Heart rate variability as a marker of autonomic nervous system activity in young people with eosinophilic and non-eosinophilic asthma

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Abstract

Objective: An imbalance in autonomic nervous system (ANS) activity may play a role in asthma, but it is unclear whether this is associated with specific pathophysiology. This study assessed ANS activity by measuring heart rate variability (HRV) in eosinophilic (EA) and non-eosinophilic asthma (NEA) and people without asthma.

Methods: HRV, combined hypertonic saline challenge/sputum induction, exhaled nitric oxide (FeNO), skin prick tests to measure atopy, and spirometry tests were conducted in teenagers and young adults (14-21 years) with (n=96) and without (n=72) generally well-controlled asthma. HRV parameters associated with sympathetic and parasympathetic ANS branches were analysed. EA and NEA were defined using a 2.5% sputum eosinophil cut-point. Airway hyperreactivity (AHR) was defined as ≥15% reduction in FEV_1 following saline challenge.

Results: HRV parameters did not differ between asthmatics and non-asthmatics or EA and NEA. They were also not associated with markers of inflammation, lung function or atopy. However, increased absolute low frequency ($LF\mu s^2$; representing increased sympathetic nervous system (SNS) activity) was found in asthmatics who used β-agonist medication compared to those who did not (median: 1611, IQR 892-3036 vs 754, 565-1592; $p<0.05$) and increased normalised low frequency (LF nu) was found in those with AHR compared to without AHR (64, 48-71 vs 53, 43-66; $p<0.05$).

Conclusion: ANS activity (as measured using HRV analysis) is not associated with pathophysiology or inflammatory phenotype in young asthmatics with generally well-controlled asthma. However, enhanced SNS activity can be detected in asthmatics with AHR or who use β-agonist medication.

Introduction

Asthma is commonly characterised by eosinophilic (1), or neutrophilic airway inflammation (2), but there is increasing evidence that inflammation is not detectable in a large proportion of cases (3). Furthermore, asthma therapies directed towards reducing airway inflammation (such as inhaled corticosteroids (ICS)) are not effective in controlling symptoms in some people (4). This has led to an increased interest in non-inflammatory mechanisms, such as neural pathways in asthma (5).

The autonomic nervous system (ANS) plays a critical role in regulation of airway smooth muscle tone (5), and historically it has been suggested that ANS dysregulation may be important in asthma (6). If true, this may provide an alternative avenue for intervention (6), particularly in asthmatics with little evidence of airway inflammation or for whom current medication is ineffective. Whereas direct assessment of autonomic activity is difficult, it can be assessed indirectly through analysis of heart rate variability (HRV). HRV data is commonly evaluated using frequency domain analyses (7). This assigns the distribution of periodicities in HR fluctuation into frequency bands, including high frequency (HF) and low frequency (LF), considered to reflect parasympathetic nervous system (PNS, principally vagus nerve activity) and sympathetic nervous system (SNS) modulation, respectively. The LF/HF ratio is considered to reflect the balance between the two (8).

To date, relatively few studies have conducted HRV analysis in asthma and the results have been mixed. Increased PNS activity in asthma (i.e. increased HF) was found in some studies (9-11) but not others (12,13). Furthermore, some studies have reported an association between increased PNS activity and poor asthma control (11) and airway hyperresponsiveness (AHR) (14), while increased SNS activity (increased LF) was associated

with improved control (11) and β-agonist use (15). However, most studies have been conducted in adults or people with severe asthma, with few studies in young adults or children with mild-to-moderate asthma. Additionally, previous HRV studies have not considered the heterogeneity underlying different asthma pathologies or inflammatory phenotypes e.g. eosinophilic asthma (EA) and non-eosinophilic asthma (NEA) (16). This may have contributed to some of the mixed results reported previously. In particular, we have previously shown that NEA but not EA exhibit heightened sensory nerve reactivity (17). Other neural pathways might therefore also be important in the pathology of this phenotype.

