared using analysis of variance (ANOVA). The non-parametric Kruskal-Wallis test was used for non-normally distributed quantitative variables, yielding median, minimum, and maximum values for each group. Multivariate analysis included possible confounding and/or interaction variables. Statistical significance was set to 5% (α=0.05) and SPSS (version 22.0) for Windows (SPSS, Inc., Chicago, Il, USA) was used for the statistical analysis.

Results

Demographic and disease characteristics

The study included 30 patients with asthma (53.3% women), mean (SD) age 49.4 (12.8), 80% atopic, and 6.7% active smokers. Most had elevated type-2 biomarkers at baseline, and mean eosinophils and FeNO were 311 cells/mL and 45 ppb. Baseline demographics and disease characteristics are reported in Table 1.

Asthma control and HRV

Ten patients with uncontrolled asthma (ACT$\leq$19) were predominantly female (70%) and overweight (mean (SD) body mass index (BMI) 30 (4.6) vs 26 (3), p=0.02), and all required combination inhalers with a long-acting beta2 agonists (LABAs), long-acting muscarinic antagonists (LAMAs), and SABAs ($p\leq0.05$). Compared to patients with controlled asthma, these 10 patients also had poorer lung function (FEV_1 1.9L [0.4L] vs 3L [0.8L]; FEV_1 72% [18%] vs 92% [8.5%];

197 FEV_1/FVC 60.3% [13%] vs 69.9% [9.5%]), poorer QoL (MiniAQLQ 4.4 [1.2] vs
198 6.2 [0.8]), and experienced greater mood disorders (HADS 14.4 [7.8] vs 7.5 [9.1])
199 ($p \leq 0.05$) (Table 2). The 10 patients with uncontrolled asthma also had
200 significantly lower scores for SDNN (26.5 [8.2] vs 42.7 [29.7], p=0.03), RMSSD
201 (14 [6] vs 24 [20], p=0.05), pNN50 (0.6 [1.5] vs 6.2 [11.8], p=0.05), TP (0.0005 vs
202 0.0014, p=0.02), and Pr (0.0003 vs 0.0007, p=0.01) (Table 3).

203 *Asthma, mood disorders, and HRV*

204 Regarding the classification according to mood disorder severity, 13 patients had
205 borderline or clinically problematic HADS ≥ 8 (Table 4). Compared to patients
206 without depression-anxiety, patients at risk for depression-anxiety were
207 predominantly overweight (BMI 29.6 [4.8] vs 25.9 [2.4], p=0.02). These patients
208 needed combination inhalers with LABA (100%) ($p \leq 0.05$); differences due to
209 LAMA and SABA use were non-significant (76.9% vs 52.9%, p=0.1; 84.6% vs
210 41.1%, p=0.1). These patients also presented greater airway obstruction (FEV_1
211 2.2L [0.79L] vs 3.09L [0.79L], p=0.001; FEV_1 77.6% [19%] vs 91.8% [6.5%],
212 p=0.02; FEV_1/FVC (60% [13%] vs 71% [7%], p=0.01), lower ACT scores (18 [3.8]
213 vs 23 [2], p=0.001), and lower MiniAQLQ scores (4.8 [1.3] vs 6.3 [0.9], p=0.002).
214 Finally, they also had reduced SDNN (26.5 [7.9] vs 45.6 [31.3], p=0.04), RMSSD
215 (13.4 [6.5] vs 26 [20], $p \leq 0.05$), pNN50 (0.75 [1.4] vs 7.12 [12.6], p=0.05), TP
216 (0.0005 vs 0.0012, p=0.02), and Pr (0.0008 vs 0.0003, p=0.01) (Table 5). Only 2
217 patients with severe uncontrolled asthma were out of risk of mood disorders with
218 the following clinical features, mean (SD): age 59 (10.6), one male; BMI 28 (2.8);
219 both no smoker; both required combined LABA and LAMA treatment, and SABA
220 use; FEV_1 2.03L (0.24L); FEV_1 89% (2.5); FEV_1/FVC 65% (9.5); FeNO 100ppb
221 (78.5); blood eosinophils 280 mm3 (84); total immunoglobulin E 336 UI/ml (132),

eosinophils and neutrophils in induced sputum 17.5% (15.5) and 69 (10) respectively; ACT score 18.5 (0.5); MiniAQLQ score 4.1 (1); HADS 3 (1); SDNN and RMSSD 16.8 (20.8), pNN50 0.27 (0.39), TP 0.0007 (0.003), and Pr 0.0001 (0.0007).

