Enhanced sensory nerve reactivity in non-eosinophilic asthma

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ABSTRACT

Background: Neural mechanisms may play an important role in non-eosinophilic asthma. This study compared airway sensory nerve reactivity, using capsaicin challenge, in eosinophilic and non-eosinophilic asthma and non-asthmatics.

Methods: Thirty-eight asthmatics and nineteen non-asthmatics (aged 14-21 years) underwent combined hypertonic saline challenge/sputum induction, exhaled nitric oxide (FeNO), atopy, and spirometry tests, followed by capsaicin challenge. Eosinophilic (EA) and non-eosinophilic asthma (NEA) were defined using a sputum eosinophil cut-point of 2.5%. Airway hyperreactivity (AHR) was defined as a ≥15% drop in FEV_1 during saline challenge. Sensory nerve reactivity was defined as the lowest capsaicin concentration that evoked 5 (C5) coughs.

Results: Non-eosinophilic asthmatics (n=20) had heightened capsaicin sensitivity (lower C5) compared to non-asthmatics (n=19) (geometric mean C5: 58.3μM, 95% confidence interval 24.1-141.5 vs 193.6μM, 82.2-456.0; $p<0.05$). There was a similar (but non-significant) difference in capsaicin sensitivity in NEA compared with EA (n=18), (58.3μM, 24.1-141.5 vs 191.0μM, 70.9-514.0; $p=0.07$). FEV_1 was significantly reduced from baseline following capsaicin inhalation in both asthmatics and non-asthmatics but no differences were found between subgroups. No associations with capsaicin sensitivity and atopy, sputum eosinophils, blood eosinophils, asthma control, or treatment were observed.

Conclusion: Non-eosinophilic asthma, but not eosinophilic asthma, showed enhanced capsaicin sensitivity compared with non-asthmatics. Sensory nerve reactivity may therefore play an important role in the pathophysiology of non-eosinophilic asthma.

1 INTRODUCTION

Asthma is generally regarded as a TH2-mediated disease associated with allergic airway inflammation.[1] However, some studies have suggested that <50% of asthma cases are attributable to airway eosinophilia,[2] and that approximately 50% have no overt signs of either eosinophilic or neutrophilic inflammation.[3] In the absence of inflammation, the mechanisms underlying non-eosinophilic asthma (NEA) remain poorly understood but it is plausible that neural pathways may be involved.[4]

Historically, asthma was considered a neurological disorder,[5] and although this view fell out of favour in the 1950s, there is increasing contemporary literature supporting a role for neural involvement. There are reports of altered autonomic regulation, with changes in vagal tone and reduced sympathetic tone observed in stable asthma[4] and evidence of associations between vagal tone and both asthma severity[6] and airway hyperreactivity.[4] Other studies have shown that airway sensory nerves are activated during asthma exacerbations, with associated increases in airway neurokinin A and substance P,[7] suggesting that airway sensory nerve activation may play a key role in asthma pathogenesis.[8]

To date, evidence of altered airway sensory nerve reactivity in asthma, often measured using capsaicin challenge to induce cough by specifically targeting the transient receptor potential (TRP) vanilloid 1 (TRPV-1) channel on sensory C-fibres, is equivocal.[8] In one study, an increase in capsaicin sensitivity was observed amongst adult asthmatics,[9] whilst two further studies found no difference between asthmatics and non-asthmatics.[10, 11] Other studies measuring associations between capsaicin response and atopy, inflammatory mediators, fractional exhaled nitric oxide (FeNO),[12] or blood eosinophil percentages in asthma,[13, 14] have also shown conflicting results. However, no studies have examined capsaicin responses across inflammatory asthma phenotypes assessed using induced sputum, which is considered representative of "actual" airway pathophysiology.[13]

We hypothesise that these inconsistencies may be due to inflammatory asthma phenotypes expressing differential sensory nerve reactivity. This study compared sensory nerve reactivity (using capsaicin challenge) between young (14-21 years) asthmatics and non-asthmatics and across different asthma inflammatory phenotypes. We also examined the associations between sensory nerve reactivity and clinical, demographic, and inflammatory characteristics.