We hypothesised that an ANS imbalance may be important in NEA, for which there is little evidence of airway inflammation, and the pathophysiological basis is largely unknown (16). The aim of the study therefore was to assess ANS activity in asthma by measuring HRV in EA and NEA in young (14-21 years) people with and without asthma. The reason for choosing this specific age-group was because sputum induction and airway hyperreactivity testing is difficult in young children and previous studies have shown that pubertal status significantly affects heart rate variability measurements (18). We also examined the associations between HRV parameters and clinical and inflammatory characteristics.

Material and methods

Study Population

We recruited 96 asthmatic and 72 non-asthmatic participants aged 14–21 years from Wellington, New Zealand, either from a previous birth cohort study (19) (29 with and 70 without asthma) or through separate community-based recruitment (67 with and 2 without asthma). All participants completed a respiratory symptom questionnaire based on the ISAAC Phase II survey (20). Asthma was defined on the basis of a positive response to: '*have you had wheezing or whistling in the chest in the past 12 months?*', and/or '*have you taken asthma medication in the past 12 months'*. β-agonist use was defined as any short or long acting β-agonist use, either in the last 12 months or the last 7 days; ICS use was defined as any ICS use in the last 12 months. Participants without asthma reported no respiratory symptoms or asthma medication use. Informed consent was obtained from all participants and their parents, and the study was approved by the Northern B Health and Disability Ethics Committee (15/NTB/2).

Clinical assessments

Participants underwent the clinical assessments described below. Asthma control status was based on the asthma control questionnaire (ACQ7), with a value of ≤ 0.75 representing controlled asthma, 0.76–1.49 representing partially-controlled asthma, and ≥ 1.5 representing uncontrolled asthma (21). Participants with symptoms resembling a respiratory infection within 1 month of assessment returned when symptom-free and those with FEV_1%-predicted <75% were excluded. Prior to testing, all asthma medication and antihistamines were withheld for at least 12 and 24 hours, respectively.

HRV measurement

HRV parameters were measured using a computerized ECG data acquisition device with 16 analogue input channels sampled at 1000 Hz (PL3516 PowerLab 16/35, ADInstruments Pty Ltd. New South Wales, Australia). Measurements were conducted with participants seated and motionless; they were asked to breathe naturally and avoid talking during recording. Following a 2-minute stabilisation period, R-R intervals (between two consecutive R waves) were recorded for 10 minutes. Computation of frequency-domain parameters and R–R interval filtering of artefacts/ectopic beats were performed using LabChart Software (v. 8.1.13, ADInstruments Pty Ltd. New South Wales, Australia). Parameters used included total power (TP), HF power (0.15-0.40 Hz), LF power (0.04-0.15 Hz), and LF to HF ratio (LF/HF). The power density of LF and HF parameters was calculated and expressed in absolute (μs^2) and normalised units (nu) to account for total power and very low frequency (VLF) band (0.0033–0.04 Hz) using the following equations: "(LF/TP-VLF) x 100" and "(HF/TP-VLF) x 100", respectively. HF (nu) was not reported as it can be determined from LF (nu) using the equation "(mean (HF nu) =100 – mean (LF nu))" (8).

Atopy

Skin prick tests (SPT) were conducted using a panel of aeroallergens (22): house dust mite, tree mix, grass mix, cat and dog dander, *Alternaria tenuis* and *Penicillium mix* (Stallergenes Greer, Sydney, Australia). Atopy was determined by the presence of at least one weal >3mm.

Exhaled nitric oxide (FeNO) and spirometry

Spirometry and FeNO were measured using an Easyone spirometer (NDD Medizintechnik AG, Zurich, Switzerland) and Hypair FeNO analyser (Medisoft, Sorinnes, Belgium) as described previously (22,23).