Discussion

Our main finding is that, compared to patients with controlled asthma, patients with poorly controlled asthma had poorer lung function, were overweight, had a poorer QoL, and a more depressed PANS.

We demonstrated that objective data obtained from HRV measurement could be a non-invasive means of discriminating uncontrolled from controlled asthma. We also found that depression-anxiety were associated with reduced HRV parameters in patients with poorer lung function. Our results suggest that the PANS pathway could play a role in asthma pathogenesis, given the alteration in PANS activity in patients with asthma, most especially in patients with uncontrolled asthma and depression-anxiety.

A strength of the study is that we used an algorithm to stratify patients with asthma (as described in our recent study[28]) that, in other studies, has performed well in analysing HRV.[41-43] The most important result was, for the uncontrolled asthma group compared to the controlled asthma group, a reduction detected in vagal components, i.e., in RMSSD, pNN50, Pr, and TP. This finding contributes to paediatric findings by Lufti et al.,[44,45] who reported that poor asthma control in children and adolescents was associated with depressed HRV modulations, and that patients with better ventilatory functions had better HRV than patients with uncontrolled severe asthma.

The reduction in HRV vagal components observed in patients with uncontrolled asthma would suggest that there is a complex relationship between inflammation and neural airway control. Regarding impaired autonomic control, it is known that changes in bronchomotor tone in asthma occur rapidly. Decades ago it was suggested that people with asthma may have abnormal autonomic neural airway control, with an imbalance between the excitatory and inhibitory pathways resulting in overly reactive airways.[2] However, other studies of severe asthma point to increased vagal dominance in response to autonomic challenge (deep breathing, the Valsalva manoeuvre, and standing up from the recumbent position) and during sleep.[19,20,46] Hence, it is possible that the PANS in patients with severe asthma may become depressed during inactivity or relaxation, with bronchoconstriction occurring when the vagal pathways are activated or overrespond to stimuli.[47]

Our patients with controlled asthma obtained better results for all PANS parameters, although statistically non-significant differences were found for PLFnr, which reflects the sympathetic branch. Likewise, patients at risk for depression-anxiety showed depressed PANS for all parameters, while no differences were found for PLFnr. Compared to patients with controlled asthma, patients with poor asthma control and obstructive spirometry ($FEV_1 \leq 70\%$ predicted) showed more depressed HRV, independently of inflammation as measured by FeNO, induced sputum, peripheral blood eosinophilia, or total IgE (Table 1). Note that, although mood disorders like depression-anxiety are inherent to patients with severe asthma,[10,11,14-17] a strength of our study is that the ANS results were objective, and so can complement information obtained

from self-administered questionnaires that are subjective and difficult to interpret.[37-40]

We found significant differences between the groups in terms of mood disorders as measured by the HADS questionnaire: patients with severe asthma with depression-anxiety had poorer lung function, poorer asthma control, poorer QoL, and a depressed PANS compared to patients not experiencing mood disorders. Note, however, that although patients with uncontrolled asthma are known to experience mood disorders,[48] in our study, depression-anxiety were related to PANS alteration, so the question remains as to whether depression-anxiety are a consequence or an independent comorbidity of severe uncontrolled asthma.

To date, several studies have been published on HRV and anxiety or depression disorders[23,24,48]. The reasons given for altered HRV in mood disorders, according to a model of neurovisceral integration, nerve fibers that moderate parasympathetic activity and inhibition of the vagus nerve, a dysregulation is related to pathologies such as diabetes type II, cardiac and neurodegenerative diseases, and depression.[49-52] This model of neurovisceral integration is also characterized by specific neural structures that allow people to respond adaptively to physiological, environmental, cognitive, and emotional influences. Therefore, a healthy cardiorespiratory system is characterized, in the cardiac period, by oscillations (high HRV), while an unhealthy cardiorespiratory system shows few oscillations (low HRV).[24] Which is related to our findings in this study.