2 METHODS

2.1 Study population

Adolescents and young adults (14-21 years) were recruited from Wellington, New Zealand; either from a birth cohort study[15] or through separate community-based recruitment for a larger ongoing study. All participants completed a respiratory symptom questionnaire based on the ISAAC Phase II survey.[16] Asthma was defined as wheezing/whistling in the chest and/ or asthma medication use in the last 12 months. Non−asthmatics reported no asthma symptoms, no other respiratory conditions or asthma medication use. Informed consent was obtained from participants/parents, and the study was approved by the Northern B Health and Disability Ethics Committee (15/NTB/2).

2.2 Clinical assessments and participant selection

Participants took part in a maximum of three clinical assessments (the first involving all tests described below except capsaicin challenge). To confirm inflammatory phenotype stability, asthmatics underwent an additional hypertonic saline challenge/sputum induction at a second assessment 3-6 months later. Capsaicin challenge was conducted at a final assessment (2nd visit for non-asthmatics, 3rd for asthmatics) for a proportion of non-smoking participants identified as either eosinophilic asthma (EA), NEA, or non-asthmatics during previous visits, with recruitment being random within each subgroup. Asthma control was assessed using the Asthma Control Questionnaire (ACQ7).[17] 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.

2.3 Spirometry

Spirometry was conducted using an Easyone spirometer (NDD Medizintechnik AG, Zurich, Switzerland) as described previously.[3]

2.4 Exhaled nitric oxide (FeNO)

FeNO was measured using a Hypair FeNO analyser (Medisoft, Sorinnes, Belgium) as described previously.[18]

2.5 Atopy

Skin prick tests (SPT) were conducted using a panel of aeroallergens as described previously: house dust mite, tree mix, grass mix, cat and dog dander, *Alternaria tenuis* and *Penicillium* mix (Stallergenes Greer, Sydney, Australia). Atopy was determined by presence of at least one weal >3mm. Histamine (1%) and saline were used as positive and negative controls, respectively.[3]

2.6 Blood eosinophils

Blood was collected using BD-vacutainers (BD, Auckland, New Zealand) for a complete blood count. A high blood eosinophil count (blood EOS-high) was defined as ≥250 eosinophils/mm3.[19]

2.7 Combined hypertonic saline challenge and sputum induction

Hypertonic saline challenge/sputum induction was conducted as described previously.[20] 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, which was processed according to a well−characterised protocol, and the resulting cell suspension used to prepare cytospin slides stained using a Diff-Quik® fixative/stain set (Dade Behring, Deerfield, IL). Using light microscopy, EA was identified as ≥2.5% eosinophils at any visit and NEA as <2.5% eosinophils at both visits. Airway hyperreactivity (AHR) was defined as a ≥15% drop in FEV_1 from baseline.[20]

2.8 Capsaicin challenge

Capsaicin challenge was conducted as described previously[21] with minor modifications. Capsaicin (Sigma-Aldrich, Castle Hill, Australia) was solubilised in ethanol/Tween 80.[21] Participants inhaled single breaths of aerosolised capsaicin solution in doubling concentrations (0.98 to 500μM) from a jet nebuliser (model 646, DeVilbiss, Langen, Germany) controlled by a KoKo dosimeter (nSpire Health Inc, Louisville, CO, USA). One-minute intervals were maintained between different concentrations. The lowest concentration eliciting 2 (C2) and 5 (C5) coughs during a 30-second interval between each concentration was manually recorded by a nurse. The procedure was terminated if/when the C5 threshold

was reached. If C2 or C5 was not reached, a value of 1000μM was assigned for analysis. Lung function was measured before and after capsaicin challenge.

2.9 Statistical analysis

Statistical analyses were performed using STATA version 11.0 (STATA Corp, College Station, TX, USA) and GraphPad Prism 7.0 (Graphpad Software Inc, La Jolla, CA, USA). C2 and C5 values were expressed as geometric means (GM) with 95% confidence interval (CI), and C5 used as the primary outcome.[21] Mann-Whitney *U* tests and unpaired t-tests were used as appropriate to assess differences between groups. Chi-square tests were used to analyse dichotomous data. Comparisons were made between asthmatics and non-asthmatics, and EA and NEA. To examine associations with demographic/clinical factors, linear regression analyses were conducted using log-transformed C5. As a result, regression coefficients were exponentiated and presented as relative difference i.e. ratio (per unit increase for continuous variables and compared to the reference category for categorical variables). If significant associations were found, sensitivity analyses (excluding subgroups with or without these factors) were conducted to assess robustness of findings.