Combined hypertonic saline challenge and sputum induction

Combined hypertonic saline challenge/sputum induction was conducted as described previously (24). Aerosolised hypertonic saline (4.5%w/v) was produced using an ultrasonic nebuliser (DeVilbiss Ultraneb 2000, Langen, Germany) and administered orally through a mouthpiece (Hans-Rudolph Inc, Kansas City, USA) for increasing intervals from 0.5-4 minutes to a total of 16 minutes. Spirometry was conducted between intervals, and salbutamol was administered if FEV_1 dropped to ≤75%-predicted. Participants were subsequently encouraged to produce sputum into a sterile plastic container. The resulting cell suspension was used to prepare cytospin slides stained using a Diff-Quik® fixative and stain set (Dade Behring, Deerfield, IL). Using light microscopy, EA was identified as ≥2.5% eosinophils and NEA as <2.5% eosinophils. Airway hyperreactivity (AHR) was defined as a reduction of ≥15% in FEV_1 from baseline (24).

Blood eosinophils

Blood was collected using BD-vacutainers (BD, Auckland, New Zealand) and a complete blood count was obtained.

Statistical analysis

Data analyses were performed using STATA version 11.0 (STATA Corp, College Station, TX, USA) and Prism 5 (Graphpad Software Inc, La Jolla, CA, USA). Data are expressed as mean/standard deviation (SD), median/interquartile ranges (IQR), or frequency (percentage) as appropriate. Mann-Whitney *U* tests or unpaired t-tests were used as appropriate to assess differences between groups. Chi-square tests were used to assess differences between groups for dichotomous data. Comparisons were made between people with and without asthma, and

those with EA and NEA. Absolute and normalised HRV indices were used as the primary outcome variables.

Linear regression analyses (either unadjusted or adjusted for age, sex and ethnicity) were used to assess associations between demographic/clinical factors and normalised LF (nu), LF/HF ratio and LF (μs^2) and HF (μs^2) in asthma. Prior to regression, LF (μs^2) and HF (μs^2) values were log-transformed as data were not normally distributed. Regression outcomes were reported as regression coefficient for (non-log-transformed) LF (nu) and LF/HF ratio data, and a relative difference (i.e. ratios per unit increase for continuous variables; compared to reference for categorical variables) for (log-transformed) LF (μs^2) and HF (μs^2) data. To assess the robustness of our findings, which relied on an asthma definition solely based on symptoms, we conducted sensitivity analyses including only asthmatics who also had AHR. Further stratified analyses were also conducted as appropriate.

Results

Participant characteristics

Three people with and nine without asthma were excluded due to either poor quality or no sputum sample; 93 participants with asthma and 63 without asthma were therefore included in analyses. Participants with asthma were slightly younger than those without asthma but there were no differences in sex, ethnicity, or lung function (Table 1). As expected, atopy and AHR were more prevalent, and sputum eosinophil percentages higher, in asthma. Among those with asthma, 18% were classified as uncontrolled, 30% as partially controlled and 52% as well-controlled.

Inflammatory phenotypes

Forty-four percent (n=41) of participants with asthma were classified as having EA and 56% (n=52) as NEA. Compared to NEA, those with EA were more likely to be atopic, have AHR, and have higher FeNO and ACQ7 scores (Table 1). Neutrophilic or mixed granulocytic asthma (25) were not detected, and sputum neutrophil levels were higher in people without asthma compared to those with asthma.

HRV parameters and inflammation

There were no differences in absolute or normalised HRV parameters between participants with and without asthma, or between those with EA and NEA (Table 2). Results remained similar when analyses were restricted to asthmatics with AHR (supplementary Table 1). There were also no significant associations observed between HRV parameters and inflammatory markers including sputum eosinophils and neutrophils, blood eosinophils, FeNO, or atopy in linear regression analyses, either unadjusted (Supplementary Table 2) or adjusted for age, sex and ethnicity (Table 3).