All patients were on asthma medication, especially the patients with uncontrolled asthma that was so severe as to require more than one inhaler. Patients with asthma were being treated with LABAs in combination with inhaled corticosteroids and with LAMAs; those with mild and moderate asthma were only

295 on inhaled corticosteroids, without LABAs or LAMAs. SABAs were avoided in the
296 hours prior to recording. While LABA or LAMA use might suggest that our results
297 may have been influenced by those medications, the literature is not entirely clear
298 as to LABA or LAMA influence on HRV, as contradictory results are reported. An
299 HRV study of patients on LABA found that its use was associated with
300 sympathetic nervous system (SNS) dominance,[53] while another study
301 demonstrated that salbutamol was associated with decreased parasympathetic
302 nervous system (PNS) and increased SNS activity.[54] Although the underlying
303 mechanism is not entirely clear, it is possible that LABAs bind to β2
304 adrenoceptors at efferent sites in the cardiac SNS, or that the peripheral
305 vasculature may directly stimulate SNS activity. Another more direct study of the
306 potential effect of LABAs on HRV reports that there was no change in time-
307 domain parameters (mean RR and SDRR) when fenoterol was administered
308 immediately before and immediately after HRV analysis, which would suggest
309 sympathetic activation.[55] However, studies in patients with asthma show that
310 different LABAs have different effects on cardiac autonomic control. Thus,
311 Eryonucu et al.[56] reported that fenoterol inhalation had no effect on sympathetic
312 activation (mean RR and SDRR) in regularly treated patients, while Zahorska-
313 Markiewicz et al.[46] showed that salbutamol and terbutaline tended to increase
314 SNS parameters. Yao-Kuang Wu et al.,[57] who studied the effects of LAMA on
315 HRV in patients with stable chronic obstructive pulmonary disease, found no
316 significant change in HRV parameters other than a significant decrease in the HF
317 component and increase in the LF component after 1 month of continued LAMA
318 treatment, but not after 3 months. Overall, they found no change in HRV
319 parameters that was of sufficient magnitude to explain the increased HRV.

However, since we found significant differences between patients with severe controlled asthma treated with LABAs or LAMAs and patients with uncontrolled asthma, we do not believe that LABAs or LAMAs had a direct effect on HRV results. Given the lack of clarity, nonetheless, further studies are needed of the pharmacological effects of LABAs or LAMAs and their influence on HRV outcomes in asthma.

The main limitations of our study are the small number of subjects and the lack of a control group, both typical features of proof-of-concept studies. Necessary to confirm our results is an extended study that includes more subjects, other physiological factors, and a control group. However, a study strength is that our asthma population is very well characterized, with objective evidence of asthma status (such as bronchodilator reversibility, lung function, inflammation biomarkers, and allergy status).

This study points to the potential role that the PANS may play in asthma control and its relationship with depression-anxiety. No study, as far as we are aware, has focused on that role of the PANS, despite the existence of studies addressing the ANS response to pharmacological intervention and bronchial provocation. In this sense, and if confirmed with other studies, it could underscore the pathophysiological role of the PNS in the control of asthma associated with depression-anxiety, justifying the development of future research to identify new pharmacological therapeutic targets in the parasympathetic system, even for the development of a potential complementary clinical tool, objective and non-invasive in specialist consultations focused on severe asthma, which could contribute to remote or continuous monitoring with wireless devices or mobile

applications, providing a comprehensive approach to the current ones for the evaluation of asthma control and mood disorders.

In conclusion, variables derived from the PANS show depressed HRV in patients with uncontrolled asthma and depression-anxiety, as compared to patients with controlled asthma and without mood disorders. PANS evaluation by analysing non-invasive cardiorespiratory parameters may be a useful means for, and contribute to, follow-up of asthma control and associated depression-anxiety. Further studies using HRV analysis are needed to be able to comprehensively evaluate the PANS in patients with uncontrolled asthma and depression-anxiety.

Table 1. Baseline demographics asthma characteristics.

Variables	All sample n=30	Mild & moderate asthma n=10	Severe asthma n=20	P
Demographic/clinical data				
- Age, mean (SD), y	49.4 (12.8)	48 (10)	49.70 (14)	0.9
- Body Mass Index, mean (SD), kg/m2	27.5 (4)	27.2 (2.7)	30.2 (4.6)	**0.01**
- Female (%)	53.3	40	60	0.4
- Atopy (%)	80	90	75	0.5
- Active smoker (%)	6.7	10	5	0.6
- SABA use/week (%)	60	30	75	**0.002**
- Combined LABA treatment (%)	86.7	60	100	**0.007**
- Combined LAMA treatment (%)	66.7	0	100	**0.000**
- Asthma Control Test, mean (SD)	20.8 (3.9)	23 (1.7)	19.70 (4.2)	**0.000**
- MiniAQLQ, mean (SD)	5.6 (1.3)	6.6 (0.2)	5.1 (1.4)	**0.001**
- HADS, mean (SD)	9.83 (9.2)	7.8 (11.2)	10.8 (8.1)	0.1
Pulmonary function				
- FEV_1, mean (SD), L	2.7 (0.8)	3.2 (0.83)	2.4 (0.8)	**0.001**
- Reference FEV_1 (%)	85.7 (15)	95.6 (9.7)	80.7 (15.4)	**0.001**
- FEV_1/FVC (%)	66.7 (11.6)	74.7 (6.3)	62.7 (11.7)	**0.01**
- FeNO, mean (SD), ppb	45.8 (49.1)	26.3 (13.3)	55.6 (57.4)	0.1
Laboratory				
- Blood eosinophils, mean (SD) mm3	311 (171)	289 (95.9)	232 (200.2)	0.8
- Total immunoglobulin E, mean (SD), UI/ml	309 (419)	283.9 (385.2)	323.2 (446.3)	0.1
Induced sputum (%)				
- Eosinophils	4.9 (7)	3.5 (4.6)	5.7 (7.9)	0.6
- Neutrophils	54.3 (19)	56.7 (21.3)	53.2 (18.3)	0.8
- Macrophages	34.5 (19)	37.5 (19.7)	33 (19.4)	0.5
- Lymphocytes	1.4 (0.8)	1.2 (0.74)	1.5 (0.8)	0.3