3 RESULTS

3.1 Population characteristics

Thirty-nine asthmatics and 21 non-asthmatics were recruited, either from the previous birth cohort study[15] (12 asthmatics and 20 non-asthmatics), or through community-based recruitment (27 asthmatics and 1 non-asthmatic). One asthmatic and two non-asthmatics were excluded due their FEV_1 being ≤75%, leaving 38 asthmatics and 19 non-asthmatics. Asthmatics were slightly younger than non-asthmatics, but no differences in sex, ethnicity, or FeNO were observed (Table 1). Prevalence of atopy, AHR, and sputum eosinophil percentages were higher in asthmatics. Of the asthmatics, 21.1% were untreated, 18.4% used short-acting β-agonists only, 15.7% used inhaled corticosteroids (ICS) only, and 44.8% used a combination of both in the last 12 months. Eighteen percent were classified as uncontrolled, 26.3% as partly controlled and 55.4% as well-controlled.

3.2 Inflammatory phenotypes

Fifty−three percent (n=20) of asthmatics were NEA at both visits 1 and 2, with the remaining 47% (n=18) EA. Two participants changed from EA at visit 1 to NEA at visit 2 with no asthma medication change (data not shown). EA were more likely to be atopic, and have AHR, higher FeNO and more poorly controlled asthma than NEA (Table 1). There were no significant differences in cough symptoms between the groups (Table 1). Neutrophilic asthma or mixed granulocytic asthma as described previously[22] were not detected, and sputum neutrophil levels were not significantly different between asthmatics and non-asthmatics, or EA and NEA (Table 1).

3.3 Capsaicin response and inflammatory phenotypes

Capsaicin response did not differ between asthmatics and non-asthmatics (Fig 1A and Fig 1B). However, NEA had significantly greater capsaicin sensitivity (i.e. reached C5 at a lower dose) than non-asthmatics (GM 58.3μM, 95% CI 24.1-141.5 vs 193.6μM, 82.2-456.0; Fig 1B). There were similar (but non-significant) differences between NEA and EA (58.3μM, 24.1-141.5 vs 191.0μM, 70.9-514.0; p=0.07). Results for C2 showed no differences between groups (Fig 1A). When subjects were stratified by atopy (Supplementary Fig 1A and B) or blood eosinophils (Supplementary Fig 2A and B) rather than EA/NEA, we found no significant differences in C2 or C5 between groups.

3.4 Capsaicin response and demographic/clinical characteristics

In asthmatics and non-asthmatics, no associations were found between capsaicin sensitivity and demographic parameters, asthma control, lung function, inflammatory markers, treatment or AHR (Table 2). In EA, capsaicin sensitivity was significantly lower for Europeans (n=14) compared to non-Europeans (n=4) i.e. they required a capsaicin concentration 10.23 times higher to elicit 5 coughs compared to non-Europeans (Table 2). Capsaicin sensitivity was also significantly lower for those with (n=11) compared to without AHR (n=7; ratio=7.94, $p<0.05$). In NEA, C5 was inversely associated with FeNO (ratio=0.9 per unit increase, $p<0.05$) and positively associated with FEV_1/FVC% predicted (ratio=1.12 per unit increase, $p<0.05$; Table 2).

3.5 Sensitivity analyses

Post-hoc sensitivity analyses were conducted for demographic and clinical characteristics that were independently associated with capsaicin response. Limiting analysis to only asthmatics with AHR, we found that capsaicin sensitivity was significantly greater in NEA (15.6μM, 2.6-95.0) compared with non-asthmatics (193.6μM, 82.2-456.2) and EA (441.0μM, 127.0-1533.0; Fig 2A). Excluding all non-European New Zealanders (n=9) showed significantly increased capsaicin sensitivity in NEA (50.3μM, 18.0-139.0) compared with non-asthmatics (206μM, 84-507), and EA (320μM, 104-989; Fig 2B). Similar sensitivity analyses including only participants with FEV_1% predicted <95%, or excluding participants with high FeNO in NEA, showed similar results between NEA and non-asthma (supplementary Fig 3A and B). To exclude the possibility that NEA was in fact EA with suppressed eosinophilia due to ICS,[23] we conducted an analysis excluding all NEA who received ICS in the last 14 days (n=4). This did not have an appreciable effect on the main findings (although results were no longer statistically significant; Supplementary Fig 3C). Finally, as females have previously been shown to have enhanced capsaicin sensitivity,[24] we also conducted a sensitivity analysis excluding males (n=20). This analysis showed significantly increased sensitivity in NEA (41.7 μM, 13.2-131.7) compared with EA (222.7μM, 81.3-610.5); a borderline statistically significant difference was also found comparing NEA with non-asthmatics (146.6μM, 57.3-375.2; supplementary Fig 4A and B).