HRV parameters and clinical characteristics

No associations were observed between lung function parameters and HRV indices, in either unadjusted (Supplementary Table 2) or adjusted (Table 3) regression analyses. However, participants with AHR had higher LF (nu) (median 63.7, IQR 48.4-71.0 vs 53.2, 43.3-65.5; Regression Coefficient (RC) 9.8, 95% CI 3.7-16.0; $p<0.05$)) and LF/HF ratio (1.8, 0.9-2.4 vs 1.1, 0.7-1.9; ratio 0.7, 0.1-1.2; $p<0.05$) compared to those without AHR, and asthmatics who used β-agonists had higher LF (μs^2) (1611.0, 892.0-3036.0 vs 753.7, 565.2-1592.0; ratio=1.9, 95% CI 1.3-2.7; $p<0.05$) compared to those who did not (Table 3). Borderline significant ($p<0.1$) positive associations were found between absolute LF (μs^2) and β-agonist use in the last 7 days (ratio=1.39, 95% CI 0.9-2.0) or ACQ7 score (ratio=1.31, 95% CI 0.8-2.2; Table 3). No association was found with ICS use and as none of the participants used ipratropium bromide (IB), the association between IB use and HRV could not be assessed.

As β-agonist use and AHR were each associated with HRV parameters, we attempted to further clarify the nature of these associations by comparing HRV data in asthmatics with: AHR and β-agonist use (Group A; n=33); AHR and no β-agonist use (Group B; n=7); no AHR and β-agonist use (Group C; n=35); and no AHR and no β-agonist use (Group D; n=18). Group A had higher normalised LF (nu) and LF/HF ratio compared to groups C and D (i.e. those without AHR; Fig 1A and 1B). Groups A and C had higher absolute LF (μs^2) compared to group D (Fig 1C). No differences in absolute HF (μs^2) were observed across groups (Fig 1D). As β-agonist use has been shown to have a short-term effect on HRV parameters (26), we repeated analyses using β-agonist use in the last 7 days; this showed similar results (Supplementary Fig 1).

Discussion

This study found no evidence of an imbalance or difference in ANS activity (as measured by HRV analysis) between people with and without asthma or between EA and NEA. However, increased absolute and normalised LF (representing increased SNS activity) was found in asthmatic participants who used β-agonist medication or had AHR. Differences in autonomic activity may therefore be associated with some clinical characteristics (i.e. β-agonist treatment and AHR) but appear independent of inflammatory pathology or phenotype.

Although increased HF (representing PNS predominance) in asthma has been reported (11), we found no evidence of autonomic imbalance between people with and without asthma, which is consistent with previous findings (13). We also found no significant associations between HRV indices and demographic characteristics that have previously been reported, such as age (27), gender (28), ethnicity (27), or BMI (28); or with baseline FEV_1 (11). It is possible that this is due to differences in the populations studied. In particular, we recruited young participants from the general population with relatively well-controlled asthma, whereas most previous studies have assessed either older (12) or pre-pubertal populations (9) or more severe asthma in a tertiary setting (9-11). Alternatively, mixed findings between studies may be due to methodological differences in HRV measurement (e.g. short-term vs long-term measurements), hampering valid comparisons between studies (8). Finally, as speculated (see introduction), mixed results may be due to the heterogeneity underlying different asthma pathologies or inflammatory phenotypes. However, when inflammatory phenotypes were considered (to our knowledge this is the first study to do so), we found no association; likewise, no associations were found with the inflammatory markers studied. This suggests that mixed results are unlikely to be related to asthma phenotypes. It also suggests that, at least in younger people with well-controlled asthma, there is no evidence that

autonomic regulation, as assessed by using HRV analysis, is associated with airway inflammation. However, other neural pathways (not assessed in this study) may still play a role; indeed, we have previously shown that heightened sensory nerve reactivity may be involved, particularly in NEA (17).