Abbreviations: FeNO, fraction of exhaled nitric oxide; $FEV_{1,}$ forced expiratory volume in the first second; FVC, forced vital capacity; HADS, Hospital Anxiety and Depression Scale; LABA, Long-Acting Beta2 Agonist; LAMA, Long-Acting Muscarinic Antagonists; MiniAQLQ, Mini Asthma Quality of Life Questionnaire; SD, Standard Deviation; SABA, Short Acting Beta-2 Agonists.

Table 2. Demographic and clinical characteristics for patients with controlled and uncontrolled asthma.

Variables	**Controlled asthma n=20**	**Uncontrolled asthma n= 10**	**P**
Demographic/clinical data			
- Age, mean (SD), y	49 (12)	49 (13)	0.9
- Body Mass Index, mean (SD), kg/m2	26 (3)	30 (4.6)	**0.02**
- Female (%)	30	70	0.1
- Atopy (%)	85	70	0.8
- Active smoker (%)	5	10	0.3
- SABA use/week (%)	40	100	**0.000**
- Combined LABA treatment (%)	80	100	**0.000**
- Combined LAMA treatment (%)	50	100	**0.000**
- Asthma Control Test, mean (SD)	23 (2.1)	16 (2.8)	**0.001**
- MiniAQLQ, mean (SD)	6.2 (0.8)	4.4 (1.2)	**0.001**
- HADS, mean (SD)	7.5 (9.1)	14.4 (7.8)	**0.04**
Pulmonary function			
- FEV_1, mean (SD), L	3.09 (0.8)	1.9 (0.4)	**0.001**
- Reference FEV_1 (%)	92.2 (8.5)	72.7 (18)	**0.001**
- FEV_1/FVC (%)	69.9 (9.5)	60.3 (13.3)	**0.03**
- FeNO, mean (SD), ppb	34.3 (37)	68.9 (63)	0.06
Laboratory			
- Blood eosinophils, mean (SD) mm3	303 (190)	328 (132)	0.6
- Total immunoglobulin E, mean (SD), UI/ml	219 (298)	510 (582)	0.08
Induced sputum (%)			
- Eosinophils	4.2 (4.7)	6.5 (10.4)	0.4
- Neutrophils	54 (18)	54 (21)	0.9
- Macrophages	37 (17)	28 (21)	0.2
- Lymphocytes	1.3 (0.6)	1.7 (1.06)	0.1

Abbreviations: FeNO, fraction of exhaled nitric oxide; $FEV_{1,}$ forced expiratory volume in the first second; FVC, forced vital capacity; HADS, Hospital Anxiety and Depression Scale; LABA, Long-Acting Beta2 Agonist; LAMA, Long-Acting Muscarinic Antagonists; MiniAQLQ, Mini Asthma Quality of Life Questionnaire; SD, Standard Deviation; SABA, Short Acting Beta-2 Agonists.

Table 3. HRV indices in studied asthmatics with controlled and uncontrolled asthma.