3.6 Capsaicin challenge and spirometry

FEV_1%-predicted and FVC%-predicted were significantly reduced following capsaicin challenge in both asthmatics and non-asthmatics. However, this was not different between subgroups, including EA and NEA (Table 3).

4 DISCUSSION

This study found enhanced airway sensory nerve reactivity in NEA compared with non-asthmatics. In contrast, no difference in capsaicin response between EA and non-asthmatics was found. This suggests that sensory nerve reactivity may play an important role in the pathophysiology of NEA, but not EA.

Although our findings are consistent with some previous reports showing no difference in capsaicin response between asthmatics and non-asthmatics,[10, 11] other studies found a heightened capsaicin response in asthma.[9, 24] These inconsistencies may be due to demographic and methodical differences, or alternatively, as suggested here, airway sensory nerve reactivity may be specific to inflammatory phenotypes, with differences masked for comparisons with general asthma.

To our knowledge, a direct relationship between sensory nerve reactivity and NEA has not previously been shown. However, some recent studies have suggested an association with non-atopic asthma, which, like NEA, may be driven by non-TH2 mechanisms.[2, 24] For example, one study reported that capsaicin-induced cough was more pronounced in non-atopic asthmatics compared to atopic asthmatics or non-asthmatics.[24] Other studies suggest that heightened capsaicin sensitivity is associated with poor asthma control and frequent hospitalisation in non-atopic asthmatics.[25] However, data are equivocal and studies in healthy non-asthmatics have found no association with atopy.[21] This suggests that atopy does not reliably predict capsaicin response. In agreement, we observed no differences between non-atopic and atopic asthmatics, or between atopic or non-atopic individuals in general. However, our study was not specifically powered to examine capsaicin response in non-atopics, who made up a small proportion (16%) of asthmatics, as is typical in New Zealand.[3]

Few studies have assessed associations between airway inflammation and sensory nerve reactivity, and yielded inconsistent results, possibly due to asthmatic airway inflammation heterogeneity.[2, 22] Three studies showed no association between capsaicin response and sputum eosinophilia;[26-28] however, in these studies capsaicin response was assessed in allergic asthmatics or following allergen challenge, which likely excluded individuals with TH2-low inflammation and/or NEA. Other studies used FeNO[12, 14] or blood eosinophils[24, 25] as indicators of TH2-mediated airway inflammation, and again, results varied.[14, 29] In the present study, we used multiple TH2-indicators; both systemic (atopy, blood eosinophils) and

airway-specific (FeNO, sputum eosinophils), but an increased capsaicin response was observed only in NEA. The reasons are unclear, but it is possible that, whilst all are TH2 markers, they may not be specific enough to accurately identify airway inflammatory patterns, and in particular, NEA (e.g. in our study 75% of NEA were atopic).

The causes of enhanced sensory nerve reactivity in NEA are unknown. However, viruses and irritants, previously identified as potential triggers of asthma,[8] and NEA in particular,[2] may play a role. These may result in sensory nerve TRPV1 and TRP subfamily A (TRPA) ion channel activation or increased ion channel expression, leading to increased cough response, even in the absence of inflammation.[8] Similar hyperresponsive capsaicin-sensitive phenotypic changes have been reported in sensory nerves in vasomotor rhinitis, despite no evidence of nasal mucosal inflammation.[30] Alternatively, increased capsaicin response may be due to alterations in the afferent sensory pathways or neuronal networks regulating airway responses upstream of initial TRPV1 activation.[8]

Although we found no statistically significant associations with characteristics previously associated with capsaicin sensitivity such as age,[28] gender,[24] asthma control,[25] or treatment,[9] we observed an association with ethnicity in EA. There are few studies examining associations between either sensory nerve reactivity or inflammatory phenotypes and ethnicity, and of the former, no association has been found.[31] However, it has been suggested that different environmental exposures or variations in innervation density between ethnic groups may impact sensory response to capsaicin, albeit in a dermal rather than respiratory setting.[32] Alternatively, as our finding was based on small numbers of non-Europeans (n=4 in the EA groups), it may be due to chance. Whilst no association was observed between capsaicin sensitivity and gender, we found that limiting analyses to females only (previously shown to have enhanced capsaicin sensitivity compared to males)[24] led to a statistically significant (or borderline significant) difference in C5 between EA, NEA and non-asthmatics. The reasons for this are not clear.