Previous studies have reported that increased HF was associated with poor asthma control (11) or severity (9). In the present study, a borderline significant positive association was found between absolute LF and ACQ7. However, when conducting multivariate regression analyses adjusting for β-agonist medication, which was associated with both ACQ7 and LF (Table 3), the association with ACQ7 disappeared (data not shown), suggesting that the association was confounded by β-agonist use. The observation that β-agonist use was associated with higher absolute LF is consistent with two clinical studies showing a shift towards increased LF (SNS dominance) following β-agonist administration. In particular, Jartti *et al* reported that salbutamol administration within two hours of (29), or two weeks preceding (15) HRV analysis was associated with decreased PNS and increased SNS activity in asthma. Although the underlying mechanism is not entirely clear, it is possible that β-agonists binding β_2-adrenoceptors in cardiac efferent SNS sites or peripheral vasculature may directly stimulate SNS activity (30).

Relatively few studies are available assessing the association between HRV parameters and AHR in asthma, but those that did reported increased PNS activity (14,31). One previous study of 53 people with untreated asthma (14) found that normalised HF was significantly higher in asthmatic subjects with AHR compared to those without, suggesting increased PNS activity. In contrast, our data showed a positive association between AHR and normalised LF and LF/HF ratio, suggesting increased SNS activity. However, most asthmatic participants

with AHR in our study were undergoing β-agonist treatment, and it is possible that the effect of the latter (see above) may have masked any associations with AHR. In an attempt to clarify this, we conducted a stratified analysis grouping on the basis of β-agonist use and/or AHR in asthma. This showed that normalised LF was reduced in those without AHR and absolute LF was reduced in subjects who did not use β-agonists. As absolute and normalised LF showed contrasting findings, we speculate that the association we observed between normalised LF and AHR may possibly be due to the process of data normalisation. Similar discrepancies between normalised and absolute HRV indices in asthma have previously been reported (10).

While the present study did not find evidence of autonomic imbalance in asthma (or between asthma phenotypes), this may be because HRV analysis is not the most appropriate tool for evaluating autonomic airway regulation. Although widely accepted as a surrogate measure of autonomic function (32), HRV is at best a proxy, and does not directly evaluate autonomic respiratory control (8). Furthermore, it is unclear whether some HRV frequency bands are truly representative of distinct ANS components (32). In particular, there has been debate about interpretation of the LF component, which is considered by some as solely a marker of sympathetic control (33), while others have suggested that it is a marker of both sympathetic and parasympathetic control (34). This is in part due to evidence suggesting that absolute LF values are determined by baroreflexes mediated by both PNS and SNS; therefore, LF may effectively reflect both PNS *and* SNS activity (11).

Ultimately, the complexity of the ANS is such that there is currently no single "gold standard" test to accurately assess respiratory autonomic activity. To avoid further ambiguous or equivocal results when attempting to characterise ANS activity in asthma, we suggest that

using a battery of tests, rather than relying on one single test may provide clearer results. An example could be the Ewing test battery; this consists of five tests assessing different aspects of ANS control and is often used in the diagnosis of diabetic neuropathy (35).

This study has some limitations. Firstly, although almost all participants in the asthma group reported a doctor's diagnosis and/or recent symptoms, we did not use objective tests (such as bronchodilator reversibility or AHR) to confirm diagnosis. It is possible that some misclassification may have occurred. However, we consider that any bias introduced as a result will be minimal as this approach, also used in many other studies (36-38), generally compares well with clinical diagnoses (36) and has been shown to be better than some objective measures (37). Secondly, as mentioned above, those with asthma in the present study were young with well-controlled asthma. It is currently unclear how generalisable these findings are to other age groups, or in more severe or uncontrolled asthma. Thirdly, this was a cross-sectional study, and therefore only HRV data representing a single timepoint are available. While studies in coronary artery disease have found that HRV is relatively stable over time (39), it remains unclear if this is the case in asthma, which (as discussed above) is highly variable. Finally, breathing frequency (which has been shown to affect HRV analysis) (40) was not recorded in this study. However, to minimise any potential effect, participants were advised to breathe normally during HRV measurement.