Variables	Controlled asthma n=20	Uncontrolled asthma n= 10	P
HRV parameters (PANS-related)			
- SDNN, mean (SD)	42.7 (29.7)	26.5 (8.2)	**0.03**
- RMSSD, mean (SD)	24 (20)	14.1 (6.5)	**0.05**
- pNN50, mean (SD)	6.2 (11.8)	0.6 (1.5)	**0.05**
HRV frequency domain			
- TP, mean (SD)	0.0014 (0.00085)	0.0005 (0.00046)	**0.02**
HRV respiratory component			
- Pr, mean (SD)	0.0007 (0.00060)	0.0003 (0.00025)	**0.01**
- PLFnr, mean (SD)	0.0001 (0.00014)	0.0001 (0.00022)	0.5

Abbreviations: HRV, heart rate variability; PLFnr, non-respiratory-related HRV power; pNN50, percentage of consecutive normal-to-normal (NN) intervals that differ by more than 50ms; Pr, respiratory-related HRV power; RMSSD, root mean square of the successive differences; SD, Standard Deviation; SDNN, standard deviation of the difference between NN intervals; TP, total power (PLFnr and Pr summed).

Table 4. Clinical characteristics of patients with asthma with and without risk of clinical stress and anxiety.

Variables	**HADS ≤7** **n= 17**	**HADS ≥8** **n= 13**	**P**
Demographic/clinical data			
- Age, mean (SD), y	50 (10)	48 (15)	0.7
- Body Mass Index, mean (SD), kg/m2	25.9 (2.4)	29.6 (4.8)	**0.02**
- Female (%)	23	76	0.1
- Atopy (%)	85	84	0.2
- Active smoker (%)	0	15	**0.001**
- SABA use/week (%)	41.1	84.6	0.1
- Combined LABA treatment (%)	76.5	100	**0.000**
- Combined LAMA treatment (%)	52.9	76.9	0.1
- Asthma Control Test, mean (SD)	23 (2.4)	18 (3.8)	**0.001**
- MiniAQLQ, mean (SD)	6.3 (0.9)	4.8 (1.3)	**0.002**
- HADS, mean (SD)	2.94 (1.9)	18.85 (6.7)	**0.001**
Pulmonary function			
- FEV_1, mean (SD), L	3.09 (0.79)	2.2 (0.79)	**0.001**
- Reference FEV_1 (%)	91.8 (6.5)	77.6 (19.7)	**0.02**
- FEV_1/FVC (%)	71.7 (7)	60 (13.3)	**0.01**
- FeNO, mean (SD), ppb	44 (53.1)	48.3 (45.3)	0.8
Laboratory			
- Blood eosinophils, mean (SD) mm3	292 (184)	336 (155)	0.4
- Total immunoglobulin E, mean (SD), UI/ml	206 (246)	455 (566)	0.1
Induced sputum (%)			
- Eosinophils	5.7 (8)	3.9 (5.5)	0.4
- Neutrophils	56.7 (17.6)	51.3 (21)	0.4
- Macrophages	34.1 (17.6)	35 (21.9)	0.9
- Lymphocytes	1.5 (0.67)	1.3 (0.9)	0.5

Abbreviations: FeNO, fraction of exhaled nitric oxide; $FEV_{1,}$ forced expiratory volume in the first second; FVC, forced vital capacity; HADS, Hospital Anxiety and Depression Scale; LABA, Long-Acting Beta2 Agonist; LAMA, Long-Acting Muscarinic Antagonists; MiniAQLQ, Mini Asthma Quality of Life Questionnaire; SD, Standard Deviation; SABA, Short Acting Beta-2 Agonists.

Table 5. HRV indices in studied asthmatics with and without risk of clinical stress and anxiety.

Variables	**HADS ≤7** **n= 17**	**HADS ≥8** **n= 13**	**P**
HRV parameters (PANS-related)			
- SDNN, mean (SD)	**45.6 (31.3)**	**26.5 (7.9)**	**0.04**
- RMSSD, mean (SD)	**26.4 (20.8)**	**13.4 (6.5)**	**0.03**
- pNN50, mean (SD)	**7.12 (12.6)**	**0.75 (1.4)**	**0.05**
HRV frequency domain			
- TP, mean (SD)	**0.0012 (0.00087)**	**0.0005 (0.00048)**	**0.02**
HRV respiratory component			
- Pr, mean (SD)	**0.0008 (0.00062)**	**0.0003 (0.00027)**	**0.01**
- PLFnr, mean (SD)	**0.0002 (0.00014)**	**0.0001 (0.0019)**	**0.3**

Abbreviations: HRV, heart rate variability; PLFnr, non-respiratory-related HRV power; pNN50, percentage of consecutive normal-to-normal (NN) intervals that differ by more than 50ms; Pr, respiratory-related HRV power; RMSSD, root mean square of the successive differences; SD, Standard Deviation; SDNN, standard deviation of the difference between NN intervals; TP, total power (PLFnr and Pr summed).