Consistent with other studies,[9, 25] baseline lung function was not associated with capsaicin response. However, following capsaicin challenge, FEV_1%-predicted and FVC%-predicted were slightly decreased across all groups with no differences between subgroups. This is in agreement with previous studies showing that capsaicin does not cause clinically significant bronchoconstriction in asthmatics.[24] Our results suggest that whilst capsaicin challenge

produces an increased tussive response in NEA, it is not associated with clinically significant AHR in this (or any other) group.

The observation that increased sensory nerve reactivity is associated with NEA or non-inflammatory asthma may have significant implications. As we have reported previously, NEA in adolescents/young adults makes up >50% of asthma,[3] and NEA is less responsive to ICS,[23] the mainstay drug in asthma management. There is therefore a substantial and unmet need in the therapeutic management of this group. If sensory nerve reactivity plays a major role in the pathology underlying NEA and is therefore a potential treatable trait,[33] then accurately identifying individuals with increased airway sensory reactivity, and developing appropriate therapeutic approaches specifically targeting this, will be of considerable importance. Of particular interest, recent reports suggest that anticholinergics which are effective in some but not all asthma[34] (but are not widely used) may markedly reduce airway reactivity to a variety of stimuli including capsaicin.[4] Tiotropium bromide in particular reduces both cough and cough-reflex sensitivity in patients with asthma refractory to ICS/LABA.[35] Alternatively, P2X3 antagonists, which have previously shown promise in the treatment of refractory chronic cough may be of potential benefit.[36] However, it is currently unclear whether these will be effective in NEA, which in this study was not associated with self-reported cough symptoms. In addition to results being relevant to treatment, our findings also suggest that capsaicin challenge, in conjunction with other methods such as sputum induction, AHR, FeNO, and atopy testing, may be a useful tool to differentiate between asthma phenotypes. Finally, it may provide important clues regarding the causal (non-allergenic) exposures, for which our knowledge is currently limited.

This study has limitations. Firstly, the number of participants, particularly when stratified by phenotype, were relatively small (this may explain why there was a significant difference between NEA and non-asthmatics, and a very similar difference between NEA and EA that did not reach statistical significance, involving slightly smaller numbers). Secondly, asthmatics were generally well-controlled and identified using an epidemiological rather than clinical definition, which although previously shown to correlate strongly with clinical diagnosis,[37] may not be representative of all specific subgroups (e.g. severe asthma). Despite this, in sensitivity analyses including only asthmatics with objective markers that have previously been used to identify asthma such as AHR,[20] the main study findings remained unchanged. Thirdly, due to the cross-sectional nature of the study, capsaicin challenge was

not repeated and reproducibility of capsaicin response in inflammatory phenotypes remains unstudied. However, a high degree of reproducibility of capsaicin response in asthmatics has been documented previously.[38] Fourthly, there is a possibility that at least some of the NEA cases may be EA in which airway eosinophilia has been suppressed by ICS treatment.[23] However, *post hoc* analysis, excluding the 4 NEA participants who used ICS in the last 14 days, did not have an appreciable effect on the main findings (although results were no longer statistically significant). Finally, although often used, it has been suggested that the C2 and C5 end-point is insufficiently sensitive, and that a non-linear fix-modelling procedure may be more appropriate.[24] However, in the current study, capsaicin challenge was terminated when the C5 threshold was reached (to avoid further discomfort to participants). Hence, non-linear fix-modelling was not feasible.

In conclusion, our study suggest that sensory nerve reactivity may play an important role in the pathophysiology of NEA. Furthermore, it suggests that sensory nerve reactivity may represent a novel therapeutic target in NEA for whom current asthma medications are less effective.