In conclusion, our study suggests that autonomic imbalance (as measured using HRV analysis) is not associated with pathophysiology or inflammation in asthma, or with any inflammatory phenotype, such as NEA, in young people with generally well-controlled asthma. However, altered ANS activity can be detected in asthmatic subjects with AHR or

using β-agonist medication. Further studies using a more comprehensive battery of tests may be required to adequately evaluate autonomic activity in asthma.

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Table 1. Population characteristics

	Non-asthma (N=63)	Asthma (N=93)	Eosinophilic asthma (N=41)	Non-eosinophilic asthma (N=52)
Age	20.2 (1.1)	18.1 (2.0) **	17.9 (2.0)	18.4 (2.0)
Males- n (%)	23 (37%)	43 (46.2%)	21 (51.0 %)	22 (42.0 %)
Height (cm)	170.1 (9.2)	169.0 (9.0)	169.0 (9.0)	169.0 (9.0)
Weight (Kg)	66.3 (14.8)	66.6 (16.2)	64.9 (16.3)	68.1 (16.1)
Ethnicity				
European- n (%)	57 (90.5%)	70 (75.3%)	31 (75.6%)	39 (75.0%)
Non-European- n (%)	6 (9.5 %)	23 (24.7%)	10 (24.4%)	13 (25.0%)
Airway hyperreactivity[b]- n (%)	3 (4.7 %)	40 (43.0 %) **	22 (54.0 %) ††	18 (35.0 %)
FEV_1% predicted	101.27 (12.0)	99.5 (14.4)	97.3 (14.3)	101.2 (14.4)
FVC% predicted	100.4 (9.9)	102.3 (12.9)	101.1 (13.0)	103.2 (13.0)
FEV_1/FVC% predicted	100.8 (7.8)	97.1 (8.0)	95.9 (7.4)	98.0 (8.1)
ACQ7 score		1.0 (0 .68)	1.3 (0.7) ††	0.8 (0 .6)
FeNO (ppb)	28.2 (19.4)	63.0 (69.3) **	92.3 (76.4) ††	39.9 (53.4)
Atopy[a]- n (%)	24 (38.1%)	77 (83.0%) **	37 (90.2%)	40 (77.0%)
β-agonist use last 12 months n (%)		68 (73.1%)	36 (88.0%) ††	32 (61.5%)
β-agonist use last 7 days n (%)		49 (53.0%)	28 (68.3%)	21 (40.4%)
ICS use n (%)		44 (47.0%)	22 (53.6%)	22 (42.3%)
Sputum eosinophils %	0.6 (2.7)	6.5 (10.7) **	14.0 (12.6) ††	0.5 (0.7)
Sputum neutrophils %	23.2 (18.0)	15.9 (15.4) **	14.0 (13.0)	17.6 (17.2)
Blood eosinophils (mm^3)	100 (100-200)	400 (200-600) **	500 (400-800) ††	250 (100-500)

T-test or Mann-Whitney test and Chi-square tests were used. Data are presented as mean (SD), or number (percentages) as appropriate. * P<0.05; ** P<0.01 asthmatics versus the reference population, † P<0.05; †† P<0.01 non-eosinophilic versus eosinophilic asthmatics.

[a] Positive SPT against one or more common allergens.

[b] ≥15% drop in FEV1 from baseline following hypertonic saline challenge.

SPT, skin prick test, FENO, Fractional exhaled nitric oxide, ICS, inhaled corticosteroid includes monotherapy and combination therapy in the last 12 months.

Eosinophilic asthma defined as ≥2.5% sputum eosinophils.