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Table 1. Population characteristics

	Non-asthma (N=19)	Asthma (N=38)	Eosinophilic asthma (N=18)	Non-eosinophilic asthma (N=20)
Age	21.0 (2.0)	19.0 (2.0) **	18.3 (2.0)	19.3 (2.0)
Males- n (%)	6 (32.0 %)	14 (37.0 %)	6 (33.0 %)	8 (40.0 %)
Height (cm)	170.0 (8.3)	167.4 (9.0)	165.0 (8.1)	168.7 (9.5)
Weight (Kg)	67.0 (12.6)	67.4 (15.4)	62.2 (12.1)	72.2 (17.0)
Ethnicity				
European-NZ (%)	18 (94.7 %)	30 (78.9 %)	14 (77.8 %)	16 (80.0 %)
Non-European-NZ (%)	1 (5.3 %)	8 (21.1 %)	4 (22.2 %)	4 (20 %)
Passive smoking[a]	2 (10.5 %)	3 (8.0 %)	1 (6.0%)	2 (10%)
Asthma medication[a]				
No asthma medication- n (%)		8 (21.1%)	3 (17.0 %)	5 (25.0 %)
ICS alone- n (%)		6 (15.7%)	4 (22.2 %)	2 (10.0 %)
β-agonist alone- n (%)		7 (18.4%)	2 (11.1 %)	5 (25.0 %)
ICS & β-agonist - n (%)		17 (44.8%)	9 (50.0 %)	8 (40.0 %)
Sleep disturbance due to cough[a]	0 (0.0 %)	14 (36.8 %) **	7 (39.0 %)	7 (35 %)
Dry cough at night[b]	0 (0.0 %)	13 (34.0 %) **	7 (39.0 %)	6 (30.0 %)
ACQ7 score		0.8 (0.3-1.3)	1.4 (0.7-1.7) ††	0.6 (0.2-0.9)
FeNO (ppb)	41.5 (38.1)	66.6 (76.1)	82.3 (75.2) †	53.0 (76.0)
Atopy[c]- n (%)	10 (53 %)	32 (84.2 %) *	17 (94.4 %)	15 (75.0 %)
Airway hyperreactivity[d]- n (%)	0 (0.0 %)	15 (39.5 %) **	11 (61.1 %) ††	4 (20.0 %)
Sputum eosinophils %	0.0 (0.0-0.3)	2.2 (0.0-10.7) **	12.0 (9.0-40) ††	0.0 (0.0-0.8)
Sputum neutrophils %	13.0 (7.0-33.0)	8.3 (4.3-24.0)	7.8 (5.0-24.0)	8.5 (4.1-24.4)
Blood eosinophils (mm^3)	200 (100-300)	500 (200-800) **	600 (500-900) ††	200 (100-400)

Means (standard deviation), median (interquartile range) or frequency (%), Mann-Whitney test and Chi-square tests were used 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] In the past 12 months

[b] In the past 12 months without cold or respiratory infection

[c] Positive SPT against one or more common allergens

[d] ≥15% drop in FEV_1 from baseline following hypertonic saline challenge

SPT, skin prick test; FeNO, fractional exhaled nitric oxide

Eosinophilic asthma defined as ≥2.5% sputum eosinophils

Table 2. Associations between demographic and clinical characteristics and capsaicin response (C5)