Table 2. HRV parameters

	Non-asthma (N=63)	**Asthma (N=93)**	**Eosinophilic asthma (N=41)**	**Non-eosinophilic asthma (N=52)**
Total power (TP)	4554 (2845-8455)	4635 (2337-8380)	4635 (2242-10100)	4558 (2490.5-7053.5)
Low Frequency (μs^2)	1389 (877.8-2347)	1364 (729.7-2723)	1386 (738.2-3322)	1324.5 (690.3-2495.5)
High Frequency (μs^2)	1008 (468.8-2704)	1097 (509.2-2242)	1067 (408.8-2922)	1142 (527.1-1837)
LF (nu)	60.28 % (44.01-67.90)	54.10 % (43.70-66.86)	58.40 % (47.80-68.80)	53.30 (43.60-64.10)
LF/HF % ratio	1.51 (0.78-2.12)	1.20 (0.77-2.02)	1.41 (0.91-2.21)	1.14 (0.77-1.80)

T-test or Mann-Whitney test were used. Data are presented as median (IQR), or percentages, as appropriate.

Table 3. Association of HRV parameters with clinical characteristics in asthmatics (adjusted for age, sex and ethnicity).

	LF (nu)	LF/HF ratio	Log LF (μs^2)	Log HF (μs^2)
	Regression coefficient [95% CI]		Relative difference, or ratio [95% CI]	
Atopy (n=77, yes vs no)	0.913 [-8.014,9.836]	-0.120 [-0.898,0.657]	0.835 [0.507,1.378]	0.815 [0.418,1.59]
AHR (n=40, yes vs no)	9.849 [3.688,16.010] **	0.660 [0.110,1.209] *	1.211 [0.842,1.741]	0.79 [0.485,1.286]
β-agonist use[a] (n=68, yes vs no)	1.552 [-5.712,8.815]	0.266 [-0.365,0.897]	1.863 [1.264,2.734] **	1.714 [1.005,2.922]
β-agonist use[b] (n=49, yes vs no)	1.031 [-5.475,7.538]	0.120 [-0.446,0.687]	1.398 [0.976,2.001] †	1.331 [0.820,2.160]
ICS use (n=44, yes vs no)	1.276 [6.869,9.420]	0.039 [-0.670,0.750]	0.997 [0.632,1.572]	0.935 [0.508,1.719]
FeNO (ppb)	0.017 [-0.029,0.063]	-0.000 [-0.004,0.004]	1.00 [0.9975,1.003]	1.00 [0.997,1.003]
FEV_1% pred	0.088 [-0.148,0.324]	0.008 [-0.012,0.029]	0.997 [0.984,1.010]	0.993 [0.98,1.006]
FVC% pred	0.013 [-0.254,0.279]	0.002 [-0.021,0.025]	1.000 [0.985,1.015]	0.999 [0.981,1.018]
Sputum eosinophils %	-0.014 [-0.319,0.290]	-0.004 [-0.031,0.023]	1.011 [0.992,1.029]	1.012 [0.989,1.035]
Blood eosinophils %	-2.704 [-13.134,7.724]	-0.314 [-1.20,0.572]	1.194 [0.626,2.277]	1.361 [0.604,3.069]
ACQ7	4.147 [-1.499,9.792]	0.145 [-0.296,0.586]	1.305 [0.986,1.727] †	1.159 [0.793,1.693]

Data presented as regression coefficient and 95% confidence limit for LF (nu) and LF/HF ratio and as ratios (per unit increase in case of continuous variables and compared to the reference category in case of categorical variables (yes/no)) for log transformed LF (μs^2) and HF (μs^2). † P<0.1, * P<0.05; ** P<0.01

[a] β-agonist use in the last 12 months

[b] β-agonist use in the last 7 days

Figure legends

Figure 1. Normalised LF (nu) (A), LF/HF ratio (B), Absolute LF (μs^2) (A) and absolute HF (μs^2) (B) in asthmatics in (Group A) AHR and β-agonist use in the last 12 months; (Group B) AHR and no β-agonist use in the last 12 months; (Group C) no AHR and β-agonist use in the last 12 months; and (Group D) no AHR and no β-agonist use in the last 12 months. Solid line represents median. Mann-Whitney test was used. * $p<0.05$