	C5 response[#]			
	Non-asthma (N=19)	**Asthma (N=38)**	**Eosinophilic asthma (N=18)**	**Non-eosinophilic asthma (N=20)**
	Ratio (95%CI)	Ratio (95%CI)	Ratio (95%CI)	Ratio (95%CI)
Continuous variables				
Age (years)	1.10 [0.70,1.72]	0.89 [0.70,1.09]	0.79 [0.51,1.25]	1.26 [0.80,1.98]
FEV_1% predicted	0.99 [0.91,1.09]	1.02 [0.98,1.07]	1.07 [0.98,1.17]	1.02 [0.94,1.12]
FVC% predicted	0.96 [0.83,1.09]	1.00 [0.91,1.09]	1.05 [0.96,1.15]	0.96 [0.87,1.05]
FEV_1/FVC% predicted	1.10 [0.92,1.31]	1.07 [0.98,1.17]	1.07 [0.94,1.23]	1.12* [1.03,1.23]
FeNO (ppb)	0.99 [0.97,1.01]	1.00 [0.99,1.01]	1.01 [1.00,1.02]	0.99* [0.98,1.00]
Sputum eosinophil %	0.91 [0.76,1.09]	1.02 [0.98,1.07]	0.99 [0.95,1.04]	0.63 [0.26,1.56]
Sputum neutrophil %	1.00 [0.94-1.10]	0.96 [0.93,1.00]	0.96 [0.91,1.01]	0.98 [0.93,1.02]
Blood eosinophil/mm^3	0.99 [0.99,1.00]	1.01 [0.99,1.00]	1.00 [0.99,1.01]	0.99 [0.99,1.00]
ACQ7 score	-	1.74 [0.71,2.13]	1.59 [0.41,6.14]	0.40 [0.04,3.8]
Dichotomous variables				
Female (vs male)	0.81 [0.13,4.94]	1.59 [0.41,6.14]	3.98 [0.66,24.21]	0.63 [0.10, 3.84]
Ethnicity (Eur vs non-Eur)	3.23 [0.53,19.68]	2.04 [0.53,7.91]	10.23* [1.68,62.23]	0.48 [0.12,1.85]
Dry cough at night (yes vs no)	-	0.32 [0.08,1.22]	0.14 [0.02,0.91]	0.9 [0.14,5.81]
Sleep disturbance due to cough (yes vs no)	-	1.27 [0.32,4.98]	1.70 [0.19,14.53]	0.80 [0.15,4.26]
AHR (yes vs no)	-	2.51 [0.65,9.73]	7.94* [1.31,48.31]	0.20 [0.03,1.21]
Atopy (yes vs no)	1.26 [0.21,7.66]	0.63 [0.10,3.84]	0.40 [0.01,23.12]	0.40 [0.07,2.42]
Treated (yes vs no)	-	0.33 [0.07, 1.72]	0.23 [0.01, 4.21]	0.25 [0.04, 1.60]
ICS use (yes vs no)	-	0.81 [0.23, 2.88]	1.14 [0.17, 7.64]	0.41 [0.08, 2.21]
β-agonist use (yes vs no)	-	1.02 [0.28, 3.80]	1.14 [0.17, 7.64]	1.02 [0.20,5.70]

[#] As analyses were conducted on-log transformed C5 values, regression coefficients are shown as ratios (per unit increase in case of continuous variables and compared to the reference category in case of categorical variables (yes/no)). * $p<0.05$

Table 3. Changes in lung function following capsaicin challenge

	Non-asthma (N=19)	Asthma (N=38)	Eosinophilic asthma (N=18)	Non-eosinophilic asthma (N=20)
FEV_1 % predicted	**Mean (SD)**	**Mean (SD)**	**Mean (SD)**	**Mean (SD)**
Baseline	97.6 (7.9)	96.0(10.7)	91.5 (11.1) †	99.6 (9.0)
Post	95.0 (8.0)	92.2 (11.2)	88.4 (11.1) †	96.0 (10.3)
Δ	-2.7 (3.6) #	-3.7 (-2.7) #	-3.1 (2.7) #	-4.3 (2.7) #
FVC % predicted				
Baseline	97.7 (7.0)	100.0 (11.0)	98.0 (11.4)	102.0 (9.6)
Post	95.3 (7.5)	95.5 (10.9)	94.0 (11.2)	97.0 (11.0)
Δ	-2.4 (2.5) #	-3.3 (5.1) #	-4.0 (4.2) #	-4.6 (6.0) #
FEV_1/FVC % predicted				
Baseline	101.2 (4.4) *	97.3 (7.7)	94.9 (7.4) †	99.5 (7.4)
Post	100.0 (5.0)	97.0 (7.4)	94.5 (7.3) †	99.3 (7.0)
Δ	-1.4 (3.0)	-0.3 (4.4)	-0.4 (3.1)	-0.2 (5.3)

Data presented as mean (SD). t-test: * $p<0.05$; ** $p<0.01$ asthmatics versus the reference population; † $p<0.05$, †† $p<0.01$ non-eosinophilic versus eosinophilic asthmatics; # $p<0.01$ baseline versus post capsaicin challenge.

Figure legends

Figure 1. Concentrations (μM) of capsaicin eliciting (A) 2 coughs (C2) or (B) 5 coughs (C5) in participants with and without asthma, and eosinophilic asthma (EA) and non-eosinophilic asthma (NEA). Dashed lines at 1000 μM represent values assigned to those participants who did not achieve C2 or C5 during testing. Solid line represents geometric mean. Mann-Whitney test was used. * $p<0.05$

Figure 2. Concentration (μM) of capsaicin eliciting 5 (C5) coughs in participants with AHR (A) and Europeans only (B). Dashed lines at 1000 μM represent values assigned to those participants who did not achieve C2 or C5 during testing. Solid line represents geometric mean. Mann-Whitney test was used. * $p<0.05$